Pollinisation intégrée des cultures : intégrer les mécanismes liés à la température pour évaluer l'offre et la demande en pollinisation

Stan Chabert

THESE POUR OBTENIR LE GRADE DE DOCTEUR
DE L'UNIVERSITE D'AVIGNON ET DES PAYS DE VAUCLUSE
École doctorale 536 *Agrosciences & Sciences*
Discipline *Sciences Agronomiques*

Présentée et soutenue publiquement par
Stan CHABERT
Le 17 décembre 2018 en Avignon

Pollinisation intégrée des cultures : intégrer les mécanismes liés à la température pour évaluer l'offre et la demande en pollinisation

Sous la direction de
Mohamed EL MAATAOUI et Bernard VAISSIERE
Unité de Recherche 406 *Abeilles & Environnement*

Devant le jury composé de :

Anne-Laure JACQUEMART *Professeure, Université Catholique de Louvain, Belgique*	**Rapportrice**
Alexandra JULLIEN *Professeure, AgroParisTech, France*	**Rapportrice**
Jean-Christophe CONJEAUD *Responsable projets R&D, ANAMSO, France*	**Examinateur**
Michael GARRATT *Senior Researcher, University of Reading, United Kingdom*	**Examinateur**
Michel RENARD *Directeur de Recherche, INRA centre de Rennes, France*	**Examinateur**
Mohamed EL MAATAOUI *Professeur, Université d'Avignon, France*	**Directeur de thèse**
Bernard VAISSIERE *Chargé de Recherche, INRA centre d'Avignon, France*	**Co-directeur de thèse**

« Je ne cherche pas à connaître les réponses,
je cherche à comprendre les questions. »
Confucius (-551 ; -479)

« En vérité, le chemin importe peu,
la volonté d'arriver suffit à tout. »
Albert CAMUS
Le Mythe de Sisyphe, 1942

« Patience et longueur de temps
font plus force, ni que rage… »
Jean DE LA FONTAINE
Le Lion et le Rat, 1668

RESUME

Pollinisation intégrée des cultures : intégrer les mécanismes liés à la température pour évaluer l'offre et la demande en pollinisation

Les insectes contribuent à la pollinisation de 70% des espèces cultivées aujourd'hui à travers le monde. Avec l'intensification de l'agriculture au début du XX^{ème} siècle, les agriculteurs se sont mis à introduire des colonies d'abeilles mellifères dans leurs cultures entomophiles pour assurer le service de pollinisation. Avec la reconnaissance croissante du rôle majeur joué par les insectes sauvages dans la pollinisation des cultures, le concept de *pollinisation intégrée des cultures* a récemment vu le jour, encourageant à combiner insectes pollinisateurs introduits et sauvages en adaptant les pratiques agricoles pour assurer une pollinisation durable des cultures. Mais l'introduction d'insectes pollinisateurs d'élevage est une pratique encore très empirique qui manque de références techniques pour pouvoir être mise en œuvre avec précision. L'objectif général de cette thèse était de fournir des éléments pour mettre au point une méthode objective pour définir la charge en unités opérationnelle d'abeilles mellifère à introduire par unité de surface de culture cible pour complémenter la faune pollinisatrice sauvage pour que la pollinisation ne soit pas un facteur de production limitant.

Nous avons travaillé sur des lignées mâle fertile et mâle stérile de colza (*Brassica napus* L.), les productions de semence hybride dépendant entièrement des insectes pour la pollinisation chez cette espèce. Nous avons pu déterminer (*i*) le nombre minimum de grains de pollen viables devant être déposés par stigmate pour que la grenaison puisse être complète en fonction de la température, (*ii*) la durée après anthèse pendant laquelle le pollen doit être déposé sur le stigmate pour que la grenaison puisse être complète en fonction de la température, (*iii*) la durée pendant laquelle une fleur sécrète du nectar en fonction de la température pour chacune des deux lignées, et (*iv*) la vitesse de cette sécrétion nectarifère en fonction de la température pour chacune des deux lignées. Nous avons également validé une méthode d'évaluation rapide de la taille des cheptels d'abeilles mellifères introduits dans les cultures entomophiles, couramment utilisée dans certains pays, en tenant compte de la température.

A partir de ces éléments, nous avons proposé d'introduire les concepts d'*offre* et de *demande* en pollinisation d'une culture cible, afin de quantifier les déficits de pollinisation et le nombre d'insectes pollinisateurs à introduire pour compléter ces déficits. Ces premiers éléments fournissent une base pour construire un modèle mécaniste de gestion intégrée de la pollinisation, pour prédire le nombre d'insectes pollinisateurs à introduire dans une culture étant donné son contexte climatique, paysager et variétal.

Mots clés : *pollinisation intégrée des cultures, colza (Brassica napus), température, offre en pollinisation, demande en pollinisation, sécrétion nectarifère, abeilles mellifères (Apis mellifera), production de semence hybride, période effective de pollinisation*

ABSTRACT

Integrated crop pollination: integrate temperature mechanisms to assess pollination supply and demand

Insects contribute to the pollination of 70% of the species cultivated today around the world. With the agriculture intensification in the early twentieth century, farmers began to introduce honey bees colonies into their entomophilous crops to provide pollination service. With the growing recognition of the major role played by wild insects in crop pollination, the concept of *integrated crop pollination* recently emerged, encouraging the combination of introduced and wild pollinating insects by adapting agricultural practices to ensure sustainable pollination of crops. But the introduction of managed pollinating insects is still an empirical practice that lacks technical references to be implemented with precision. The general objective of this thesis was to provide elements to develop an objective method to define the operational unit load of honey bees to be introduced per unit area of target crop to complement wild pollinating fauna so that pollination is not a limiting factor for production.

We worked on a male fertile and a male sterile oilseed rape (*Brassica napus* L.) lines, hybrid seed productions being entirely dependent on insects for pollination in this species. We were able to determine (*i*) the minimum number of viable pollen grains to be deposited on stigma so that seed set was complete depending on the temperature, (*ii*) the duration after anthesis during which the pollen must be deposited on the stigma so that seed set was complete depending on the temperature, (*iii*) the length of time a flower secretes nectar as a function of temperature for each of the two lines, and (*iv*) the rate of this nectar secretion as a function of temperature for each of the two lines. We also validated a method for rapid assessment of the size of honey bee stocking rate introduced into entomophilous crops, commonly used in some countries, taking into account temperature.

From these elements, we proposed to introduce the concepts of *supply* and *demand* in pollination of a target crop, in order to quantify the pollination deficits and the number of pollinating insects to introduce to complete these deficits. These first elements provide a basis for constructing a mechanistic model of integrated pollination management, to predict the number of pollinating insects to be introduced into a crop given its climatic, landscape and varietal context.

Keywords: *integrated crop pollination, oilseed rape (Brassica napus), temperature, pollination supply, pollination demand, nectar secretion, honey bees (Apis mellifera), hybrid seed production, effective pollination period*

REMERCIEMENTS

TABLE DES MATIERES

RESUME…….… **5**

ABSTRACT…… **6**

REMERCIEMENTS **7**

INTRODUCTION GENERALE **13**

1. Origine évolutive et évolution de la pollinisation **15**

1.1. Des Cryptogames aux Phanérogames, fixation du mégagamétophyte sur le sporophyte : naissance de la pollinisation 15

1.2. La pollinisation chez les Préspermatophytes, Gymnospermes et Chlamydospermes 15

1.3. Innovation dans la pollinisation chez les Angiospermes : le carpelle 16

1.4. Définir la pollinisation et la fécondation 19

2. Vecteurs de pollen et attractifs chez les Angiospermes **20**

2.1. De la dominance de l'anémophilie chez les Préspermatophytes, Gymnospermes et Chlamydospermes à la dominance de l'entomophilie chez les Angiospermes 20

2.2. Attractifs primaires chez les Angiospermes : du pollen au nectar 21

2.3. Développement d'attractifs secondaires chez les Angiospermes : co-radiation évolutive avec les pollinisateurs 22

3. De la reconnaissance de la reproduction sexuée chez les végétaux dans l'Antiquité à la pollinisation intégrée des cultures aujourd'hui **23**

3.1. Découverte de la reproduction sexuée chez les végétaux, de la pollinisation et de l'entomophilie chez les Angiospermes : de l'Antiquité à la fin du XIX^{ème} siècle 23

3.2. Reconnaissance de l'entomophilie dans l'agriculture et premières introductions de colonies d'abeilles mellifères au début du XX^{ème} siècle 26

3.3. L'entomophilie dans l'agriculture aujourd'hui 27

3.4. Introduction de colonies d'abeilles mellifères dans les cultures entomophiles et charge en colonies à l'hectare 27

3.5. La pollinisation intégrée des cultures, nouveau paradigme du XXI^{ème} siècle 30

4. Contexte, problématique et objectifs du travail de thèse **32**

4.1. Contexte sociétal 32

4.2. Contexte scientifique 33

4.3. Contexte expérimental 34

4.4. Choix du modèle biologique 35

4.5. Orientation du travail de thèse 35

4.6. Objectifs de la thèse 36

CHAPITRE I : Mesure de la période effective de pollinisation et de la période de sécrétion nectarifère en fonction de la température ***41***

Abstract…… **44**

1. Introduction **45**

2. Materials and methods **49**

2.1. Identification of flower cohorts 49

2.2. Duration of the pistil receptivity period 50

2.3. Duration of the nectar secretion period 52

2.4. Data analysis 54

3. Results……. **56**

3.1. Duration of the pistil receptivity period 57

3.2. Duration of the nectar secretion period 57

3.3. Synchrony of nectar secretion with pistil receptivity ... 60
3.4. Synthesis on a common thermal time scale ... 60

4. Discussion… ... **64**
4.1. Ageing rate versus developmental rate ... 64
4.2. Duration of the pistil receptivity period ... 65
4.3. Duration of the nectar secretion period and synchrony with that of the pistil receptivity ... 66

5. Conclusions. ... **67**

Contributions. ... **68**

Funding ... **68**

Acknowledgements ... **68**

Appendix A. Supplementary data ... **69**

CHAPITRE II : Évaluation du nombre minimum de grains de pollen viables devant être déposés par stigmate en fonction de la température pour que la pollinisation ne soit pas un facteur limitant pour la grenaison ... ***73***

Abstract ... **76**

1. Introduction ... **77**

2. Materials and Methods ... **80**
2.1. Hand pollinations, pollen loads and seed set ... 81
2.2. Data analysis ... 83

3. Results ... **88**
3.1. Dose-response relationship between pollination intensity and seed set ... 89
3.2. Predicted effect of successive stigmatic pollen depositions in sequence on the threshold of pollen limitation ... 93

4. Discussion… ... **96**

Acknowledgements ... **99**

Author contributions ... **99**

Appendix A – Supplementary tables and figures ... **100**

Appendix B – Methodology for extraction, staining and filtration of pollen loads from dry stigmas ... **104**

Appendix C – Photography and image computational analysis of stigmatic pollen loads recovered on filter cloths ... **106**
C.1. Photography taking ... 106
C.2. Automated image analysis with ImageJ ... 106
C.3. Selection of the ImageJ procedure parameter values, and assessment of the error made by this procedure ... 107

Appendix D – Macro in Java language used by running the ImageJ software to count the number of pollen grains deposited on the filter cloths ... **110**

Appendix E – Calculations of the primitive function of Eqn 5 and of the associated threshold value P_0 ... **112**

Appendix F – Calculation of the pollen reproductive success rate *R* for each model ... **115**

Appendix G – Calculations of the expected number of pollen tubes reaching the ovary *B* and of the threshold values P_{tot} and P_d for Eqns 6a and 6b in the case of a sequence of successive pollen depositions ... **118**

CHAPITRE III : Mesure de la vitesse de sécrétion nectarifère en fonction de la température *123*

Résumé..... **126**

Abstract..... **127**

1. Introduction..... **128**
1.1. Origine évolutive des nectaires floraux..... 128
1.2. La sécrétion nectarifère florale est-elle forcément synonyme d'entomophilie ?..... 128
1.3. La particularité des productions de semence hybride..... 129
1.4. Mesurer la sécrétion nectarifère..... 129
1.5. Les lignées MS sont-elles aussi nectarifères que les lignées MF ?..... 131

2. Matériel et méthodes..... **132**
2.1. Identification de cohortes de fleurs..... 132
2.2. Mesure du taux de sécrétion brut de nectar..... 135
2.3. Analyses statistiques..... 138

3. Résultats..... **138**

4. Discussion..... **140**
4.1. L'effet unimodal de la température sur la sécrétion nectarifère..... 140
4.2. La lignée MS sécrète deux fois moins vite que la lignée F1..... 140
*4.3. Une méthode rigoureuse pour comparer la sécrétion nectarifère de différentes lignées ou variétés*141
4.4. Perspectives : quels autres facteurs prendre en compte ?..... 142

Remerciements..... **143**

CHAPITRE IV : Validation d'une méthode simple et rapide d'évaluation de la taille de population adulte des colonies d'abeilles mellifères *145*

Abstract..... **148**

1. Introduction..... **149**

2. Materials and methods..... **151**
2.1. Study site, biological model, and hive type..... 151
2.2. The IFB method – counting the number of inter-frames covered with adult honey bees..... 152
2.3. Measure of the bee population size..... 152
2.4. Relationship between IFB and bee population size..... 154
2.5. Reliability of estimating the bee population size from IFB..... 155
2.6. Assessment of robustness – climate effect..... 157
2.7. Assessment of robustness – effect of hive type..... 158
2.8. Assessment of objectivity – experienced versus naive observers..... 159
2.9. Application for ecological issues of colony loss and crop pollination..... 159

3. Results..... **160**
3.1. Relationship between IFB and bee population size..... 160
3.2. Reliability of estimating the bee population size from IFB..... 161
3.3. Assessment of robustness..... 163
3.4. Application for ecological issues of colony loss and crop pollination..... 166

4. Discussion..... **168**

5. Conclusion..... **171**

Author contributions..... **171**

Funding..... **171**

Acknowledgements..... **172**

Appendix A. Tables and figures..... **173**

Appendix B. Calculations to get Eqs. (1), (2), (3) and (4) **181**

Appendix C. Calculations to get Eqs. (8), (9), (10) and (11) **183**

DISCUSSION GENERALE ***185***

1. Proposition d'un nouveau concept : l'offre et la demande en pollinisation **186**

1.1. La demande en pollinisation 186

1.2. L'offre en pollinisation, l'offre en pollinisateurs 188

2. L'offre et la demande en pollinisation comme cadre conceptuel quantitatif pour la pollinisation intégrée des cultures **189**

2.1. Lacune dans le concept de pollinisation intégrée des cultures 189

2.2. Vers un modèle de gestion intégrée de la pollinisation 190

2.3. La température, facteur prépondérant 191

2.4. Optimiser l'offre en abeilles mellifères 192

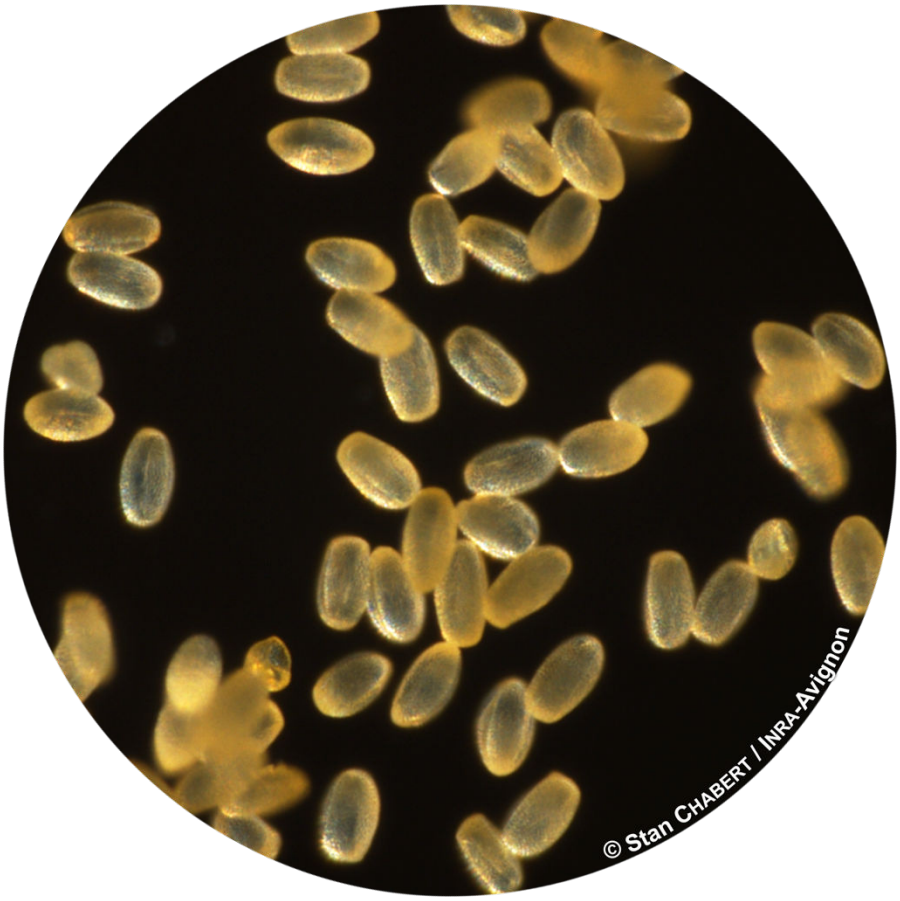
3. Le nectar, « entremetteur » entre offre et demande en pollinisation **192**

3.1. Sécrétion nectarifère et fréquentation en production de semence 193

3.2. Hypothèse pour augmenter les transferts de pollen en production de semence 194

3.3. Apiculture : ajuster la demande à l'offre en nectar 195

BIBLIOGRAPHIE ***197***



INTRODUCTION GENERALE

1. Origine évolutive et évolution de la pollinisation

1.1. Des Cryptogames aux Phanérogames, fixation du mégagamétophyte sur le sporophyte : naissance de la pollinisation

La pollinisation est le processus qui précède la fécondation, fusion d'un anthérozoïde (= gamète mâle) avec l'oosphère (= gamète femelle), dans la reproduction sexuée des Phanérogames[1] (Faegri & van der Pijl, 1979 ; Dumas & Zandonella, 1984 ; Ramawat *et al.*, 2014). A l'instar des Cryptogames[2], cette étape correspond à la phase de dissémination des spores dans l'environnement, une fois libérées du sporange porté par le sporophyte[3]. La différence est que chez les Phanérogames, le mégagamétophyte (= gamétophyte[4] femelle), qui contient l'oosphère, est inclus et fixé dans le mégasporange du sporophyte, tandis que chez les Cryptogames, la mégaspore (= spore femelle) est, à l'instar de la microspore (= spore mâle), libérée dans l'environnement. Chez les Phanérogames, le mégagamétophyte étant fixé sur le sporophyte dans une structure appelée ovule, c'est au microgamétophyte (= gamétophyte mâle), appelé pollen, d'être transporté au niveau du mégagamétophyte (Friedman, 1993). Cette situation nécessite un transport ciblé et précis du grain de pollen vers l'ovule. Chez les Cryptogames, les gamétophytes mâle et femelle sont tous deux libérés dans l'environnement et peuvent donc être dispersés à proximité l'un de l'autre. Les anthérozoïdes ciliés libérés par le microgamétophyte peuvent alors compenser la distance restante entre les deux gamétophytes par leur mobilité.

1.2. La pollinisation chez les Préspermatophytes, Gymnospermes et Chlamydospermes

Chez les Préspermatophytes, le pollen est réceptionné par une gouttelette micropylaire, solution aqueuse sucrée, qui, en se résorbant, achemine le pollen au niveau du nucelle (= mégasporange) (Friedman, 1987 ; Gelbart & von Aderkas, 2002 ; Little *et al.*, 2014 ; Lora *et al.*, 2016). Seule la réception de pollen conspécifique permet une résorption complète de la gouttelette micropylaire (Lora *et al.*, 2016). Le pollen germe ensuite en tube pollinique qui parasite le nucelle, puis libère deux anthérozoïdes flagellés libres qui nagent vers le

[1] Du grec *phaneros*, « apparent », et *gamos*, « union ». Groupe de plantes qui comprend les Préspermatophytes (*Ginkgo*, *Cycas*), les Gymnospermes (= conifères), les Chlamydospermes (*Ephedra*, *Gnetum*, *Welwitschia*) et les Angiospermes.
[2] Du grec *cryptos*, « caché », et *gamos*. Groupe de plantes qui comprend les Mycophytes (= Champignons), les Phycophytes (= Algues), les Bryophytes (= Mousses) et les Ptéridophytes (= Fougères, Prêles, Lycopodes et Psilotes).
[3] Appareil végétatif, stade diploïde des plantes dans leur cycle de vie.
[4] Stade haploïde des plantes dans leur cycle de vie.

mégagamétophyte et l'oosphère que l'un d'eux féconde : c'est la zoïdogamie (Friedman, 1987, 1993 ; Little *et al.*, 2014). Chez les Gymnospermes et les Chlamydospermes, le pollen est toujours réceptionné par une gouttelette micropylaire, qui, en se résorbant, achemine le pollen au niveau du nucelle (Owens *et al.*, 1998 ; Little *et al.*, 2014 ; Lora *et al.*, 2016). Et de même, seule la réception de pollen conspécifique permet une résorption complète de la gouttelette micropylaire (Lora *et al.*, 2016). Mais le tube pollinique issu du pollen traverse complètement le nucelle, pénètre le col de l'archégone (= gamétange femelle) et libère deux cellule spermatiques (= gamètes mâles) non mobiles au niveau de l'oosphère que l'un d'eux féconde : c'est la siphonogamie (Friedman, 1993 ; Gelbart & von Aderkas, 2002 ; Fernando *et al.*, 2005 ; Little *et al.*, 2014). Toutes les espèces de ces trois groupes sont strictement soit dioïques, soit monoïques, ce qui permet de limiter l'autofécondation (= fécondation de l'ovule par du pollen provenant de la même plante).

1.3. Innovation dans la pollinisation chez les Angiospermes : le carpelle

Chez les Angiospermes (= plantes à fleurs), l'ovule est enveloppé dans un carpelle, ou plusieurs carpelles lorsqu'il y a plusieurs ovules. Le carpelle ou l'ensemble des carpelles est appelé gynécée ou pistil. Cette structure est développée par le sporophyte, et comprend l'ovaire, sous-structure enveloppant l'ovule, le stigmate, surface qui réceptionne le pollen, et le style, tissu qui relie l'ovaire au stigmate (Endress & Igersheim, 2000 ; Friis *et al.*, 2011a ; Lora *et al.* 2016). Lorsque le pollen est réceptionné au niveau du stigmate, le pollen est hydraté et germe en tube pollinique. Ce tube pollinique traverse le style, puis l'ovaire, pénètre dans le micropyle de l'ovule, traverse le nucelle, puis pénètre dans une des deux cellules synergides où il libère deux cellules spermatiques non mobiles (Figure I) (Kessler & Grossniklaus, 2011 ; Dresselhaus & Franklin-Tong, 2013 ; Qu *et al.*, 2015 ; Lora *et al.* 2016). Il y a donc également siphonogamie chez les Angiospermes. Une cellule spermatique fusionne avec l'oosphère pour former l'embryon, diploïde, tandis que la seconde fusionne avec la cellule centrale de l'ovule contenant deux noyaux polaires pour former une cellule triploïde : c'est la double fécondation, propre aux Angiospermes (Raghavan, 2003, 2006), à quelques rares autres exceptions près chez les Chlamydospermes (ex. Friedman, 1990 ; Frideman & Carmichael, 1996). La cellule centrale triploïde de l'ovule se développe ensuite en albumen de la graine, tissu nourricier pour l'embryon. Le stigmate et le style apportent les nutriments et les molécules nécessaires, comme les ions calcium Ca^{2+} et le bore, à la germination du pollen et à la croissance et au guidage du tube pollinique vers l'ovaire, sans lesquels il ne pourrait l'atteindre (Labarca & Loewus, 1973 ; Dresselhaus & Franklin-Tong, 2013 ; Higashiyama & Takeuchi, 2015 ; Qu *et al.*, 2015 ; Lora

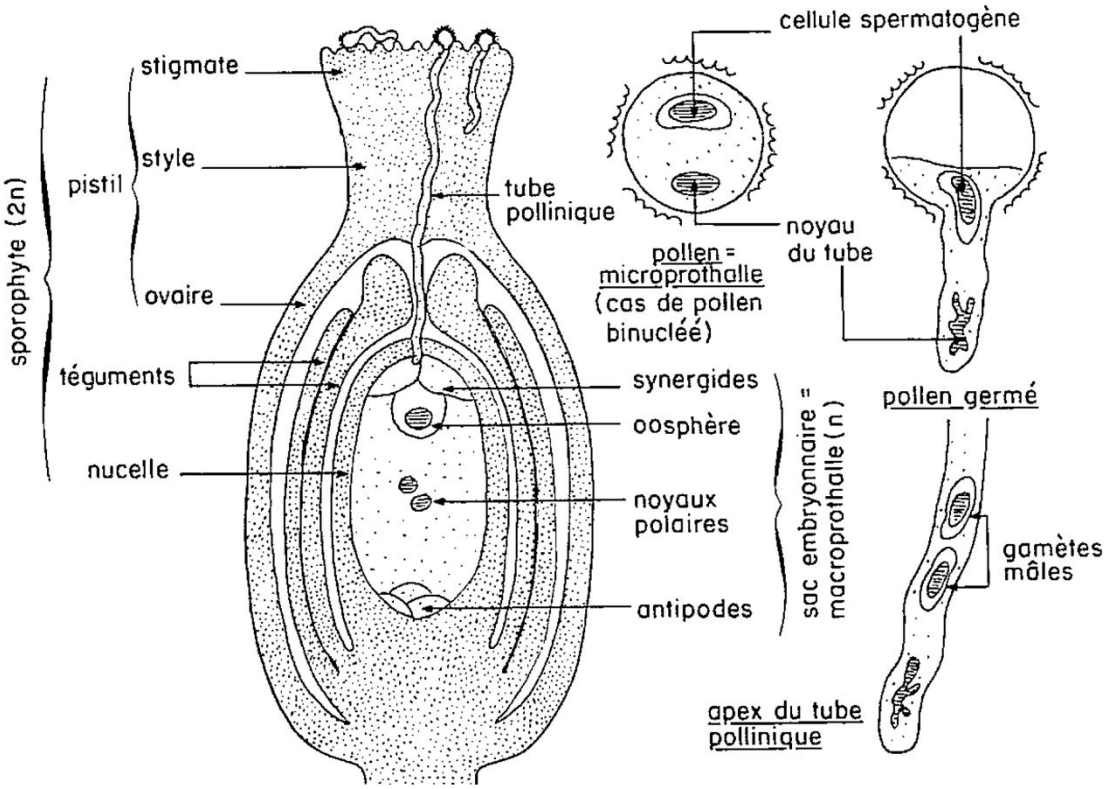


Figure I – Fécondation chez une Angiosperme. Représentation semi-schématique de coupes sagittales d'un pistil, d'un ovule (de type *Polygonum*) et de pollens (d'après Dumas & Zandonella).

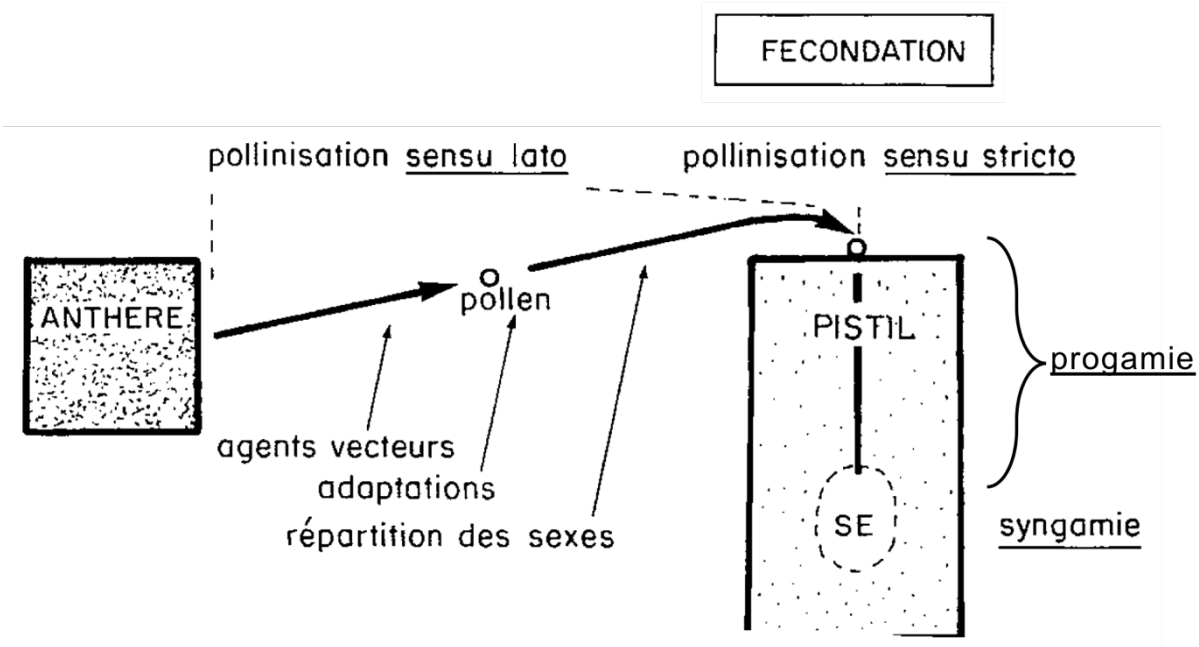


Figure II – Définition schématique de la pollinisation et de la fécondation chez une Angiosperme (d'après Dumas & Zandonella).

et al. 2016). Dans l'ovaire, les cellules synergides de l'ovule émettent des peptides qui permettent de guider le tube pollinique jusqu'à l'oosphère à travers le micropyle (Higashiyama *et al.*, 2001; Okuda *et al.*, 2009; Kessler & Grossniklaus, 2011 ; Dresselhaus & Franklin-Tong, 2013 ; Higashiyama & Takeuchi, 2015 ; Qu *et al.*, 2015).

Parallèlement, la mise en place du carpelle chez les Angiospermes permet de contrôler et de sélectionner les gamétophytes mâles pour la fécondation des ovules à travers deux mécanismes. Le premier de ces mécanismes est l'auto-incompatibilité, apparue de façon indépendante de nombreuses fois dans différentes lignées évolutives, et que l'on estime répandue chez une moitié des espèces Angiospermes (Allen & Hiscock, 2008 ; Ferrer & Good, 2012 ; Dresselhaus & Franklin-Tong, 2013 ; Gibbs, 2014). L'auto-incompatibilité permet d'empêcher l'autofécondation en empêchant la germination et la croissance de tubes polliniques issus de pollen provenant du même individu ou d'un même clone (Takayama & Isogai, 2005 ; Franklin-Tong, 2008 ; Iwano & Takayama, 2012), et peut intervenir au niveau du sporophyte, du gamétophyte, ou même après la fécondation (Gibbs, 2014). Le deuxième mécanisme est la compétition des gamétophytes mâles dans un style aux ressources en nutriments et à l'espace limités (Erbar, 2003 ; Harder *et al.*, 2016a,b). Les tubes polliniques atteignant les ovules le plus rapidement sont supposés être ceux détenant un génotype conférant une meilleure vigueur hybride à la descendance (Mulcahy, 1979), bien que cela reste encore à démontrer (Baskin & Baskin, 2015). Un pistil syncarpe (= plusieurs carpelles fusionnés) et un style long permettent de renforcer cet effet de compétition gamétophytique mâle (Mulcahy, 1979 ; Endress, 1982 ; Herrero & Hormaza, 1996). Le carpelle permet donc aux Angiospermes d'accroître le contrôle du sporophyte sur le gamétophyte (Herrero & Hormaza, 1996 ; Lora *et al.* 2016).

Originellement majoritairement hermaphrodites (Thien *et al.*, 2000 ; Endress, 2001) et auto-incompatibles (Allen & Hiscock, 2008), les fleurs d'Angiospermes ont également développé des barrières physiques qui permettent de limiter l'autogamie (= fécondation par des gamètes mâles provenant de la même fleur) (Shivanna, 2014) :

- la diclinie = monoécie lorsque le pistil et les étamines (= partie mâle de la fleur) sont disposées sur des fleurs séparées au sein d'un même individu ; dioécie lorsque les individus sont sexuellement femelles ou mâles ;
- la dichogamie = protogynie lorsque la fleur est hermaphrodite avec la phase femelle précédant la phase mâle ; protandrie lorsque la fleur est hermaphrodite avec la phase mâle précédant la phase femelle ;
- l'herkogamie = fleur hermaphrodite dont le stigmate est spatialement situé au-dessus des anthères, ou inversement.

Ceci a conduit Darwin à conclure que « *la Nature nous indique de la façon la plus catégorique qu'elle abhorre la perpétuelle autofécondation* » (Proctor *et al.*, 2012). La pollinisation croisée, ou allogamie, ou encore xénogamie (= pollinisation et fécondation par du pollen provenant d'un individu différent de celui portant les ovules), permet en effet d'augmenter la vigueur et la qualité de la descendance (ex : Schemske, 1983 ; Chautá-Mellizo *et al.*, 2012), fournissant une validation supplémentaire de la théorie de la sélection naturelle de Darwin (1859). La geitonogamie[5], qui correspond à la pollinisation et fécondation par du pollen provenant d'une autre fleur du même individu ou d'un même clone que celle qui porte le(s) ovule(s), a les même effets que l'autogamie.

1.4. Définir la pollinisation et la fécondation

Il existe deux acceptions pour définir le terme de « pollinisation » (Dumas & Zandonella, 1984 ; Figure II). Dans son acception stricte, il s'agit de la réception du pollen par la surface réceptrice du tissu femelle, c'est-à-dire soit la goutte micropylaire chez les Préspermatophytes, Gymnospermes et Chlamydospermes, soit le stigmate chez les Angiospermes. Dans son acception large, il s'agit de la libération du pollen par les sacs polliniques (= anthères chez les Angiospermes), de son transport jusqu'à la surface réceptrice du tissu femelle, la goutte micropylaire chez les Préspermatophytes, Gymnospermes et Chlamydospermes, le stigmate chez les Angiospermes, où il est réceptionné. Suit la fécondation, qui s'opère en deux étapes successives (Dumas, 1984) :

- la phase progamique, qui correspond à la pollinisation *sensu stricto* suivie de la germination du grain de pollen en tube pollinique, puis à la croissance du tube pollinique jusque dans le col de l'archégone pour les Gymnospermes et les Chlamydospermes, ou jusqu'aux cellules synergides pour les Angiospermes ;
- la phase syngamique, qui correspond à la libération des deux anthérozoïdes suivie de la fusion de l'un d'eux avec l'oosphère chez les Préspermatophytes, à la libération des deux cellules spermatiques dans le col de l'archégone suivie de la fusion de l'une d'elles avec l'oosphère chez les Gymnospermes et Chlamydospermes, ou à la libération des cellules spermatiques dans une cellule synergide suivie de la double fécondation chez les Angiospermes.

[5] Du grec *geiton*, « voisin ».

2. Vecteurs de pollen et attractifs chez les Angiospermes

2.1. De la dominance de l'anémophilie chez les Préspermatophytes, Gymnospermes et Chlamydospermes à la dominance de l'entomophilie chez les Angiospermes

Chez les Phanérogames, il existe quatre vecteurs de pollen pour que le pollen puisse atteindre la goutte micropylaire chez les Préspermatophytes, Gymnospermes et Chlamydospermes, ou le stigmate chez les Angiospermes : les animaux (zoophilie), qui incluent des insectes (entomophilie), mais aussi des oiseaux (ornithophilie), des chauve-souris (chiropterophilie), des souris, des écureuils, des serpents et des lézards (Shivanna, 2014), le vent (anémophilie), l'eau (hydrophilie), la gravité, et le contact direct entre anthères et stigmate dans le cas des Angiospermes. Le vecteur privilégié chez les Préspermatophytes, Gymnospermes et Chlamydospermes, groupes qui comportent tous exclusivement des espèces dioïques ou monoïques, est le vent (Owens *et al.*, 1998 ; Fernando *et al.*, 2005 ; Little *et al.*, 2014). Néanmoins, certaines espèces de ces trois groupes ont développé une entomophilie, les insectes pouvant être attirés à la fois par le pollen présent dans les sacs polliniques et par la goutte micropylaire sucrée sécrétée par les ovules (Thien *et al.*, 2000, 2009 ; Labandeira *et al.*, 2007 ; Peris *et al.*, 2017).

Les Angiospermes demeurent toutefois le groupe de Phanérogames qui a le plus développé la zoophilie et l'entomophilie. Considéré comme un groupe monophylétique (Friis & Endress, 1990 ; Friis *et al.*, 2011a ; Soltis *et al.*, 2018), les Angiospermes seraient apparues vers le début du Jurassique Inférieur il y a environ 200 Ma, puis se seraient soudainement diversifiées et mises à dominer les communautés végétales au cours du Crétacé Inférieur à partir d'environ -125 Ma (Friis *et al.*, 2006, 2011b ; Bell *et al.*, 2010 ; Magallón, 2010 ; Smith *et al.*, 2010 ; Doyle, 2012 ; Magallón *et al.*, 2015). Les Angiospermes ancestrales auraient été principalement entomophiles, pollinisées majoritairement par des coléoptères, diptères et thysanoptères (Thien, 1980 ; Thien *et al.*, 2000, 2009 ; Endress, 2001, 2010 ; Hu *et al.*, 2012), mais auraient également pu être ambophiles, c'est-à-dire pollinisées à la fois par les insectes et le vent (Hu *et al.*, 2012). L'anémophilie stricte, ainsi que l'hydrophilie, ne seraient en revanche que des caractères dérivés développés plus tardivement (Thien *et al.*, 2000 ; Endress, 2010 ; Hu *et al.*, 2012). L'anémophilie stricte chez les Angiospermes ne se retrouve en effet aujourd'hui essentiellement que chez quatre familles dérivées monocotylédones (Poaceae, Cyperaceae, Betulaceae et Juncaeae) (Shivanna, 2014), tandis que la zoophilie est estimée répandue chez 87,5 % des espèces Angiospermes actuelles (Ollerton *et al.*, 2011). La pollinisation par contact

direct entre anthères et stigmate existe par exemple chez les espèces dites cléistogames (du grec *kleistós*, « fermé », et *gámos*, « union »), où la fleur ne s'ouvre pas.

2.2. Attractifs primaires chez les Angiospermes : du pollen au nectar

L'attractif utilisé par les Angiospermes ancestrales pour attirer les insectes était principalement le pollen, comme ressource trophique, mais également dans une certaine mesure la thermogénèse, comme ressource énergétique pour des organismes ectothermes (Thien *et al.*, 2000, 2009). Si du nectar pouvait être sporadiquement sécrété chez certaines Angiospermes ancestrales par du tissu nectarifère situé principalement à la surface des tépales (Endress, 2001), les nectaires floraux différenciés, structure de la fleur spécialisée dans la sécrétion nectarifère, ne seraient en revanche apparus en tant que tels que vers le début du Crétacé Supérieur, il y a environ 100 Ma (Friis *et al.*, 2006). Cette période correspond à celle identifiée pour la radiation évolutive des Anthophiles (= abeilles) (Poinar & Danforth, 2006 ; Michez *et al.*, 2012 ; Branstetter *et al.*, 2017 ; Peters *et al.*, 2017), groupe monophylétique apparu il y a environ 123 Ma (Cardinal & Danforth, 2013), c'est-à-dire au moment où les Angiospermes se sont mises à dominer et à se diversifier (Cappellari *et al.*, 2013 ; et voir les références citées plus haut). Les Anthophiles sont les insectes qui butinent le plus communément les fleurs des Angiospermes actuelles (Willmer *et al.*, 2017), et, par leurs structures morphologiques spécialisées comme les poils branchus ou l'acquisition de comportements de butinage spécialisés les rendant particulièrement efficaces dans l'accumulation passive de pollen sur leur corps (Thorp, 2000 ; Michez *et al.*, 2012), et par leur comportement de constance florale au cours du butinage (Waser, 1986), ce sont également les pollinisateurs les plus efficaces dans la quantité de pollen déposée par stigmate par visite de fleur, et les plus spécifiques en ne déposant que peu de pollen hétérospécifique par stigmate par visite (Willmer *et al.*, 2017). Mais comme leur nom l'indique, ce sont des insectes spécialisés dans la consommation de pollen, et l'acquisition de leurs structures morphologiques et comportements de butinage propres leur ont permis de pouvoir récolter activement et efficacement le pollen et de l'accumuler dans des structures appelées scopae et corbiculae (Thorp, 2000 ; Michez *et al.*, 2012). Dans certaines situations, cette récolte active de pollen peut se faire au détriment de la pollinisation (Hargreaves *et al.*, 2009, 2010). L'apparition de ces insectes spécialisés a donc pu conduire les Angiospermes, dans une économie de pollen devenue nécessaire, à proposer un attractif moins coûteux - le nectar - en développant les nectaires (Takhtajan, 1980).

Le pollen et le nectar sont aujourd'hui les principaux attractifs utilisés par les Angiospermes pour attirer les pollinisateurs. Mais il existe aussi d'autres attractifs plus

marginalement utilisés, comme des huiles, des résines, de la cire, ou des composés parfumés (Armbruster, 2012 ; Shivanna, 2014). Ces attractifs sont dits « attractifs primaires », dans le sens où ils sont utilisés comme ressource par les pollinisateurs (Faegri & van der Pijl, 1979 ; Armbruster, 2012).

2.3. Développement d'attractifs secondaires chez les Angiospermes : co-radiation évolutive avec les pollinisateurs

Il existe également les attractifs dits « secondaires », également appelés signaux floraux, qui servent comme intermédiaires entre l'attractif primaire et le pollinisateur pour indiquer au pollinisateur la présence, la localisation et la qualité d'une ressource. Deux types d'attractifs secondaires ont été identifiés (Shivanna, 2014 ; Borghi *et al.*, 2017 ; Leonard & Francis, 2017 ; Valenta *et al.*, 2017) :

- les indices visuels : la couleur, la morphologie, la taille et l'ornementation des pétale ou des sépales pétaloïdes, ou parfois des bractées ou du pollen ;
- les indices olfactifs : parfums volatiles produits par les pétales, le pollen ou le nectar.

Un indice supplémentaire est utilisé par les pollinisateurs : le champs électrique émis par les fleurs (Clarke *et al.*, 2013 ; Greggers *et al.*, 2013).

Les pollinisateurs associent un attractif secondaire à une ressource disponible donnée et à une capacité d'accès à cette ressource par apprentissage ou par préférence innée (Schiestl, 2012 ; Clarke *et al.*, 2013 ; Greggers *et al.*, 2013 ; Knauer & Schiestl, 2015 ; Muth *et al.*, 2015). La mise en place de ces attractifs secondaires permet aux Angiospermes d'attirer les pollinisateurs spécifiquement adaptés à leur morphologie et à leur(s) attractif(s) primaire(s), et ainsi de maximiser quantitativement leur pollinisation, et qualitativement en minimisant le dépôt de pollen hétérospécifique et en maximisant la pollinisation croisée (Johnson, 2006 ; Schiestl, 2012). Parallèlement, les pollinisateurs ont adapté au cours de l'évolution leur propre morphologie (ex : un long proboscis) et leur propre comportement (ex : préférence de butinage pour des attractifs primaires et secondaires spécifiques) à la morphologie des fleurs et aux ressources qu'elles offrent : on parle de co-évolution ou de co-radiation évolutive (Bronstein *et al.*, 2006 ; Michez *et al.*, 2012 ; Schiestl, 2012 ; Cappellari *et al.*, 2013).

3. De la reconnaissance de la reproduction sexuée chez les végétaux dans l'Antiquité à la pollinisation intégrée des cultures aujourd'hui

3.1. Découverte de la reproduction sexuée chez les végétaux, de la pollinisation et de l'entomophilie chez les Angiospermes : de l'Antiquité à la fin du XIX^{ème} siècle

Le rôle du pollen dans la production de graines et de fruits a été reconnu pour la première fois chez le dattier palmier durant l'Antiquité, chez les Babyloniens et les Grecs (Zirkle, 1935 ; Proctor *et al.*, 2012). Ils ont également reconnu l'existence d'une reproduction sexuée chez cette espèce dioïque, par la distinction de plantes « mâles » et « femelles ». Mais cette reconnaissance du sexe chez les plantes est restée réduite uniquement au palmier dattier jusqu'au XVII^{ème} siècle. En 1676, le botaniste anglais Nehemiah Grew, reprenant une suggestion du professeur de philosophie naturelle Thomas Millington, a soutenu devant la Société Royale que les étamines jouaient un rôle, comme partie mâle, dans la production de graines, et que la plante ne produisait de graines que si du pollen atteignait le stigmate de la fleur (Proctor *et al.*, 2012 ; Crane, 1999), affirmations publiées ensuite en 1682 dans son ouvrage The Anatomy of Plants with an Idea of Philosophical History of Plants. And several other Lectures, Read before the Royal Society. Mais ces affirmations ne reposaient que sur de « l'idée », sans être vérifiées par de l'expérimentation. Les preuves expérimentales ont été apportées en 1694 par le professeur allemand de physique Rudolph Jacob Camerarius, dans une lettre intitulée Epistola de Sexu Plantarum adressée à un autre professeur de physique. Il y indiquait que lorsqu'il retirait les étamines ou les stigmates de fleurs de plantes monoïques, les fleurs femelles ne produisaient pas de graines. Il indiquait de même que lorsque les plantes femelles d'espèces dioïques se trouvaient en absence de plantes mâles, ces plantes femelles ne produisaient pas non plus de graines (Proctor *et al.*, 2012 ; Crane, 1999). Le professeur de botanique anglais Richard Bradley a ensuite retrouvé les mêmes résultats en émasculant des fleurs hermaphrodites de tulipe, rapportés dans son ouvrage New improvements of Planting and Gardening publié en 1717 (Proctor *et al.*, 2012).

Le botaniste britannique Philip Miller fut ensuite le premier à décrire dans une lettre en 1721 le phénomène de pollinisation croisée par le transport de pollen par des abeilles de tulipes non émasculées sur des stigmates de tulipes émasculées, et reporta ensuite ses observations dans ses ouvrages Gardener's and Florist's Dictionary (1724) et Gardener's Dictionary (1731). En 1750, l'irlandais Arthur Dobbs observa le transport spécifique de pollen entre fleurs

hermaphrodites par des abeilles, décrivant ainsi pour la première fois le phénomène de constance ou fidélité spécifique florale des abeilles lors de leur butinage (Crane, 1993).

Le professeur d'histoire naturelle allemand Joseph Gottlieb Kölreuter fut ensuite le premier à décrire le rôle nécessaire des insectes dans la pollinisation et la fécondation de nombreuses espèces dans ses écrits publiés entre 1761 et 1766. Il reconnut également le rôle du nectar dans l'attraction des insectes (Proctor *et al.*, 2012). L'entomophilie fut ensuite redécouverte par celui que l'on considère comme le « père » de la biologie florale et de l'écologie de la pollinisation, le naturaliste allemand Christian Konrad Sprengel. Dans son célèbre ouvrage intitulé Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen (« *Le secret de la Nature découvert dans la structure et la fécondation des fleurs* » ; Figure III) publié en 1793, Sprengel décrivit notamment le rôle du nectar dans l'attraction des insectes, le rôle de la corolle et de l'odeur de la fleur pour guider les insectes vers le nectar, les adaptations florales de plus de 500 espèces pour attirer les insectes, la protogynie, la large distribution de la protandrie, ainsi que l'anémophilie. Il alla jusqu'à conclure que « *la Nature semble réticente à ce que les fleurs soient fécondées par leur propre pollen* ».

Le botaniste anglais Thomas Andrew Knight découvrit ensuite en 1799 les effets bénéfiques de la pollinisation et de la fécondation croisées pour la qualité de la descendance (Proctor *et al.*, 2012). Les travaux de Sprengel et Knight n'ont été ensuite repris que bien plus tard, par Charles Robert Darwin dans ses deux ouvrages, On the various contrivances by which orchids are fertilised by insects (1862) et On the effects of cross and self fertilisation in the vegetable kingdom (1876). En comparant des fleurs librement visitées par les insectes et des fleurs recouvertes d'un filet pour empêcher le butinage des insectes, Darwin y a notamment confirmé que les plantes ayant reçu une pollinisation croisée ont une descendance plus vigoureuse que plantes ayant reçu uniquement de l'auto-pollen. Il a également confirmé le comportement de constance ou fidélité florale des abeilles au cours de leur butinage qui favorise la pollinisation croisée entre individus d'une même espèce, préalablement découvert par Dobbs. Les travaux de Darwin ont stimulé un regain d'intérêt pour les disciplines de la biologie florale et de l'écologie de la pollinisation dans la deuxième moitié du XIX^{ème} siècle avec une profusion de nouveaux auteurs, parmi lesquels on peut citer les naturalistes Hermann et Fritz Müller, Friedrich Hildebrand, Frederico Delpino, Anton Kerner von Marilaun (The Natural History of Plants: Their Forms, Growth, Reproduction and Distribution, 1896), Paul Knuth (Handbook of Flower Pollination, 1893-1896), et le botaniste Gaston Bonnier (Les Nectaires, Étude critique, Anatomique et Physiologique, 1879).

Figure III – Page de titre de l'ouvrage de Sprengel, le « père » de la biologie florale et de l'écologie de la pollinisation. Noter la « guêpe » ichneumon sur une fleur d'Orchidée (II), l'abeille butinant une fleur de sauge (XV) et la « guêpe » visitant une fleur de scrofulaire (XXV).

Le XIX^{ème} siècle a également connu la découverte du développement des tubes polliniques à travers le tissu du style jusque dans l'ovaire avec la libération d'un noyau spermatique par les tubes polliniques dans un ovule par Amici en 1824 (Observations microscopiques sur diverses espèces de plantes), puis par Eduard Strasburger en 1884, ainsi que celle du phénomène de double fécondation du noyau de la cellule-œuf et des noyaux polaires de la cellule centrale par les deux noyaux spermatiques délivrés par le tube pollinique simultanément par Sergeï Nawaschin en 1898 en Russie, Jean Louis Léon Guignard en 1899 en France et Ethel Sargant en 1899 en Angleterre (Raghavan, 2003, 2006).

3.2. Reconnaissance de l'entomophilie dans l'agriculture et premières introductions de colonies d'abeilles mellifères au début du XX^{ème} siècle

L'importance du rôle joué par les insectes dans la production des cultures entomophiles a en revanche été reconnue plus tardivement chez les agriculteurs, en décalage avec les découvertes scientifiques des XVIII^{ème} et XIX^{ème} siècles. Les premières locations de colonies d'abeilles mellifères d'agriculteurs auprès d'apiculteurs professionnels aujourd'hui connues remontent au début du XX^{ème} siècle, dans l'État de l'Oregon aux États-Unis, chez les producteurs de fruits à pépins[6] (Kellar, 2018). Ce type de transaction a été initié à cette période à la faveur de quatre circonstances :

- comme indiqué précédemment, le développement des connaissances scientifiques montrant l'importance du rôle joué par les insectes, et notamment les abeilles mellifères, dans la pollinisation croisée (ou xénogamie) des plantes à fleurs, et en particulier dans les vergers de fruits à pépins, dont les espèces sont auto-incompatibles (Waite, 1898) ;
- l'augmentation et la concentration de la demande en pollinisation des vergers de fruits à pépins de plus en plus vastes et de plus en plus intensifiés ;
- la diminution du nombre d'insectes pollinisateurs sauvages disponibles du fait de l'intensification de l'agriculture et du déploiement de l'usage d'insecticides ;
- la diminution du nombre de petits apiculteurs amateurs initialement largement répandus dans les zones rurales à la faveur du développement d'une apiculture professionnelle plus concentrée, et la diminution du nombre d'agriculteurs élevant leur propre cheptel apicole sur leur exploitation (Cook, 1897).

[6] Fruits à pépins = pommes et poires.

La reconnaissance du rôle joué par les insectes dans la production des fruits à pépins dans cette région a été diffuse et s'est échelonnée sur plusieurs décennies entre la fin du XIX^{ème} siècle et le début du XX^{ème} siècle. En effet, une thèse développée par un entomologiste du Missouri selon laquelle les abeilles mellifères abîmeraient les fruits, en particulier ceux à péricarpe fin comme le raisin, la pêche ou la prune en incisant leur péricarpe pour y récolter du jus sucré, s'est même répandue chez les agriculteurs jusqu'aux années 1920 (Kellar, 2018).

3.3. L'entomophilie dans l'agriculture aujourd'hui

On sait aujourd'hui que 70% (87 sur 124) des principales[7] cultures mondiales de fruits, légumes et graines sont dépendantes - au moins partiellement - de la pollinisation animale pour la production, tandis que 23% (28 sur 124) n'en dépendant pas, la zoophilie étant inconnue pour 7% des cultures (Klein *et al.*, 2007). Les cultures identifiées comme zoophiles sont pour l'essentiel entomophiles, puisque seules deux cultures parmi les 87 identifiées comme zoophiles ne sont pas pollinisées par des insectes : le feijoa est pollinisé par des oiseaux, et le durian semble être pollinisé par des chauve-souris. Les cultures non zoophiles correspondent principalement aux céréales (= Poaceae), strictement anémophiles, ainsi qu'à d'autres cultures totalement auto-fertiles[8], comme le soja, ou parthénocarpiques[9], comme la banane. Bien que minoritaires en nombre d'espèces, les cultures non zoophiles sont majoritaires en termes de production, puisqu'elles constituent 61% de la production mondiale en tonnage, contre 36% pour les cultures zoophiles. Les cultures zoophiles, par leur diversité, permettent néanmoins de maintenir un régime alimentaire humain nutritionnellement et culturellement riche, non carencé en vitamines A. Dans un scénario où les pollinisateurs disparaîtraient, la santé de nombreuses populations humaines s'en trouverait grandement affecté (Smith *et al.*, 2015).

3.4. Introduction de colonies d'abeilles mellifères dans les cultures entomophiles et charge en colonies à l'hectare

Les cultures entomophiles pouvant être couramment en déficit de pollinisation en raison d'un déficit en insectes pollinisateurs (Garibaldi *et al.*, 2016), les agriculteurs introduisent couramment des insectes pollinisateurs élevés par l'homme dans leurs cultures depuis le début du XX^{ème} siècle (Farrar, 1931 ; McGregor, 1976 ; Free, 1993 ; Delaplane & Mayer, 2000 ;

[7] Cultures conduisant à une production mondiale annuelle supérieure à 4 millions de tonnes de denrées alimentaires, soit 95% des espèces cultivées dans le monde.
[8] Espèce auto-fertile = espèce auto-compatible capable de s'auto-féconder uniquement par auto-pollinisation.
[9] Espèce parthénocarpique = espèce dont le fruit est naturellement ou artificiellement induit sans pollinisation et sans fécondation des ovules. Les fruits parthénocarpiques sont sans graines.

Garibaldi *et al.*, 2017). Ces insectes introduits correspondent le plus souvent à des introductions de colonies d'abeilles mellifères, louées auprès des apiculteurs, comme pollinisateur généraliste, mais 33 autres espèces d'abeilles sont également utilisées selon la culture cible (voire la liste la plus exhaustive dans Garibaldi *et al.*, 2017). Les ouvrages de référence (McGregor, 1976 ; Free, 1993 ; Delaplane & Mayer, 2000) donnent toute une série de recommandations permettant d'améliorer l'efficacité de l'introduction d'insectes sur la pollinisation des cultures, en particulier concernant l'introduction de colonies d'abeilles mellifères, selon la culture considérée : charge en colonies à l'hectare, taille des colonies, quand et où positionner les colonies, conduite des colonies par nourrissage au sirop ou par ajout de trappe à pollen ou de distributeur de pollen à l'entrée de la ruche.

La détermination de la charge optimale en colonies d'abeilles mellifères par hectare est encore très empirique. Rollin & Garibaldi (2018) ont examiné 795 études, toutes cultures entomophiles confondues, qui ont fait le lien entre au moins une valeur de densité d'abeilles mellifères ou de charge en colonie par unité de surface de culture et le rendement ou une composante de rendement (taux de fructification, grenaison, masse des graines ou fruits). Sur ces 795 études, seulement 22 ont comparé au moins deux valeurs de densité d'abeilles mellifères ou de charge en colonies. Parmi ces études, certaines concluent à un effet positif de l'augmentation de la densité ou de la charge en colonies d'abeilles mellifères sur le rendement ou l'une de ses composantes (Steffan-Dewenter, 2003 ; Sabbahi *et al.*, 2005 ; Eaton & Nams, 2012 ; Benjamin & Winfree, 2014 ; Cunningham *et al.*, 2016), d'autres à un effet négatif simple ou un effet négatif une fois un optimum atteint (Aizen *et al.*, 2014 ; Sáez *et al.*, 2014 ; Fulton *et al.*, 2015 ; Grass *et al.*, 2018 ; Sáez *et al.*, 2018), d'autres à un plateau une fois un maximum atteint (DeGrandi-Hoffman *et al.*, 1987 ; Aras *et al.*, 1996 ; Andrikopoulos & Cane, 2018), d'autres ne concluent à aucun patron particulier (Benedek *et al.*, 1972 ; Viana *et al.*, 2014 ; Blitzer *et al.*, 2016 ; Garratt *et al.*, 2018b). Peu d'études ont tenu compte du contexte cultural. Gaines-Day & Gratton (2016) ont par exemple tenu compte du contexte paysager. Ils ont constaté dans leur étude que l'augmentation de la charge en colonies d'abeilles mellifères par hectare a eu un effet positif sur le rendement de la canneberge uniquement dans les parcelles dont le paysage environnant dans un rayon de 1 km contenait moins de 42% de surface recouverte de bois (Figure IV). Ce résultat peut être expliqué par la dilution des abeilles mellifères dans l'environnement autour de la culture lorsque cet environnement est en mesure de proposer une ressource alternative en nectar ou pollen à celle proposée par la culture (Garibaldi *et al.*, 2017).

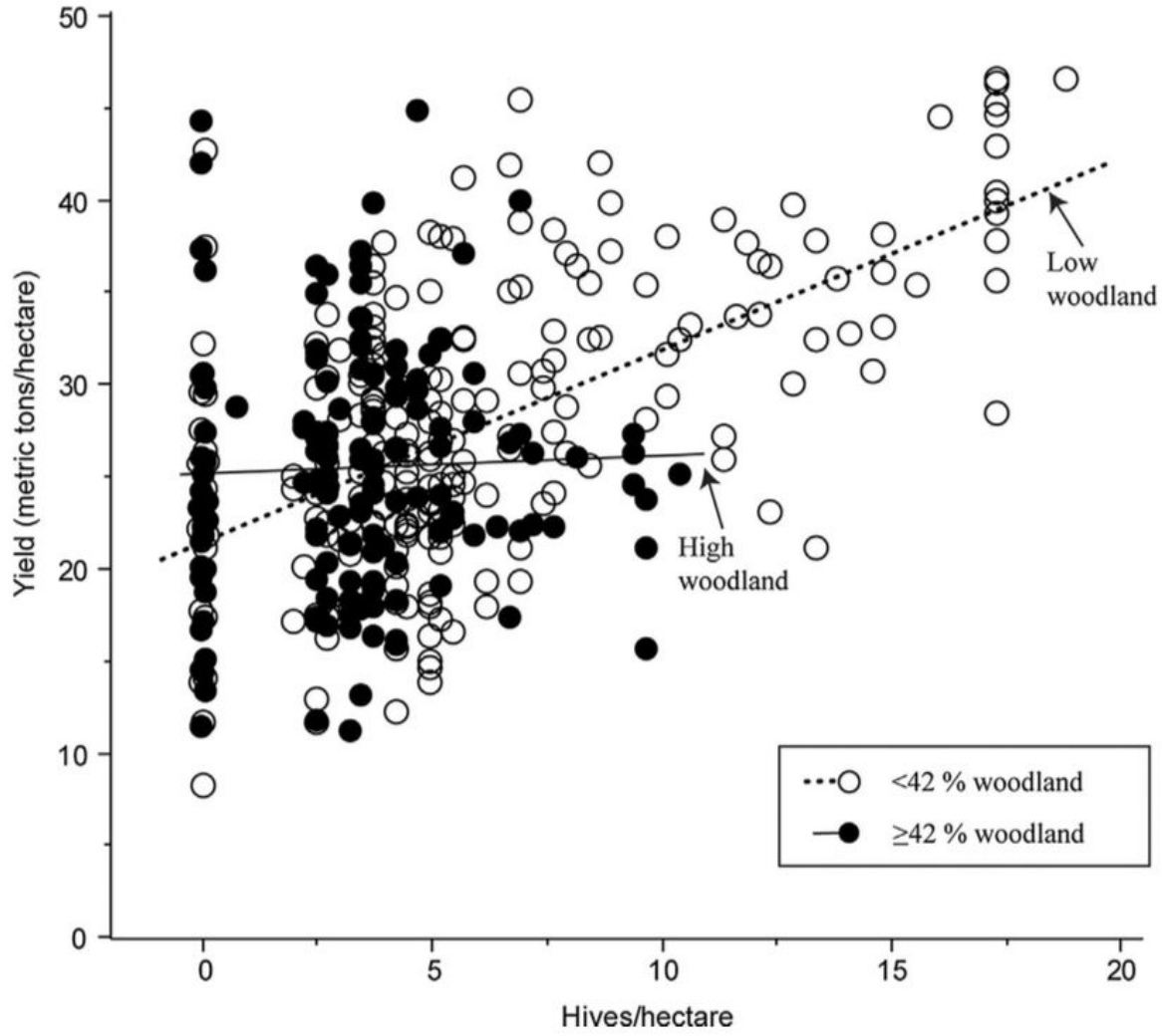


Figure IV – Relation entre le rendement de canneberge et la charge en colonies d'abeilles mellifères à l'hectare. Chaque point représente une parcelle et une année. Il y a une forte relation positive entre le rendement et la charge en colonies, mais uniquement dans les parcelles peu environnées de bois (d'après Gaines-Day & Gratton, 2016).

3.5. La pollinisation intégrée des cultures, nouveau paradigme du XXI^{ème} siècle

Le concept de pollinisation intégrée des cultures est né du constat de la différence de contribution que peuvent apporter respectivement les abeilles mellifères introduites et les abeilles sauvages dans la pollinisation du bleuet entre des petites parcelles entourées de bois et des grandes parcelles en milieu agricole intensif (Isaacs & Kirk, 2010). Isaacs & Kirk (2010) ont en effet constaté que les abeilles sauvages étaient plus abondantes dans les petites parcelles de bleuet entourées de bois, constituant 58% du total d'abeilles visitant les fleurs, alors qu'elles était à l'inverse très peu abondantes dans les grandes parcelles de milieu agricole intensif, où les abeilles mellifères constituaient 97% du total d'abeilles visitant les fleurs. Isaacs & Kirk (2010) ont estimé que les abeilles sauvages réalisaient 82% de la pollinisation du bleuet dans les petites parcelles, mais seulement 12% dans les grandes parcelles, les abeilles mellifères introduites réalisant le complément.

Il a en effet été constaté de nombreuses fois que, selon la culture considérée et selon les situations, les insectes sauvages, et en particulier les abeilles sauvages, peuvent contribuer plus fortement à la pollinisation des cultures que les abeilles mellifères (Hoehn *et al.*, 2008 ; Isaacs & Kirk, 2010 ; Russo *et al.*, 2017 ; Phillips *et al.*, 2018). Cette meilleure activité pollinisatrice peut s'expliquer soit par une meilleure efficacité pollinisatrice individuelle (Rader *et al.*, 2009 ; Park *et al.*, 2015 ; Henselek *et al.*, 2017 ; Phillips *et al.*, 2018), soit par une plus grande vitesse de butinage (Rader *et al.*, 2009 ; Garratt *et al.*, 2014 ; Couvillon *et al.*, 2015), soit par un comportement de manipulation de la fleur plus efficace (Hoehn *et al.*, 2008 ; Rader *et al.*, 2009 ; Blüthgen & Klein, 2011 ; Woodcock *et al.*, 2013 ; Park *et al.*, 2015 ; Russo *et al.*, 2017), soit par une activité temporelle de butinage plus adaptée (Hoehn *et al.*, 2008 ; Blüthgen & Klein, 2011 ; Brittain *et al.*, 2013a ; Ellis *et al.*, 2017). Par ces différences de comportements, il a été montré que les insectes sauvages peuvent être complémentaires aux abeilles mellifères introduites (Blüthgen & Klein, 2011) :

- complémentarité spatiale, les espèces n'ayant pas la même distribution géographique (Rader *et al.*, 2012 ; Kremen, 2018 ; Winfree *et al.*, 2018) ;
- contribution temporelle, les espèces n'ayant pas la même activité de butinage selon la période de la journée ou de l'année ou selon le climat considérés (Rader *et al.*, 2012 ; Brittain *et al.*, 2013a ; Fründ *et al.*, 2013 ; Rader *et al.*, 2013 ; Pisanty *et al.*, 2016 ; Ellis *et al.*, 2017) ;
- complémentarité de comportement de butinage, de manipulation de la fleur, ou de de butinage spatial intra-plante (Hoehn *et al.*, 2008 ; Brittain *et al.*, 2013a).

Bien souvent, cette complémentarité fonctionnelle spécifique ne peut être substituable par une introduction d'abeilles mellifères en supplément (Hoehn *et al.*, 2008 ; Garibaldi *et al.*, 2013 ; Button & Elle, 2014 ; Mallinger & Gratton, 2015 ; Blitzer *et al.*, 2016 ; Kremen, 2018 ; Winfree *et al.*, 2018), même s'il existe de nombreuses situations où, par son abondance, c'est l'abeille mellifère qui réalise l'essentiel de la pollinisation, et ce sans forcément nécessiter de complément (Rader *et al.*, 2009 ; Isaacs & Kirk, 2010 ; Benjamin & Winfree, 2014 ; Pisanty *et al.*, 2014 ; Russo *et al.*, 2017). Par ailleurs, dans les situations où il y a nécessité de pollinisation croisée entre deux lignées, la diversité et l'abondance des insectes sauvages peuvent augmenter l'efficacité pollinisatrice individuelle des abeilles mellifères : on parle de synergie entre abeilles mellifères et insectes sauvages pour la pollinisation (DeGrandi-Hoffman & Watkins, 2000 ; Greenleaf & Kremen, 2006 ; Carvalheiro *et al.*, 2011 ; Brittain *et al.*, 2013b ; Sapir *et al.*, 2017). Cet effet peut s'expliquer par le fait qu'en présence d'insectes sauvages diversifiés et abondants, les abeilles mellifères changent plus souvent de plante et de lignée au cours de leur butinage (Carvalheiro *et al.*, 2011 ; Brittain *et al.*, 2013b ; Sapir *et al.*, 2017).

La pollinisation intégrée des cultures est un concept émergent, basé sur un nouveau paradigme développé dès le début des années 1990 (Kevan *et al.*, 1990 ; Corbet, 1991 ; Batra, 1995), qui consiste à *utiliser des espèces pollinisatrices introduites en combinaison avec des pratiques agricoles qui soutiennent, augmentent et protègent les populations de pollinisateurs sauvages natifs pour fournir une pollinisation fiable, durable et économiquement soutenable des cultures* (Isaacs *et al.*, 2017).

4. Contexte, problématique et objectifs du travail de thèse

4.1. Contexte sociétal

Ce travail de thèse a été financé par l'ANAMSO[10], au travers des actions spécifiques du GNIS[11] et de l'UFS[12], dont la problématique est de définir les éléments permettant d'établir à la fois une charge optimale en colonie d'abeilles mellifères à introduire par unité de surface de culture adaptée aux cultures de colza et de tournesol en production de semence hybride et une conduite adaptée de ces colonies, pour que la pollinisation ne soit pas un facteur de production limitant dans ces cultures. Ce travail de thèse s'est intégré dans le programme intitulé « *Optimisation de la pollinisation d'une culture par les abeilles domestiques et sauvages : approche intégrée de la gestion du cheptel et de la conduite des colonies (POLAPIS)* » (2013-2016), en partenariat avec l'ITSAP[13] et l'ENSFEA[14] et financé dans le cadre du programme européen d'aide à l'apiculture par le Fonds Européen Agricole de GArantie (FEAGA) coordonné par FranceAgriMer et la Direction Générale des Politiques Agricole, Agroalimentaire et des Territoires du Ministère de l'Agriculture, de l'Agro-alimentaire et de la Forêt (MAAF). Le programme POLAPIS avait pour objectif, pour sa partie biologique prise en charge par l'INRA et l'ANAMSO, de *mettre au point et valider une méthode objective pour déterminer la charge en unités opérationnelles d'abeilles mellifères à introduire par unité de surface de culture cible pour complémenter la faune pollinisatrice sauvage native afin que la pollinisation ne soit pas un facteur de production limitant pour le rendement de cette culture*. C'est cette question qui a guidé et vertébré le travail de cette thèse. Le programme POLAPIS avait également un volet économique pris en charge par l'ITSAP et l'ENSFEA qui avait pour objectif d'*acquérir des références technico-économiques à l'échelle de l'exploitation sur la conduite des prestations de service apicoles en pollinisation*.

L'appel à projets de recherche émis par FranceAgriMer dans le cadre du programme communautaire pour l'apiculture pour la période 2013-2016 comprenait un item « alimentation

[10] ANAMSO = Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses. C'est l'institut technique de la filière agricole des semences oléagineuses, qui comprennent le colza, le tournesol et le soja. L'ANAMSO a la particularité d'avoir le triple rôle d'institut technique, de syndicat de filière, et d'inspection des cultures.

[11] GNIS = Groupement National Interprofessionnel des Semences et plants. C'est l'interprofession qui regroupe toutes les filières agricoles des semences. Le GNIS est subdivisé en sections. La filière semences oléagineuses est une section du GNIS.

[12] UFS = Union Française des Semenciers. C'est l'organisation professionnelle des entreprises semencières françaises.

[13] ITSAP = Institut Technique et Scientifique de l'Abeille et de la Pollinisation. C'est l'institut technique de la filière agricole de l'apiculture.

[14] ENSFEA = Ecole Nationale Supérieure de Formation de l'Enseignement Agricole.

et pollinisation » pour lequel il était attendu « *des progrès sur les modalités optimales du nourriss[age]* [des colonies d'abeilles mellifères, ndlr] *et sur le meilleur parti à tirer des variétés des plantes cultivées* » et « *de documenter et d'analyser la relation plante-abeille pour renforcer les connaissances sur les facteurs (espèces cultivées, époque de la floraison, préparation et développement des colonies au moment de cette floraison, conditions météorologiques) influençant la pollinisation sur une culture donnée dans le but de gérer et valoriser au mieux cette activité* ».

4.2. Contexte scientifique

Au niveau scientifique, la question de définir une charge en colonies d'abeilles mellifère à introduire par unité de surface d'une culture cible pour la pollinisation a été jusqu'ici approchée de façon essentiellement empirique. Les deux ouvrages de référence sur le sujet (McGregor, 1976 ; Delaplane & Mayer, 2000) ont simplement recensé pour chaque culture entomophile les valeurs de charges qui ont été utilisées dans différentes études, études pour une bonne part techniques. Keith Delaplane & Daniel Mayer (2000) ont proposé pour chaque culture une moyenne de la valeur de ces charges. La première limite de cette approche est que, comme l'ont très justement souligné Orianne Rollin & Lucas Garibaldi (2018), la plupart des références utilisées pour recommander une valeur de charge n'ont expérimenté qu'une seule valeur de charge, ne permettant donc pas de tester l'effet de cette variable sur les composantes de rendement. Pour les études qui ont testé au moins deux valeurs de densités d'abeilles mellifères par unité de surface de culture, la deuxième limite de cette approche est qu'elle est essentiellement phénoménologique : par exemple, on teste si la valeur de la pente de la relation entre le rendement et la charge en colonie est nulle ou non nulle. Ceci contraint la nature mathématique des relations entre les variables, par exemple une relation linéaire si les résidus de la variable dépendante sont distribués selon une loi normale, ou une relation exponentielle s'ils sont distribués selon une loi de Poisson etc., alors que les relations ne sont pas forcément de ces natures. Ensuite, les prédictions de ce type d'approche ne peuvent s'appliquer que dans le contexte cultural particulier de l'étude. Difficile alors d'intégrer les variations variétales, climatiques, paysagères, pédologiques…

Une autre approche consiste à décrire et paramétrer tous les processus biologiques et écologiques qui se réalisent entre la mise en place d'une charge en colonies et l'obtention du rendement et à les intégrer dans un modèle mécaniste global (ex : Sáez *et al.*, 2018b). John Free (1993) avait déjà listé un certain nombre de ces processus à caractériser (p. 53) : le taux de visite des fleurs par des insectes, le nombre de visites nécessaires par fleur pour obtenir une

pollinisation complète, l'efficacité pollinisatrice individuelle des insectes (= nombre de grains de pollen déposés sur le stigmate par visite de fleur), la période effective de pollinisation de la fleur incluant la durée de disponibilité du pollen et de réceptivité du stigmate et le temps nécessaire au tube pollinique pour atteindre l'ovule, l'auto-compatibilité ou auto-incompatibilité de la fleur et sa capacité à s'auto-polliniser ou non, le mouvement des insectes entre les fleurs, les plantes et les lignées, le nombre d'heures par jour durant lesquelles les fleurs sont butinées, le nombre de fleurs disponibles par jour et par unité de surface selon les différents protocoles de semis et les différentes conditions environnementales. John Free ajoutait également que le nombre de fois qu'une fleur est visitée par des insectes dépend largement de la quantité et de la qualité du nectar et du pollen qu'elle a à offrir. On peut séparer cet ensemble de processus en deux groupes : les processus qui relèvent de la plante ou de la fleur et qui caractérisent la *demande* en pollinisation, et ceux qui relèvent des insectes et qui caractérisent l'*offre* en pollinisateurs.

4.3. Contexte expérimental

Une série de relevés de terrain ont été réalisés dans 18 parcelles de colza en production de semence hybride entre 2014 et 2016 avec un gradient de charge en colonies à l'hectare (de 0 à 10 colonies par hectare) dans le cadre du programme POLAPIS, permettant de mesurer une bonne partie des processus cités précédemment dans cette culture, pour chacune des deux lignées (mâle fertile et mâle stérile) : quantité de nectar disponible dans des fleurs protégées du butinage des insectes, production pollinique (pour la lignée mâle fertile seulement), quantité de fleurs disponibles par unité de surface, nombre instantané d'insectes floricoles par fleur et par catégorie d'insecte (abeille mellifère, bourdon, autre abeille sauvage, syrphe ; avec présence ou non de pollen dans les corbiculae ou scopae), composition de la communauté des insectes floricoles par capture au filet, efficacité pollinisatrice individuelle pour chaque catégorie d'insecte associée à la grenaison qui en résulte, vitesse de butinage, quantité de pollen déposée par stigmate en une journée ou sur l'ensemble de la période effective de pollinisation (mesurée sur la base d'une expérimentation préliminaire) par le vent (sous tulle) ou en pollinisation libre associée à la grenaison qui en résulte, et les composantes du rendement (taux de fructification, nombre de graines, poids moyen de mille grains) et le rendement par unité de surface échantillonnée. La température, l'hygrométrie et l'intensité lumineuse ont été mesurée par des capteurs toutes les 2 min et 30 secondes, et la vitesse moyenne du vent a été mesurée par demi-journée.

4.4. Choix du modèle biologique

C'est le colza (*Brassica napus*) d'hiver qui a été choisi comme modèle d'étude plutôt que le tournesol car c'est une espèce qui est globalement plus étudiée et pour laquelle on dispose de plus de connaissances biologiques fondamentales. C'est également un modèle phylogénétiquement proche d'*Arabidopsis thaliana*, qui est le modèle de référence aujourd'hui en biologie végétale, et qui peut donc apporter un certain nombre de connaissances supplémentaires, dont certaines peuvent être directement transposables sur le colza. C'est également une espèce dont la floraison est dite indéfinie, plus longue que celle du tournesol et qui permet donc de réaliser un plus grand nombre de relevés.

Les productions de semence hybride de colza ont la particularité d'alterner deux lignées en plein champ : une lignée dite mâle fertile, qui fournit le pollen, et une lignée dite mâle stérile sur laquelle est récoltée la semence. Les lignées mâle stériles sont dotée d'anthères réduits, vides de pollen. Elles sont obtenues par croisement interspécifique entre une lignée mâle stérile sauvage de radis (*Raphanus sativus*), rendue spontanément mâle stérile par le génome mitochondrial contenu dans son cytoplasme, avec du pollen d'une lignée mâle fertile de colza. Ce premier croisement est suivi d'une série de rétrocroisements avec la lignée mâle fertile de colza. Les lignées hybrides F_1 de colza sont ensuite obtenues par croisement entre une lignée mâle stérile et une lignée mâle fertile porteuse d'un gène nucléaire dominant qui inhibe l'expression génétique de la stérilité mâle cytoplasmique. Ce gène nucléaire restaurateur de la fertilité mâle provient de lignées sauvages de radis qui ont été croisées avec des lignées de colza (Pelletier & Budar, 2015). Les lignées mâle stériles de colza n'étant, par définition, pas hermaphrodites, il ne peut y avoir auto-pollinisation par gravité (le colza étant totalement auto-compatible ; Williams, 1978 ; Williams *et al.*, 1986 ; Ouvrard *et al.*, 2017). Il faut donc un autre vecteur pour transporter le pollen de la lignée mâle fertile vers la lignée mâle stérile. Or il a été établi sous cage que ce sont principalement les insectes qui joue ce rôle de vecteur dans les productions de semence hybride de colza (Mesquida, 1981 ; Steffan-Dewenter, 2003 ; Jauker *et al.*, 2012).

4.5. Orientation du travail de thèse

Une partie des données issues des relevés exhaustifs cités précédemment a été analysée de façon descriptive et présentée succinctement dans diverses réunions de restitution aux filières agricole et apicole. Néanmoins, aucune de ces données ne sera présentée dans ce manuscrit. Il est en effet apparu en fin d'expérimentation que la plupart de ces relevés permettent de mesurer et de caractériser l'*offre* en pollinisateurs, mais nullement de caractériser la *demande* en

pollinisation de la culture. C'est pourtant cette information qu'il est nécessaire d'obtenir en premier si l'on souhaite faire correspondre ensuite une *offre* adaptée en pollinisateurs, qu'ils soient natifs ou introduits. C'est ce que nous avons voulu examiner en réalisant une expérimentation supplémentaire en 2016 et en 2017 sous des tunnels recouverts de toile anti-insectes sur le centre INRA d'Avignon.

4.6. Objectifs de la thèse

L'expérimentation supplémentaire menée en 2016 et en 2017 avait trois objectifs :

1) **Établir la durée de la période effective de pollinisation (PEP) et de la période de sécrétion nectarifère (PSN) du colza en fonction de la température.**

La PEP est importante à caractériser pour déterminer de quelle « fenêtre » temporelle un agriculteur dispose à l'échelle de la fleur pour réaliser la pollinisation. Plus cette durée est courte, plus il faut que la pollinisation soit intense, c'est-à-dire plus il faut que le butinage d'une fleur par les insectes soit fréquent, et plus il faut que l'efficacité pollinisatrice individuelle de ces insectes soit importante. Cette période a été établie pour de nombreuses espèces d'arbres fruitiers, mais à ce jour pour aucune espèce herbacée. La PSN est importante à caractériser pour indiquer si la fleur est attractive vis-à-vis des insectes sur l'ensemble de la PEP ou non. Si la PSN a une durée inférieure à celle de la PEP, alors la « fenêtre » temporelle dont un agriculteur dispose pour réaliser la pollinisation est réduite d'autant. La PSN n'a à ce jour jamais été formalisée comme telle. La longévité de la fleur étant fortement dépendante de la température, cette variable a été prise en compte dans la mesure de la durée de ces deux périodes. Enfin ces deux périodes ont été mesurées à la fois sur une lignée mâle stérile et une lignée mâle fertile de colza. Les résultats afférents à ce premier objectif sont rapportés dans le chapitre I.

2) **Établir le nombre minimum de grains de pollen qui doivent être déposés sur un stigmate de colza en fonction de la température pour que la pollinisation ne soit pas un facteur limitant pour la grenaison.**

Comme relevé précédemment par d'autres auteurs (Ne'eman *et al.*, 2010 ; Isaacs *et al.*, 2017 ; Henselek *et al.*, 2018), c'est une information essentielle si l'on veut déterminer le nombre minimal d'insectes à introduire dans une parcelle en complément de la faune sauvage native. Combinée avec la PEP, et connaissant l'efficacité pollinisatrice individuelle et la vitesse de butinage moyennes des abeilles mellifères ainsi que la quantité moyenne de fleurs à polliniser par unité de surface de culture, cette valeur peut permettre d'estimer un nombre moyen de

butineuses d'abeilles mellifères à introduire par unité de surface de culture cible. Pour obtenir cette valeur, il faut étudier la relation fonctionnelle de dose-réponse entre l'intensité de la pollinisation (= nombre de grains de pollen déposés sur le stigmate) et la grenaison (= nombre de graines contenues dans la silique) qui en résulte. Deux fonctions mathématiques ont été proposées pour caractériser cette relation. Nous en proposons une nouvelle qui intègre trois mécanismes relatifs à la fécondation, mis en évidence il y a plus ou moins longtemps : l'effet de la température sur la performance du pollen, l'effet de population du pollen à faible densité, et la distribution des tubes polliniques qui se réalise de façon bijective parmi les ovules. Cette nouvelle fonction permet d'établir un niveau d'intensité de la pollinisation seuil au-delà duquel la pollinisation n'est plus un facteur limitant pour la grenaison, ce que les fonctions précédentes ne permettaient pas. Ce niveau d'intensité seuil a été établi chez une lignée mâle stérile, mais cette valeur a également toute sa pertinence pour les lignées mâle fertiles, les deux types de lignées n'ayant *a priori* pas une biologie de la fécondation différente. Les résultats afférents à ce deuxième objectif sont rapportés dans le chapitre II.

3) Établir la vitesse de sécrétion nectarifère du colza en fonction de la température.

Différentes méthodes de mesure de la sécrétion nectarifère ont été proposées dans la littérature. Une méthode propose de mesurer la vitesse de sécrétion, en masse de sucres totaux dissous. La sécrétion nectarifère étant pourtant un processus dynamique, peu d'études mesurant la sécrétion nectarifère ont utilisé cette méthode à ce jour. Et c'est pourtant cette variable qui peut permettre d'estimer un intérêt pour les insectes à butiner plus ou moins fréquemment des fleurs pour cette ressource. C'est aussi cette variable qui permet de comparer un intérêt pour les insectes à visiter plutôt telle ou telle lignée pour cette ressource. Cette variable est donc d'importance pour les cultures de colza en production de semence hybride. En effet, si la lignée mâle stérile produit plus ou moins de nectar par rapport à la lignée mâle fertile, ceci pourra avoir une incidence sur la fréquence des insectes à passer d'une lignée à l'autre, impactant les flux de pollen de la lignée mâle fertile vers la lignée mâle stérile. Ceci peut également avoir de l'importance pour l'intérêt des insectes à butiner les fleurs de la culture cible par rapport à d'autres sources de nectar potentiellement présentes dans l'environnement de la culture, potentiellement plus intéressantes d'un point de vue approvisionnement optimal. Cette variable a été mesurée à la fois sur une lignée mâle stérile et une lignée mâle fertile. Les résultats afférents à ce troisième objectif sont rapportés dans le chapitre III.

Pour répondre à ces trois objectifs, deux tunnels recouverts de toile anti-insectes ont été semés à l'Automne 2015 et 2016 avec deux lignées de colza : la variété hybride F1 'Exocet', mâle fertile, et son parent mâle stérile. Les deux tunnels ont été semés avec trois semaines de décalage pour disposer d'un décalage de floraison entre les tunnels, et ainsi pouvoir bénéficier d'un temps supplémentaire pour les relevés. Les détails de chaque protocole sont donnés dans les différents chapitres.

Une expérimentation menée sur des colonies d'abeilles mellifères entre 2014 et 2016 a permis de répondre à un quatrième objectif :

4) **Donner l'équivalence du nombre d'inter-cadres recouverts d'abeilles mellifères adultes dans une ruche avec le nombre d'abeilles mellifères adultes contenues dans la colonie en tenant compte de la température.**

Une méthode d'évaluation de la « force » des colonies d'abeilles mellifères est en effet couramment pratiquée sur les colonies introduites dans des cultures par les apiculteurs dans le cadre de prestations de service en pollinisation pour évaluer si l'apiculteur répond ou non à la demande de l'agriculteur formulée dans un contrat (McGregor, 1976 ; Delaplane & Mayer, 2000). Dans certains pays, c'est sur la base de cette évaluation qu'est évalué le niveau de rémunération de l'apiculteur en retour pour son service. Cette méthode est basée sur le nombre d'inter-cadres recouverts d'abeilles mellifères adultes compté par ruche (Nasr *et al.*, 1990), sur un échantillon de ruches introduites. La variable du nombre d'inter-cadres recouverts d'abeilles telle qu'utilisée aujourd'hui est cependant de nature plus qualitative que quantitative. Elle permet de discriminer des colonies de « petite », « moyenne » ou « grande » taille, mais sans disposer de référentiel objectif en parallèle. L'objet de cette étude est donc de fournir un référentiel quantitatif à cette variable : la taille de la population d'abeilles mellifères adultes. Cette indication permettra de mieux connaître la taille du cheptel global d'abeilles mellifères effectivement introduite dans une culture cible, et donc de mieux doser l'*offre* en pollinisateurs à introduire pour répondre à la *demande* en pollinisation de la culture. Les résultats afférents à ce quatrième objectif sont rapportés dans le chapitre IV.

Les résultats issus de ces quatre chapitres permettront de fournir de premiers éléments pour faire correspondre l'offre en pollinisateurs à introduire dans une culture de production de semence hybride de colza en complément de la faune sauvage native pour répondre à la demande en pollinisation de cette culture. En combinant ces éléments avec les autres processus biologiques listés par John Free (1993) cités précédemment et en les élargissant aux autres

culture entomophiles, ces éléments permettront de proposer *in fine* un modèle mécaniste de prédiction de la quantité de pollinisateurs à introduire par unité de surface de culture cible, à adapter selon l'espèce et la variété cultivées, le climat régional et le contexte paysager de la culture.

Une des originalités de ce travail de thèse aura été d'intégrer systématiquement un facteur prépondérant qui affecte de façon importante les processus biologiques et écologiques autant chez les plantes que chez les insectes, qui sont des organismes ectothermes et poïkilothermes : la température. Intégrer ce facteur est important dans le cadre des variations climatiques intra- et interannuelles, et ce d'autant plus compte tenu du contexte actuel de réchauffement climatique (IPCC, 2013). Ces connaissances fondamentales de la biologie florale en lien avec la température permettront une analyse plus fine et plus approfondie des données récoltées sur le terrain entre 2014 et 2016.

CHAPITRE I :

Mesure de la période effective de pollinisation et de la période de sécrétion nectarifère en fonction de la température

Flower age expressed in thermal time: is nectar secretion synchronous with pistil receptivity in oilseed rape (*Brassica napus* L.)?

Stan Chabert[a,b,*], Taïna Lemoine[a], Mathilde Raimond Cagnato[a], Nicolas Morison[a] and Bernard E. Vaissière[a]

[a]Institut National de la Recherche Agronomique (INRA), UR406 Abeilles et Environnement, site Agroparc, domaine Saint-Paul, CS 40509, F-84914 Avignon cedex 9, France
[b]Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses (ANAMSO), Ferme expérimentale, 2485 route des Pécolets, F-26800 Étoile-sur-Rhône, France

Environmental and Experimental Botany, 2018, 155: 628-640

Abstract

Floral longevity can vary substantially with temperature in a given plant species. Thermal time has been widely used to measure developmental age of plants and poikilothermic organisms. We tested whether thermal time was suitable to express flower age to characterise the duration of pistil receptivity and that of nectar secretion in undisturbed flowers of oilseed rape (OSR), and whether these two functional processes were synchronous. Flower age was measured in a male fertile hybrid F_1 line and in its male sterile (MS) parental line to determine if the presence of pollen affected the nectar secretion period. Hand pollinations and nectar samplings were conducted throughout the lifespan of OSR flowers on several flower cohorts from plants grown under tunnels under various temperature conditions. The number of seeds produced per flower was used as a proxy to assess the level of pistil receptivity at the time of hand-pollination. Thermal time was more suitable than calendar time to express OSR flower age. A phase of maturity followed by a phase of senescence took place in the two processes, and both proceeded faster as temperature increased. Nectar was secreted during the phase of full pistil receptivity, and, in the MS line, was then reabsorbed during the gradual decline of pistil receptivity. However, the nectar secretion period was extended beyond the onset of pistil senescence in the F_1 line. OSR flowers invest in nectar secretion to attract insect pollinators at least to cover the whole receptivity period of their female part. Nectar secretion may be prolonged in pollen-laden male fertile flowers to attract insect pollinators longer to ensure as long as possible the probability of their pollen being disseminated. Flower senescence is triggered by pistil senescence, while the presence of pollen in anthers acts as an inhibitor and therefore delays flower senescence. Thermal time should henceforth be used to express more universally the age of plant organs and to model the duration of processes in relation to temperature, especially in the advent of global warming.

Keywords: pistil receptivity period, nectar secretion period, flower age, flower senescence, thermal time, oilseed rape (*Brassica napus*)

1. Introduction

Floral longevity can vary greatly across Angiosperm species, from a few hours to a few months, depending on the adaptation to a variety of ecological conditions (Kerner von Marilaun, 1896; Primack, 1985; Stead and van Doorn, 1994; Ashman, 2004). The observed longevity is the result of an optimal balance between the female and the male fitness, assessed respectively as pollen receipt and removal, over time, and the costs of floral maintenance, since bringing to anthesis a new flower may be physiologically less expensive for a plant than maintaining an existing one at anthesis for an extended period of time (Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995). The intraspecific variability of floral longevity has also been investigated, but almost only from the standpoint of the effect of pollination: pollen receipt and/or removal induce precocious floral senescence or flower closure in numerous long-lived flower and ethylene-sensitive species, thereby permitting to limit the costs of floral maintenance and unnecessary pollinator attraction (reviews in Stead, 1992; O'Neill, 1997; van Doorn, 1997; Ashman, 2004; Fründ *et al.*, 2011). But other factors that could also affect floral longevity received somewhat less attention. Temperature is one such factor and studies highlighted that longevity of unpollinated flowers decreases with the rise of temperature (Hartley *et al.*, 1995; Yasaka *et al.*, 1998; Lopez and Runkle, 2004; Vesprini and Pacini, 2005; Arroyo *et al.*, 2013; Teixido and Valladares, 2015). This more broadly ties in with the well-established fact that the lifespan of poikilothermic organisms decreases as temperature rises at the intraspecific level (Loeb and Northrop, 1917; Shaw and Bercaw, 1962; Kelly *et al.*, 2013; Aguilar-Alberola and Mesquita-Joanes, 2014; review in Keil *et al.*, 2015).

Floral longevity is often defined, sometimes implicitly, as the time from anthesis to corolla fall. On the other hand, Ashman and Schoen (1994) proposed that it corresponds to the length of time a flower remains open and functional. By focusing on this second definition, we shall consider here the longevity and synchrony of two floral functional processes: (i) the duration of pistil receptivity, upon which the female flower fitness depends directly, and (ii) the duration of nectar secretion, which is a proxy for the duration of flower attractiveness to pollinators.

The duration of pistil receptivity is often synonymous with the effective pollination period, a concept that was defined by Williams (1966) as the period during which pollination is effective to produce a fruit. This period is determined by the longevity of the ovules minus the time lag between pollen receipt upon the stigma and fertilisation. Sanzol and Herrero (2001) rightly added that this definition is true providing that the duration of stigmatic receptivity is not limiting. In fact, the duration of pistil receptivity seems to be more frequently limited by

ovule longevity (Thompson and Liu, 1973; Stösser and Anvari, 1982; Burgos and Egea, 1993; Nepi and Pacini, 1993; Steinacher and Wagner, 2010; Carbonell-Bejerano *et al.*, 2011; Guerra *et al.*, 2011). Carbonell-Bejerano *et al.* (2011) reported an increase in ethylene biosynthesis in ovules of *Arabidopsis thaliana* (Brassicaceae) unpollinated pistils just before the end of ovule longevity, thereby precipitating overall ovule and pistil senescence. And as for unpollinated flowers, ovule longevity is correlated negatively with temperature (Postweiler *et al.*, 1985; Cerović and Ružić, 1992; Cerović *et al.*, 2000).

In zoophilous flowers, nectar secretion begins usually a few hours before pollinator foraging starts (Cruden *et al.*, 1983), sometimes at the bud stage (e.g.: Pleasants, 1983; Galetto and Bernardello, 1995; Galetto *et al.*, 2000; Nepi *et al.*, 2001). Then nectar secretion is usually continuous, at least over the period of active pollinator foraging, and may cease at different times, such when a maximum amount of nectar is reached, or after a successful pollinator visit, or at flower senescence (Cruden *et al.*, 1983; Pacini and Nepi, 2007). Then nectar is reabsorbed, as was shown for the first time by Bonnier (1879), either concomitantly with secretion, or after pollination, or at flower senescence, allowing the recovery of some of the resources invested in nectar production (reviewed in Nepi and Stpiczyńska, 2008). Pacini *et al.* (2003) identified two kinds of nectaries: (i) those provided with parenchyma that store starch, allowing secretion of large quantities of nectar sugars over a short time at any hour of the day or night (Pacini and Nepi, 2007), and (ii) those whose starch is, at least in part, directly derived from photosynthesis through phloem sap. In this latter case, nectar sugars are produced in small quantities during the day and this process can last over a long period until flower senescence. Brassicaceae, and *Brassica napus* in particular, belong to this latter category. Their nectaries are directly connected to phloem sap through sieve tubes, without starch storage in nectary parenchyma (Davis *et al.*, 1986; Davis *et al.*, 1998). Indeed, the nectar secretion lasts until flower senescence takes place in this species, as observed by Burquez and Corbet (1991).

Senescence of plant organs is a tightly regulated and genetically programmed active process of living cell suicide that does enable nutrient recycling and redistribution elsewhere in the plant as organs become useless or too costly to maintain (Gan and Amasino, 1997; Fischer, 2007; Reid and Chen, 2007; Guiboileau *et al.*, 2010; Rogers, 2015; Rogers and Munné-Bosch, 2016). This view has led to the concept of 'developmental senescence' in the sense that the onset of senescence and its progression seem basically governed by the developmental age of the organ. Therefore senescence of plant organs can be considered as the final phase of their own development after a phase of maturity.

Cumulative temperature units, also known as growing degree.days, thermal time or

physiological time, are commonly used to measure developmental age, also called phenological age, of plants and poikilothermic organisms (Bonhomme, 2000; Trudgill *et al.*, 2005; Damos and Savopoulou-Soultani, 2012; Soltani and Sinclair, 2012). The first record of use for this kind of unit was Réaumur (1735), who found that adding up the mean daily air temperatures was more suitable to characterise the elapsed time between crop sowing and harvesting than the mere adding of days. Then Bonnet (1779) was the first to use it for insects. The theoretical background is that the activity of enzymes involved in the development of poikilothermic organisms has a thermal optimum. Consequently, the developmental rate of these organisms changes with temperature according to a non-linear reaction norm with three cardinal temperatures: (i) a base temperature below which there is no development, (ii) an optimum temperature at which the developmental rate is maximum, and (iii) a ceiling temperature above which development ceases (Yan and Hunt, 1999; Bonhomme, 2000; Trudgill *et al.*, 2005; Shi and Ge, 2010; Damos and Savopoulou-Soultani, 2012; Soltani and Sinclair, 2012).

As mentioned before, numerous studies showed that the lifespan of poikilothermic organisms and unpollinated flowers decreases as temperature rises at the intraspecific level. But none of these studies, excepted only one on flower longevity (Lopez and Runkle, 2004), quantified lifespan in thermal time. There are, however, good reasons to believe that thermal time would be more relevant than calendar time to measure lifespan in this kind of organisms and in plant organs, as a more universal unit.

Indeed, the most commonly accepted physiological mechanism of ageing is the production of reactive oxygen species (ROS) by aerobic metabolism that cause molecular and cellular damages, called oxidative stress, that gradually accumulate in the cell with age (Harman, 1956; Beckman and Ames, 1998; Allen and Balin, 2002; Speakman and Selman, 2011; Liochev, 2013). In the post-reproductive phase of an organism, the gradual increase in oxidative stress leads finally to the loss of the overall functions in the cell, leading to the death of cell, tissues, and ultimately of the whole organism. Yet the metabolic rate is strongly positively correlated with temperature at the intraspecific level in poikilothermic organisms, whether at the organism, cellular or mitochondrial level (Miquel *et al.*, 1976; McArthur and Sohal, 1982; Van Voorhies and Ward, 1999; review in Schulte, 2015). And the ROS production, and thus the oxidative stress, seems to be also correlated with temperature at the intraspecific level (Farmer and Sohal, 1987; Abele *et al.*, 2002; Schulte, 2015), although this relationship has been little studied. Oxidative stress seems to explain well the variations of lifespan with temperature in these organisms, at least at the intraspecific level. Likewise, oxidative stress plays a role in plant programmed cell death (Foyer and Noctor, 2005; Van Breusegem and Dat, 2006; de Pinto *et*

al., 2012; Rogers, 2015), as in the trigger of leaf and flower senescence (Zentgraf, 2007; Zentgraf and Hemleben, 2008; Rogers, 2012; Rogers and Munné-Bosch, 2016). Therefore if the oxidative stress generation rate increases with temperature from a base temperature in plants, thermal time can be expected to be a relevant unit to measure the longevity of plant organs, allowing to quantitatively predict it as a function of temperature. On the other hand, contrary to the developmental rate, the oxidative stress generation rate would not admit an optimum temperature, neither a ceiling temperature.

In *Brassica napus*, floral longevity seems to be governed by pollen removal (Bell and Cresswell, 1998). Indeed, pollen removal hastens flower senescence while pollen receipt do not. This can be explained by the fact that when flowers are foraged by insects, the ovules are gradually fertilized, in other words the female sexual function is gradually completed, with a certain time in advance compared to the gradual completion of the male sexual function, i.e. the gradual remove of pollen from the anthers by insect foragers as and when the anther dehisce (see Bell and Cresswell, 1998, Fig. 5). We can therefore expect that nectar secretion may last as long as pollen is not completely removed by insects up to a time when flower senescence is triggered in any case. Precisely, we still do not know what triggers the end of nectar secretion, even in male sterile lines (Pelletier and Budar, 2015) in which pollen is absent by genetic construction. We still do not know either the duration of the pistil receptivity in *B. napus*. If pollen removal governs floral longevity, it may be that the presence of pollen in anthers acts as an inhibitor of flower senescence. If it is only pollen removal that drives floral longevity, then in male sterile lines we can expect that flower senescence starts at the onset of flower anthesis, and therefore that the nectar secretion is interrupted at the same time (hypothesis I). Another possible hypothesis is that flower senescence is triggered by ovule senescence, and that the presence of pollen in anthers acts merely as a delayer of flower senescence until the time when pollen is too old to play its role as a senescence inhibitor (hypothesis II). Under hypothesis II, the nectar secretion should last as long as pistil is receptive in male sterile lines, and the anthers should be not be completely dehisced before the onset of pistil senescence in male fertile lines.

We tested the assumption that thermal time was more suitable than calendar time to express flower age after anthesis for measuring the duration of pistil receptivity and that of nectar secretion in undisturbed oilseed rape (OSR) flowers. The durations of these two floral processes were measured on two kinds of cultivated lines of this species, a commercial F_1 hybrid that is male fertile, and its male sterile parent (MS). We could therefore expect that, under hypothesis II, the maturity phase and the senescence phase for these two floral processes occurred simultaneously in the MS line, and that nectar secretion period was extended in the F_1 line, to

attract insect pollinators longer to ensure as long as possible the probability of pollen dissemination.

Knowing the effect of temperature on the durations of these two floral processes, i.e. their thermal reaction norm (Angilletta, 2009), is important for the pollination management of entomophilous crops, particularly in hybrid seed production where self-pollination is not possible in the male sterile lines that produce the hybrid seed and where pollinator visits are therefore necessary prior to fertilization. Given that the sexual reproductive phase is the stage most sensitive to temperature stress in plants (Hedhly, 2011; Sage *et al.*, 2015), it is also one more element to take into account in the agricultural and ecological consequences of global warming (Schmidhuber and Tubiello, 2007; Hedhly *et al.*, 2009; Wheeler and von Braun, 2013).

2. Materials and methods

This study was carried out from Winter 2016 to Spring 2017 under two tunnels located at the INRA centre of Avignon (France; 43°54'53.5"N, 4°52'39.2"E). The 22 m long x 8 m wide tunnels were covered with insectproof HD polyethylene crystal screen with 950 x 800 µm openings (Diatex, France; www.diatex.com) to prevent insect foraging and to have undisturbed flowers, i.e. flowers where pollen and nectar have not been previously removed. Seeds were sown in four rows 18 m long with two winter OSR lines (*Brassica napus* L.): the male fertile F_1 hybrid line cv 'Exocet', thereafter called F_1 line, and its male sterile parent, thereafter referred to as MS line, both provided by Monsanto®. Winter OSR F_1 hybrid lines are largely self-fertile (Williams, 1978; Williams *et al.*, 1986; Ouvrard *et al.*, 2017).

The two tunnels were sown three weeks apart, the first one on 21 Nov. 2016 and the second one on 12 Dec. 2016, with the goal to have a difference in the flowering time of both tunnels and thus a more extended flowering period for measurements. The plants were watered daily with a drip system. Ambient instant temperatures were recorded every 150 seconds by a sensor HOBO® Pro v2 (Onset® Computer Corporation, USA; www.onsetcomp.com) placed in a meteorological shelter in the centre of each tunnel at average vegetation height. The average, minimum, and maximum daily temperatures are given in Table A.1.

2.1. Identification of flower cohorts

Measurements were taken on nine flower cohorts, from 7 Apr. to 30 May 2017, to have the largest range of temperature conditions throughout OSR flowering. Mean of average daily temperatures, and minimum and maximum instant temperatures recorded for each cohort are

provided in Table 1 for the two functional processes measured and the two lines used. Based upon the mean of average daily temperatures, the cohorts were divided into three temperature groups: those for which the mean of average daily temperatures during the floral life was less than 15°C, those for which it was between 15°C and 20°C, and those for which it was greater than 20°C.

To identify a flower cohort, a first flower marker Cherry (Filpack® agricole, France; www.filpack-agricole.com) was placed under the lowest bud on a set number of racemes chosen at random for each line on all available flowering plants between 1600 and 1800 h GMT. Flowering occurs acropetally, i.e. from bottom to top, on OSR racemes, but not strictly so and one can find a few flowers at anthesis above some flower buds. The peduncles of all flowers at anthesis that were located above the marked bud were cut to retain only flowers in the bud stage above the marker. The next day at 0800 h GMT, a second flower marker was placed on these same racemes above the highest flower at anthesis. All flowers still at bud stage located below the second marker were then sectioned at the peduncle in order to retain only flowers on their first day of anthesis between the two markers. At this stage, there were one to around ten such flowers per raceme, depending on the developmental stage and the level of branching of the raceme, the temperature and the line. These flowers constituted one flower cohort of about the same age, irrespective of the raceme they were on. A jeweller's tag was attached on the stem of the raceme to record the day of the anthesis and the treatment of its flowers (see below).

2.2. Duration of the pistil receptivity period

To measure the duration of pistil receptivity on the MS line, stigmas of flower cohorts from three racemes were hand pollinated at saturation with a fine brush twice a day throughout floral life until corolla fall. These hand pollinations took place between 0800 and 1200 h GMT and between 1200 and 1600 h GMT, except on the first day of anthesis for which pollination was done only once in the afternoon. The pollen used for these hand pollinations was harvested just before use by brushing the anthers of flowers on their first day of anthesis of ten different plants of the F_1 line chosen at random, and mixed the resulting pollen in a metal cup with the brush. The resulting pods were harvested forty days after flower anthesis, when seeds were developed enough to be accurately counted visually after pod dissection. All viable ovules do not necessarily develop into seeds even when stigmas are pollinated at saturation (Stephenson, 1981; Pechan, 1988), but the number of seeds obtained could nevertheless be used as a proxy of the minimum number of viable ovules that were available for fertilization and seed production when the pollen tubes penetrated the ovary after pollination.

Table 1 Number of winter oilseed rape flower cohorts sampled for each functional process and line with their features

Functional process	Line[a]	No. flower cohorts	Dates[b]	Duration[b] (days)	No. sampled flowers	Mean of average daily temperatures[c] (°C)	Extrema of temperatures[d] (°C)	
							Minimum	Maximum
Pistil receptivity	MS	4	7 Apr - 9 Apr	3	103	15.8	5.5	28.2
			25 Apr - 29 Apr	5	55	*12.6*	3.9	26.7
			1 May - 5 May	5	44	*13.5*	5.3	26.3
			16 May - 17 May	2	92	**22.4**	12.8	33.8
	F_1	2	18 Apr - 22 Apr	5	79	*13.2*	3.7	27.3
			16 May - 17 May	2	27	**22.4**	12.8	33.8
Nectar secretion	MS	7	7 Apr - 9 Apr	3	31	15.8	5.5	28.2
			14 Apr - 16 Apr	3	37	17.8	11.1	28.9
			25 Apr - 28 Apr	4	78	*12.0*	5.7	24.3
			1 May - 4 May	4	104	*13.0*	5.3	22.9
			8 May - 10 May	3	20	16.0	8.6	25.5
			16 May - 17 May	2	25	**22.4**	12.8	33.8
			23 May - 24 May	2	47	**24.7**	11.5	34.9
	F_1	7	7 Apr - 10 Apr	4	44	16.1	5.5	29.5
			18 Apr - 23 Apr	6	66	*13.8*	3.7	27.3
			25 Apr - 30 Apr	6	135	*12.5*	3.9	26.7
			1 May - 6 May	6	113	*13.4*	5.3	26.3
			8 May - 11 May	4	65	15.9	8.6	25.5
			23 May - 24 May	2	70	**24.7**	11.5	34.9
			29 May - 30 May	2	50	**23.7**	12.1	36.4

[a]The studied lines were the male sterile parental line (MS) and the male fertile F_1 line. [b]Dates and durations include the periods from flower anthesis until the end of pistil receptivity or the absence of nectar. [c]Mean of average daily temperatures were pooled into three categories: less than 15°C (italic), between 15° and 20°C (normal font), and greater than 20°C (bold). [d]Extreme temperature values are averaged or directly taken from Table A.1.

To measure the duration of this functional process on the F_1 line, hand pollinations were done in the same way as on MS flowers with the exception that self-pollination was prevented in these flowers with small glass tubes 8 mm long inserted on pistils and sealed with adhesive putty (Fig. 1). These small glass tubes were directly obtained by cutting 100 µL ringcaps® (Hirschmann® Laborgeräte, Germany; www.hirschmann-laborgeraete.de) with a file. They were inserted on pistils between 0700 and 0800 h GMT at anthesis before the anthers opened. These glass tubes were removed at the time of hand pollination.

The number of flower cohorts used per line and the number of sampled flowers per cohort are given in Table 1.

2.3. Duration of the nectar secretion period

To measure the duration of nectar secretion, nectar was sampled throughout the whole floral life until no more nectar remained. Flowers were sampled only once in their lifetime, allowing to measure the apparent secretion rate (Corbet, 2003). For each flower cohort, between two and five racemes were sampled per day and line, based on the available time, for each flower cohort, with an interval of ca. two hours between raceme sampling between 0800 and 1800 h GMT. Nectar was sampled with microcaps® (Drummond Scientific Company, USA; www.drummondsci.com) of 1, 2 or 3 µL depending on the volume of nectar available in the flower. Only nectar secreted by lateral nectaries was sampled since these nectaries produce ca. 95% of the total amount of nectar secreted by OSR flowers (Davis *et al.*, 1986; Davis *et al.*, 1998). When nectar was too viscous, that is in situations of high nectar concentrations and low temperatures (Nicolson and Thornburg, 2007), microcaps® were left in contact with nectaries three to five minutes. As pollen removal from the anthers hastens senescence of OSR male fertile flowers (Bell and Cresswell, 1998), nectar was sampled with caution to avoid dropping pollen in these flowers.

The volume of nectar secreted per flower was calculated by measuring the height of the column of liquid in the microcaps®, and then converting it in µL based on the total volume of the microcaps® used. The nectar concentration in dissolved solids was then measured with a refractometer Eclipse 45-81 (0-50% Brix) or 45-82 (45-80% Brix), adapted for reading small nectar volumes (Bellingham and Stanley Ltd, UK; www.bellinghamandstanley.com). Nectar volume and concentration were then converted into sugar mass per flower with the conversion formula of Cruden and Hermann (1983):

$$M = V\,C\,(0.000046\,C + 0.009946)$$

Fig. 1. Winter oilseed rape male fertile flower of F_1 line cv 'Exocet', with a glass tube inserted on the pistil to prevent autonomous self-pollination. White bar, 4 mm. © Stan Chabert / INRA

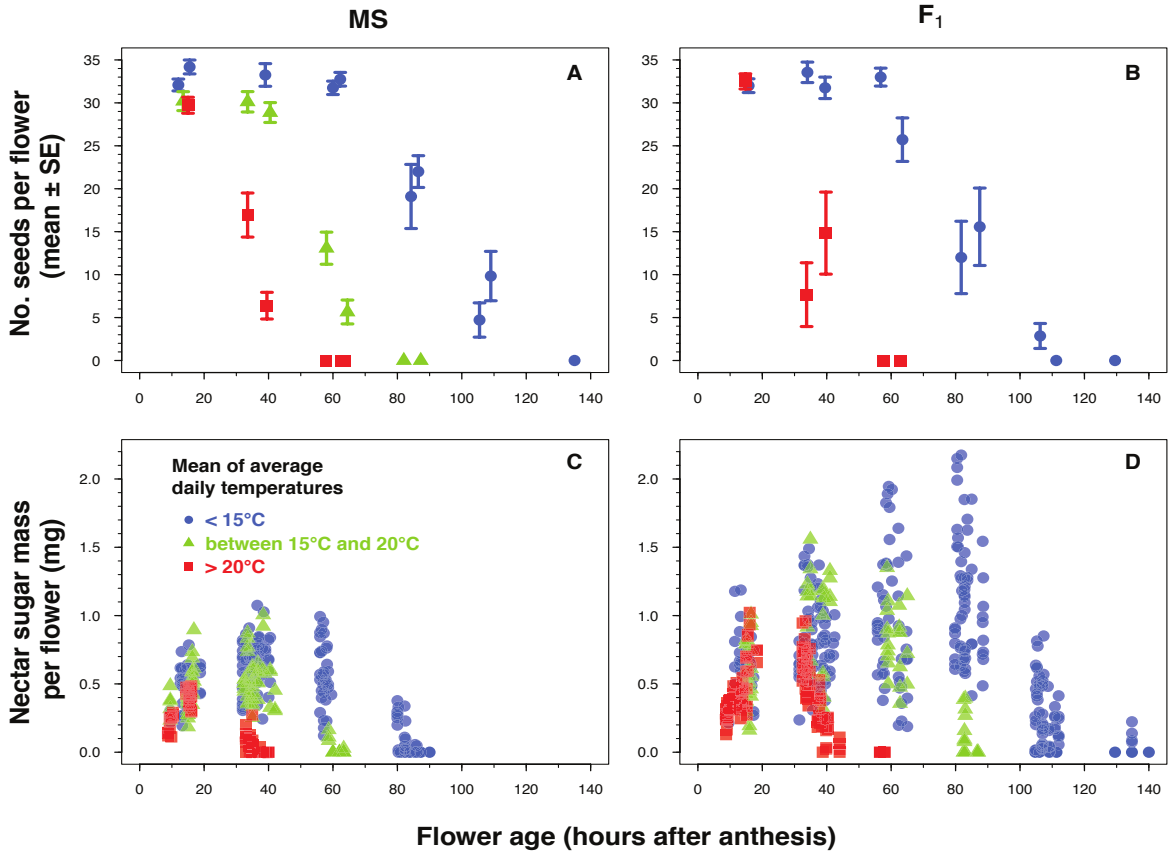


Fig. 2. (A, B) Evolution of pistil receptivity (assessed by the seed content produced per flower following saturating hand pollination) and (C, D) evolution of nectar apparent secretion rate with flower age expressed in calendar time in winter oilseed rape. (A, C) Male sterile parental line; (B, D) male fertile F_1 line. Numbers of sampled flower cohorts and of sampled flowers per cohort are given in Table 1. Each of the three colour gives the data for one category of mean of average daily temperatures for each sampled flower cohort (given in Table 1).

where M is the mass of sugar in mg, V the volume of nectar in µL, and C the concentration of nectar in % Brix (g of sugar per 100 g of solution).

2.4. Data analysis

To express the levels of pistil receptivity and nectar amount in relation to flower age in the two winter OSR lines, one piecewise polynomial function was made with two breakpoints for each functional process (Bolker, 2008).

To describe the level of pistil receptivity, the proxy of the number of seeds per flower y was expressed according to the flower age x as follows:

$$\begin{aligned} &\text{if } x > b_2,\ y = 0 \\ &\text{if } b_1 < x < b_2,\ y = s_2\,(b_2 - x) \\ &\text{if } x < b_1,\ y = s_2\,(b_2 - b_1) \end{aligned} \qquad (1)$$

where b_1 is the flower age at which ovule senescence begins, b_2 is the flower age at which all ovules are dead, s_2 is the slope of the rate of progression of ovule senescence on the assumption that this rate is constant until the death of the last ovule, and $s_2\,(b_2 - b_1)$ is the total mean number of ovules contained in OSR ovary.

To describe the level of nectar amount, the mass of nectar sugar per flower z was expressed according to the flower age x as follows:

$$\begin{aligned} &\text{if } x > b_2,\ z = 0 \\ &\text{if } b_1 < x < b_2,\ z = s_2\,(b_2 - x) \\ &\text{if } x < b_1,\ z = s_2\,(b_2 - b_1) - s_1\,(b_1 - x) \end{aligned} \qquad (2)$$

where b_1 is the flower age at which nectar secretion ceases and reabsorption begins, b_2 is the flower age at which all nectar is reabsorbed, s_1 is the slope of the apparent secretion rate on the assumption that this rate is constant until the end of secretion, s_2 is the slope of the rate of nectar reabsorption on the assumption that this rate is constant until all nectar is reabsorbed, $b_1 - (s_2\,(b_2 - b_1) / s_1)$ is the flower age at which nectar secretion begins before flower anthesis on the assumption that nectar apparent secretion rate is constant before and after flower anthesis, $s_2\,(b_2 - b_1) - s_1\,b_1$ is the sugar mass contained in flower at anthesis, and $s_2\,(b_2 - b_1)$ is the total sugar mass produced by flower when secretion ceases and reabsorption begins.

In these two kinds of mechanistic models, x is the age of the flower starting at anthesis. As OSR flower buds open throughout the day (Eisikowitch, 1981), and as the marked buds could open on the evening after placing the first flower markers, or in the morning before placing the second flower markers, the mean time of anthesis of flower cohorts was assumed to occur at 0000 h GMT on the first day of anthesis. Flower age x was expressed either in hours as unit of measure of calendar time, or in degree.hours as unit of measure of thermal time. Degree.hours is a newly proposed cumulative temperature unit that is noticeably more accurate than degree.days (Gu, 2016), and is also more relevant in flowers of OSR that last only a few days. The statistical inference method used to estimate the parameters of the two polynomial functions (1) and (2) was the nonlinear least squares (Bates and Watts, 1988).

As thermal time expression depends on a base temperature T_{base} unknown for the onset of OSR flower senescence, equations (1) and (2) were computed 101 times by varying T_{base} from 0°C to 10°C with an increment of 0.1°C for the calculation of x in degree.hours. The four T_{base} values were selected based on which coefficient of determination R^2 values were the largest (Nakagawa and Schielzeth, 2013). The assumptions were made here that (i) there is no optimum temperature at which the rate of flower ageing is maximum, (ii) there is no ceiling temperature above which flower ageing ceases, and (iii) the rate of flower ageing increases in a linear fashion with temperature above the base temperature. For the calculation of degree.hours, the mean hourly temperature was first calculated for each hour with the 24 instantaneous recordings available per hour. Then, to convert flower age expressed in a number of hours into a number of degree.hours, all hourly mean temperatures above the selected T_{base} values were added up after prior subtracting of their respective T_{base} values.

To test if thermal time was more suitable than calendar time to express the durations of OSR pistil receptivity and nectar secretion, equations (1) and (2) were compared with x expressed either in hours or in degree.hours with Akaike information criterion (AIC) values (Akaike, 1973; Burnham and Anderson, 2002). AIC is a "trade-off statistic" that allows one to select the most parsimonious model in a given set of hypotheses in balance. These models were also compared with null models for which the levels of pistil receptivity and nectar amount did not vary with either calendar time nor thermal time.

To test if nectar secretion was synchronous with pistil receptivity in the two lines, 95% confidence intervals were estimated around the breakpoint values (Nakagawa and Cuthill, 2007). As these confidence intervals were not necessarily directly comparable when expressed in degree.hours because of the potential differences in the T_{base} values retained for each

functional process and each line, breakpoint values and their associated 95% confidence intervals were converted back into hours according to the temperature as follows:

$$b_h \pm C_i = (b_{dh} \pm C_i) / (t - \dot{T}_{base}) \quad (3)$$

where b_h is the flower age for each breakpoint value in hours, b_{dh} is the flower age for each breakpoint value in degree.hours, $\dot{T}_{base}$ is the base temperature with the best R^2 value, t is the temperature included in the range of mean of average daily temperatures in °C, and C_i is the 95% confidence interval.

When two parameters had non-overlapping 95% confidence intervals, their mean values were considered statistically different.

To be able to synthetize all the information found on a common thermal time scale, breakpoint parameter values b were converted into values b' as follows:

$$b' = b\ (t_m - T_{base}') / (t_m - \dot{T}_{base}) \quad (4)$$

where b is the breakpoint thermal time initial value with the best base temperature $\dot{T}_{base}$ value proper to each functional process and each line, b' is the new breakpoint thermal time value with the common selected base temperature T_{base}' value, and t_m is the mean temperature value of the range of mean of average daily temperatures encountered in the data.

All the statistics were computed with the software R, version 3.2.0 (R Core Team, 2015). Asymptotic 95% confidence intervals of piecewise polynomial model parameters were estimated with the package *nlstools*, version 1.0-2 (Baty *et al.*, 2015).

3. Results

We were able to cover a large array of temperatures, between 3.7°C and 36.4°C in instantaneous values and between 12.0°C and 24.8°C in mean of average daily temperatures during the flowering periods over which we conducted our measurements (Table 1). A minimum of two flower cohorts was sampled for pistil receptivity recordings in the F_1 line, up to 7 cohorts for the nectar secretion recordings for the two lines. Between 20 and 135 flowers were sampled per cohort for each functional process and each line, with a grand total of 1,285 flowers sampled.

3.1. Duration of the pistil receptivity period

The period following pollination during which seeds were produced lasted from two to five days in the two lines, depending on the temperature (Table 1, Fig. 2A and B). When equation (1) was used with the cumulative degree.hour unit as explanatory variable, the base temperatures with the best R^2 values were respectively 7.5°C and 5.9°C for the MS and F_1 lines (Fig. A.1A and B). For the two lines, the models selected with the lowest AIC values were the polynomial functions with the cumulative degree.hour unit as explanatory variable, with a great gap of AIC values when using models with the hour as explanatory variable ($\Delta AIC \geq 95$ in the two lines, Table 2). The models with the cumulative degree.hour unit as explanatory variable were relatively well supported with R^2 values of respectively 0.83 and 0.77 for the MS and F_1 lines. The pistil receptivity period was characterized by a first phase of stable receptivity after anthesis followed by a clearly distinct second phase of gradual and steady decline leading to a third phase in which there was no more receptivity at all (Fig. 3A and B, see also b_1 and b_2 parameters with 95% IC values in Table 2). The total mean estimated numbers of ovules were $s_2 (b_2 - b_1) = 31.4$ in the MS line, and 32.6 in the F_1 line.

No seed was produced from the day of anthesis until the time of hand-pollination in those flowers that were hand-pollinated after their pistil receptivity was over, but remained exposed for the whole period before that in the two lines. This absence of seed production indicates that there was no unwanted pollination due to wind in the MS line, and that the glass tubes were able to adequately prevent autonomous self-pollination in the F_1 line, thereby providing some validation to our methods.

3.2. Duration of the nectar secretion period

The period during which there was still a presence of nectar after flower anthesis lasted two to four days, depending on the temperature, in the MS line, and two to six days in the F_1 line (Table 1, Fig. 2C and D). When equation (2) was used with the cumulative degree.hour unit as explanatory variable, the base temperatures with the best R^2 were respectively 3.4°C and 6.8°C for the MS and F_1 lines (Fig. A.1C and D). For the two lines, the models selected with the lowest AIC values were the polynomial functions with the cumulative degree.hour unit as explanatory variable, with again a great gap of AIC values when using models with the hour as explanatory variable ($\Delta AIC > 247$ at least in the two lines, Table 2). The models with the cumulative degree.hour unit as explanatory variable were again relatively well supported with R^2 values of respectively 0.62 and 0.53 for the MS and F_1 lines. The period of presence of nectar was characterized in the two lines by a first phase of gradual increase of nectar in the flower

Table 2 Estimated parameter values and features of calendar time (hours) and thermal time (degree.hours) models computed for each winter oilseed rape flower functional process and line

Functional process	Line[a]	No. sampled flowers	Model[b]	k[c]	$\dot{T}_{base}$[d] (°C)	Mean parameter values (± 95% CI)				R^2	AIC	Model rank	ΔAIC
						b_1[e]	b_2[f]	s_1[g]	s_2[h]				
Pistil receptivity	MS	294	null	2							2,392	3	514
			hours	4		46.3 (± 17.8)	129 (± 28)		0.239 (± 0.113)	0.202	2,330	2	452
			degree.hours	**4**	**7.5**	**287 (± 25)**	**608 (± 20)**		**0.0979 (± 0.0108)**	**0.828**	**1,878**	**1**	**0**
	F_1	106	null	2							877	3	150
			hours	4		44.3 (± 19.1)	115 (± 17)		0.366 (± 0.146)	0.431	822	2	95
			degree.hours	**4**	**5.9**	**284 (± 76)**	**771 (± 68)**		**0.0670 (± 0.0160)**	**0.766**	**727**	**1**	**0**
Nectar secretion	MS	342	null	2							80	3	325
			hours	5		55.8 (± 7.4)	86.7 (± 6.4)	$6.78e^{-4}$ (± $2.72e^{-3}$)	0.0146 (± $4.26e^{-3}$)	0.215	3	2	248
			degree.hours	**5**	**3.4**	**433 (± 27)**	**746 (± 28)**	**$7.82e^{-4}$ (± $2.42e^{-4}$)**	**$2.09e^{-3}$ (± $3.10e^{-4}$)**	**0.620**	**-245**	**1**	**0**
	F_1	543	null	2							671	3	404
			hours	5		72.3 (± 6.0)	118 (± 7)	$6.43e^{-3}$ (± $2.39e^{-3}$)	0.0199 (± $4.97e^{-3}$)	0.205	552	2	285
			degree.hours	**5**	**6.8**	**457 (± 21)**	**683 (± 21)**	**$1.57e^{-3}$ (± $2.93e^{-4}$)**	**$4.91e^{-3}$ (± $8.45e^{-4}$)**	**0.530**	**267**	**1**	**0**

[a]The studied lines were the male sterile parental line (MS) and the male fertile F_1 line. [b]Models with calendar time and thermal time were computed from equation (1) for pistil receptivity, and from equation (2) for nectar secretion. [c]k was the number of estimated parameters per each model. [d]$\dot{T}_{base}$ were the retained base temperatures with the best R^2 values for thermal time models (calculated from Fig. A.1). [e]b_1 is the flower age at which ovule senescence begins, or at which nectar secretion ceases and reabsorption begins (in hours or degree.hours); [f]b_2 is the flower age at which all ovules are dead, or at which all nectar is reabsorbed (in hours or degree.hours); [g]s_1 is the nectar apparent secretion rate (in mg of sugar mass per hour or degree.hour); [h]s_2 is the rate of progression of ovule senescence (in ovules per hour or degree.hour), or the rate of nectar reabsorption (in mg of sugar mass per hour or degree.hour). Models in bold indicate those with the lowest AIC value.

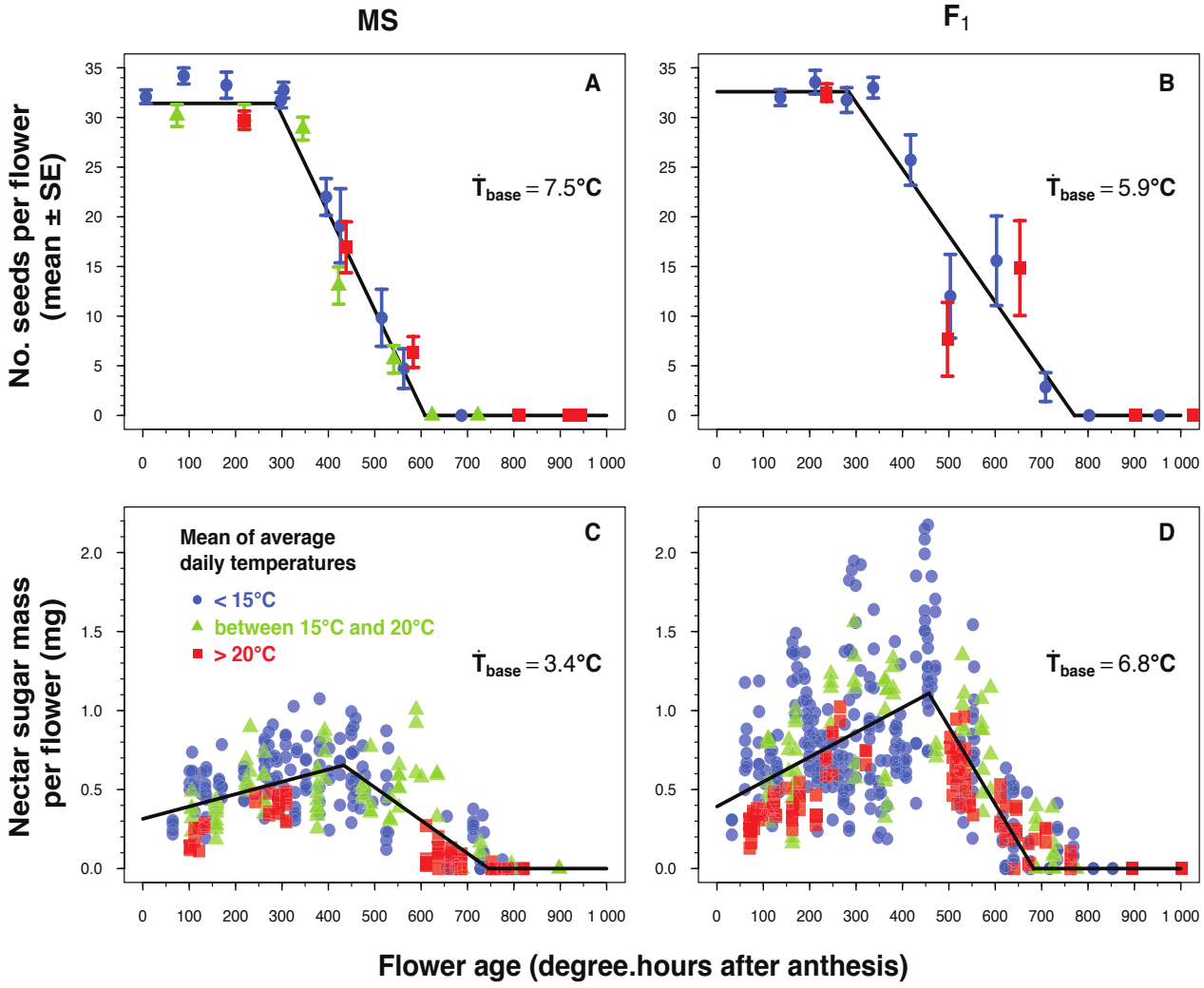


Fig. 3. (A, B) Evolution of pistil receptivity (assessed by the seed content produced per flower following saturating hand pollination) and (C, D) evolution of nectar apparent secretion rate with flower age expressed in thermal time in winter oilseed rape. (A, C) Male sterile parental line; (B, D) male fertile F_1 line. Numbers of sampled flower cohorts and of sampled flowers per cohort are given in Table 1. Each of the three colour gives the data for one category of mean of average daily temperatures for each sampled flower cohort (given in Table 1). Solid lines give the mean values predicted by equations (1) and (2) (estimated parameter values given in Table 2) with the best base temperatures retained $\dot{T}_{base}$ (got from Fig. A.1).

after anthesis followed by a clearly distinct second phase of gradual decrease in the amount of nectar, leading to a third phase in which no more nectar was available (Fig. 3C and D, see also b_1 and b_2 parameters with 95% IC values in Table 2). The rate of reabsorption s_2 was 2.7 times higher than the rate of secretion s_1 in the MS line, and 3.1 times higher in the F_1 line. The estimated mean amounts of sugar contained in the flower at anthesis were $s_2 (b_2 - b_1) - s_1 b_1 =$ 0.315 mg in the MS line, and 0.391 in the F_1 line. The estimated total amount of sugar produced by flowers when secretion ceased and reabsorption started were $s_2 (b_2 - b_1) = 0.654$ mg in the MS line, and 1.11 mg in the F_1 line. The estimated flower ages at which nectar secretion started before flower anthesis were $b_1 - (s_2 (b_2 - b_1) / s_1) = -427$ degree.hours ($T_{base} = 3.4°C$) in the MS line, and -249 degree.hours ($T_{base} = 6.8°C$) in the F_1 line.

3.3. Synchrony of nectar secretion with pistil receptivity

The two lines did not show different durations of stable pistil receptivity along the range of mean of average daily temperatures encountered in our measurements (Fig. 4A). The end of nectar secretion was concomitant with the beginning of decline of pistil receptivity in the MS line, but occurred later in the F_1 line. Otherwise, pistil receptivity totally ended at the same time in the two lines along the range of temperatures encountered in our measurements, and nectar was totally reabsorbed at this time in the F_1 line (Fig. 4B). In the MS line, the nectar was totally reabsorbed before pistil receptivity ended, except at the high temperatures for which the end of nectar reabsorption was concomitant with the end of pistil receptivity.

3.4. Synthesis on a common thermal time scale

All previous information found were synthesized together on a common thermal time scale (Fig. 5). Breakpoint parameter values converted in common thermal time are given in Table 3. The mean temperature value over the range of mean of average daily temperatures encountered in the data t_m was 18°C. The common base temperature selected was 6.8°C, because it corresponded to the median of the best base temperatures we found (Table 2), excluding the marginal value of 3.4°C.

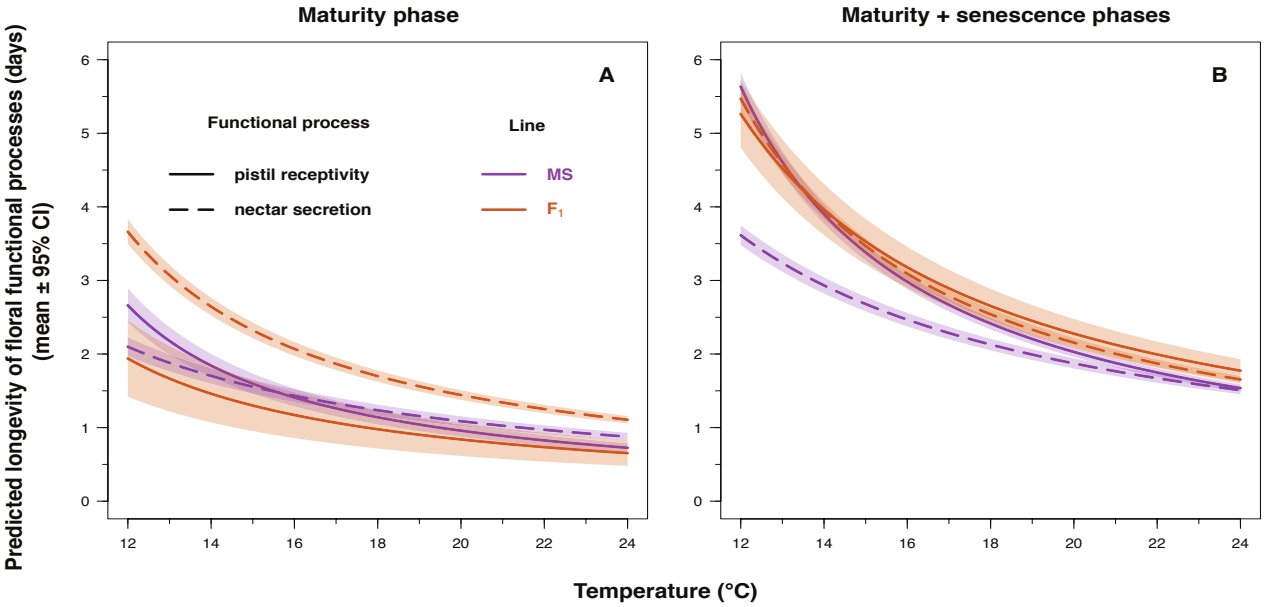


Fig. 4. Reaction norms of the predicted duration of pistil receptivity and of presence of nectar with the temperature for flowers of a winter oilseed rape male fertile F_1 line and its male sterile parental line (MS). (A) Durations of the phases of maximal stable pistil receptivity and of nectar secretion using the parameters b_1 (given in Table 2). (B) Durations of the whole periods of pistil receptivity and of presence of nectar using the parameters b_2 (given in Table 2). Shaded areas depict the 95% confidence intervals (CI) around the average predictions calculated with equation (3). The temperature range shown in the abscissa corresponds to the range of mean of average daily temperatures encountered in this study (Table 1).

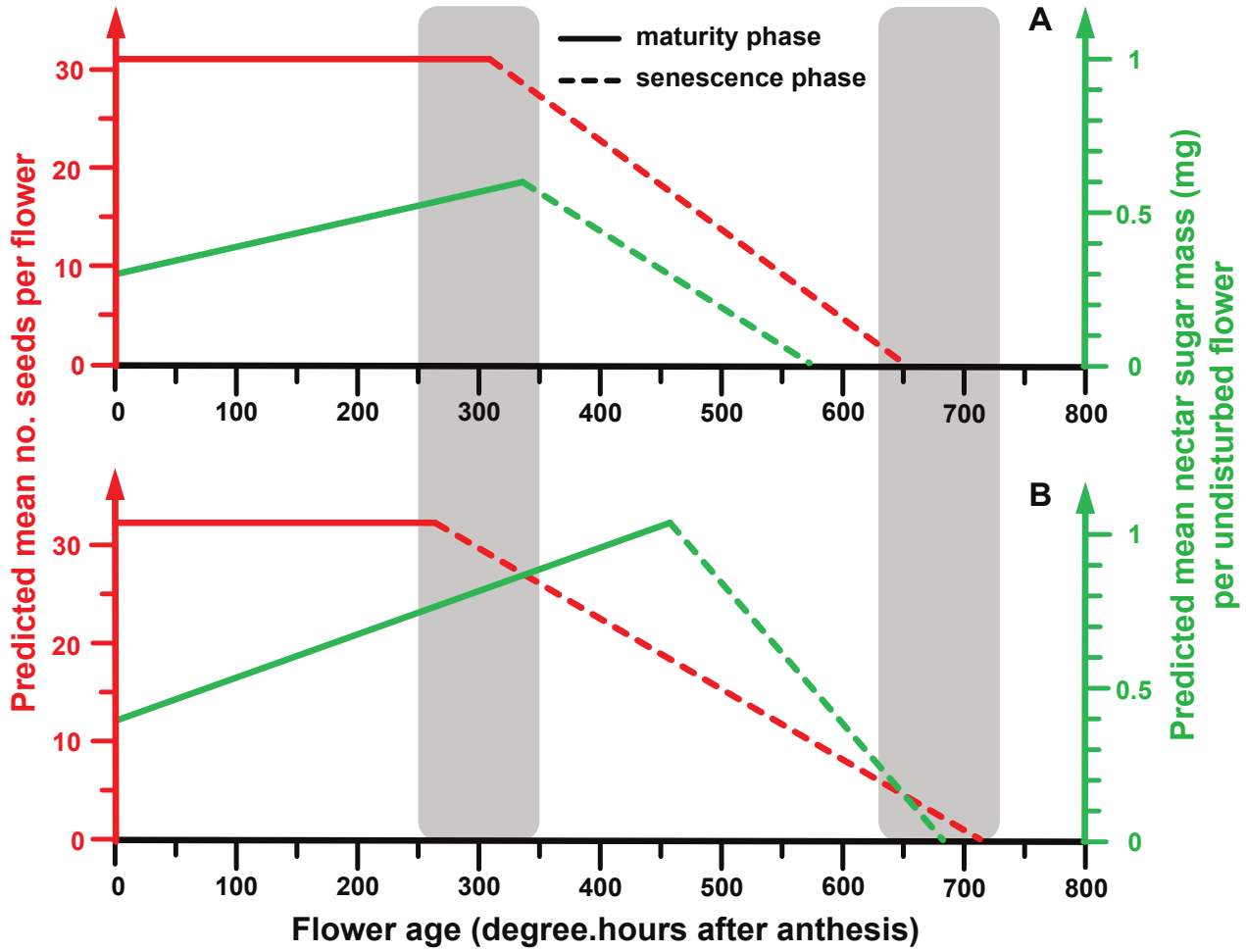


Fig. 5. Synthesis on the same thermal time scale (with the selected common base temperature of 6.8°C) of the estimated durations of pistil receptivity (in red) and of presence of nectar (in blue) in winter oilseed rape flowers. (A) Male sterile parental line; (B) male fertile F_1 line. Solid lines represent the phase of stable pistil receptivity and of nectar secretion. Dashed lines depict the senescence phase, i.e. the gradual decline of pistil receptivity and the nectar reabsorption. Shaded areas show the phase boundaries that are not statistically different at the 5% threshold (calculated from the 95% confidence intervals given in Fig. 4). Phase boundaries values converted in common thermal time scale were calculated from equation (4) and are given in Table 3. Values were predicted from equations (1) and (2). An undisturbed flower is a flower where pollen and nectar have not been previously removed.

Table 3 Breakpoint parameter values of thermal time models converted into a common thermal time scale

Functional process	Line[a]	$\dot{T}_{base}$[b] (°C)	Parameters	Parameter values (degree.hours)	
				with $\dot{T}_{base}$[b]	with T_{base} = 6.8°C
Pistil receptivity	MS	7.5	b_1[d]	287	307
			b_2[e]	608	649
	F_1	5.9	b_1[d]	284	263
			b_2[e]	771	713
Nectar secretion	MS	3.4	b_0[c]	-427	-328
			b_1[d]	433	332
			b_2[e]	746	572
	F_1	6.8	b_0[c]	-249	-249
			b_1[d]	457	457
			b_2[e]	683	683

[a]The studied lines were the male sterile winter oilseed rape parental line (MS) and the male fertile F_1 line. [b]$\dot{T}_{base}$ were the retained base temperatures with the best R^2 values for thermal time models (obtained from Fig. A.1). [c]b_0 is the flower age at which nectar secretion begins before flower anthesis; [d]b_1 is the flower age at which ovule senescence begins, or at which nectar secretion ceases and reabsorption begins; [e]b_2 is the flower age at which all ovules are dead, or at which all nectar is reabsorbed. The selected base temperature for common thermal time scale was 6.8°C and corresponded to the median of the best base temperatures got (given in Table 2 and obtained from Fig. A.1), excluding the marginal value of 3.4°C. Values converted in common thermal time were calculated with equation (4).

4. Discussion

Our results show that, as expected, expressing flower age in cumulative temperature units is more suitable than expressing it simply in calendar time for the duration of the two floral functional processes we studied, namely pistil receptivity and nectar secretion (Table 2, Figs 2 and 3). We shall now discuss the different physiological mechanisms attached to thermal time that drive ageing rate and developmental rate. To give a more visual and practical connection between the level of pistil receptivity and the amount of available nectar with the morphology of the flower, photographs at the flower at each morphological stage were taken for each line (Fig. A.2).

4.1. Ageing rate versus developmental rate

The best base temperatures we found were fairly similar - between 5.9°C and 7.5°C - except for the one that concerned the MS line nectar secretion that was 3.4°C. The observed base temperature to measure flower age here is higher than the one that is largely known for OSR plant development, which is around 0°C (Netzer *et al.*, 1986; Kirkegaard *et al.*, 2012; Soltani and Sinclair, 2012; Andreucci *et al.*, 2016). Grimm *et al.* (1994) also observed different base temperatures to quantify soybean plant age from stage R_1 (flowering) to stage R_5 (beginning of seed growth), and from stage R_5 to stage R_7 (physiological maturity), showing that the development base temperature can vary with the physiological stage of the plant.

To have a more accurate estimation of these base temperatures, it would have been necessary to be able to measure the period of these functional processes under even lower temperatures and ideally under controlled temperature conditions as in a phytotron. This would have enabled us to study the reaction norm *per se* of the ageing rate with the temperature, which we could not do under our conditions of varying uncontrolled temperatures. This reaction norm of the ageing rate with temperature has been very poorly studied, indeed, compared to the reaction norm of developmental rate with temperature. Overall, the lifespan continually decreases as temperature rises in most of the intraspecific studies of poikilothermic organisms cited by Keil *et al.* (2015), knowing that a relatively narrow range of temperatures have been studied in most of them. We can still note that the lifespan continues to decrease beyond 35°C in the studies that tested the highest temperatures (Loeb and Northrop, 1917; Pandey and Tripathi, 2008; Pakyari *et al.*, 2011). Three other studies show an exception with a decrease of lifespan at lower temperatures (Shaw and Bercaw, 1962; Klass, 1977; Wang and Tsai, 2001). All of these studies show in any case that the thermal reaction norms of the ageing rate follow

a different pattern from those of the developmental rate. This probably reflects the differences of physiological mechanisms that drive these two processes in these organisms: the developmental rate is driven by enzyme activity, while the ageing rate seems driven by oxidative stress.

4.2. Duration of the pistil receptivity period

We found two distinct phases in the duration of the two measured flower processes: a first phase of maturity followed by a second phase of senescence (Figs 3 and 5). Pistil has a full receptivity during some length of thermal time, and then it gradually declines to zero. These two phases last about the same time in the MS and the F_1 lines (Figs 4 and 5). This confirms that pistil receptivity is limited by a gradual and acropetal senescence of ovules, as showed by Carbonell-Bejerano *et al.* (2011) in *Arabidopsis thaliana*, and not by an end of the stigmatic receptivity. Otherwise, the end of pistil receptivity would have been much more sudden and without a gradual decline. Carbonell-Bejerano *et al.* (2011) showed more specifically that ovule and pistil senescence are precipitated by an increase in ethylene biosynthesis in ovules of unpollinated pistils just before the end of ovule longevity. However, they specified that this up-regulation of ethylene biosynthesis is not strictly necessary for the onset of ovule senescence. Indeed, in an ethylene-insensitive *A. thaliana* mutant, the onset of ovule senescence was delayed by a mere one day. In both cases, we still do not know what does trigger the increase of ethylene biosynthesis or the 'naturally' programmed ovule senescence, but as discussed above, oxidative stress and disruption of the redox balance seem to be the most plausible explanation.

Overall, we found that the effective pollination period decreased gradually with temperature (Fig. 4), as it has already been found a few times in other species (Vasilakakis and Porlingis, 1985; Burgos *et al.*, 1991; Tromp and Borsboom, 1994; Vuletin Selak *et al.*, 2014), or for ovule longevity as mentioned in the introduction, or for the duration of the stigmatic receptivity (Hedhly *et al.*, 2003; Hedhly *et al.*, 2005; Lora *et al.*, 2011; Vuletin Selak *et al.*, 2014). It is hoped that this work will encourage future studies to measure the effective pollination period in cumulative temperature units, and more precisely in degree.hours, to allow to predict this period according to temperature. More broadly, cumulative temperature units could also be used to measure the lifespan of plant organs and poikilothermic organisms. Insect pollinators, being also poikilothermic organisms, have a thermal optimum for their foraging activity as well (Corbet *et al.*, 1993; Willmer and Stone, 2004; Colinet *et al.*, 2015; Inouye *et al.*, 2015). This phenomenon, combined with the decreasing effective pollination period with

increasing temperature, could lead to a pollination deficit (Wilcock and Neiland, 2002; Ashman *et al.*, 2004; Aizen and Harder, 2007) increased in entomophilous crops at high temperatures.

4.3. Duration of the nectar secretion period and synchrony with that of the pistil receptivity

Nectar secretion was found synchronous with the phase of full ovule viability in the MS line, then the nectar is reabsorbed during the ovule senescence phase, with a reabsorption rate a bit faster than ovule senescence (Figs 4 and 5). The period of flower attractivity is therefore well adjusted with that of pistil receptivity in this line in agreement with our hypothesis II. Floral longevity is therefore not governed by the presence of pollen in anthers alone, but also by the ovule senescence that acts as an activator of the whole flower senescence. The senescence of the nectary tissue may be induced by the spread of ethylene from the pistil to the nectaries.

On the other hand, and again as expected under hypothesis II, the nectar secretion phase is longer than the period during which pistil remained completely receptive in the F_1 line (Figs 4 and 5). This is in agreement with the assumption that the presence of pollen in anthers act as an inhibitor and a delayer of flower senescence. Further investigations are needed, however, to test if full pistil receptivity ends, or not, when anther dehiscence is completed. From a proximal point of view, a senescence-inhibiting hormone, such as a cytokinin or gibberellin (Reid and Chen, 2007; Rogers, 2015; Shibuya and Ichimura, 2016), may be biosynthesised by anthers filled with pollen during a certain period of thermal time and spreads towards nectaries to counteract the effect of ethylene biosynthesised by the pistil. Once the pollen is too old, the anthers may stop to produce this inhibitory hormone. From an ultimate point of view, the period of flower attractivity is probably extended to attract insect pollinators longer to ensure as long as possible the probability of pollen dissemination before its viability is lost. The pollen is probably still viable at the end of the full pistil receptivity period in OSR, as it seems to be the case in *Raphanus sativus* (Dafni and Firmage, 2000), a species belonging to the Brassicaceae as OSR. Nepi and Pacini (1993) and Franchi *et al.* (2014) noted that pollen viability falls below 50% after the flowers close in six Angiosperm species studied. They proposed that the flower closure at the end of floral life may be a strategy to prevent pollination with pollen of low viability. Another ultimate explanation of the break of nectar secretion, if pollen viability is still high at this time in OSR, may be that costs of floral maintenance exceed that of constructing a new flower (Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995). Indeed, the amount of sugar contained in undisturbed male fertile OSR flowers at the end of nectar secretion was

about 1 mg (Fig. 3D). Further investigations would be necessary to evaluate the pollen viability level of OSR at the end of the nectar secretion period.

The secretion of nectar in OSR flowers begins at the bud stage, about 300 degree.hours (T_{base} = 6.8°C, Table 3) before flower anthesis for the two lines, which is about 30 hours at 18°C. This corresponds closely to the stage 12 according to Smyth *et al.* (1990) classification of *A. thaliana* flower stages, being the last stage just before flower anthesis, when nectaries are still growing. This onset of nectar secretion before flower anthesis should perhaps promote cross-pollination at flower anthesis with a certain amount of nectar already available to attract insect pollinators. Indeed, despite its apparent homogamy, OSR flowers are first predominantly functionally female, before being predominantly functionally male (Bell and Cresswell, 1998).

The sugar mass was about 1.2 times higher in the F_1 line compared to the MS one at flower anthesis, and about 1.7 times higher at their respective maxima. This is in agreement with the observation of Pierre *et al.* (1999) that three OSR MS lines produced 16 to 70% less nectar in volume compared to their isogenic male fertile counterparts.

5. Conclusions

B. napus flower ageing was quantified in thermal time, a unit still almost never used until now to quantify ageing, neither for flowers, nor more broadly for that of poikilothermic organisms, to measure the duration of pistil receptivity and that of nectar secretion. Two distinct phases were identified in both processes, a maturity one and a senescence one, and both of them proceeded faster as temperature increased. The decrease of the pistil receptivity period with increase temperature was examined quantitatively on the basis of the mechanisms attached to thermal time, enabling us to predict the duration of this process in relation to temperature. The nectar secretion period was also formalised, and thereby the period of flower attractiveness to pollinators. It provides also the tools to predict the duration of this period in relation to temperature, a feature which may be of major significance for zoophilous plants at a time of decreasing pollinator populations and of climate change. The data supported that flower senescence was triggered by the pistil senescence, and that the presence of pollen in anthers acted as an inhibitor and a delayer of flower senescence. Flowers invested therefore in nectar secretion to attract insect pollinators at least to cover the entirety of the receptivity period of their female part. Nectar secretion could be prolonged in pollen-laden male fertile flowers to attract insect pollinators longer to ensure as long as possible the probability of pollen dissemination.

This knowledge should help us to quantify better the investment of zoophilous flowers to attract pollinators in regards to their pollination requirements. It should also help us to better manage the pollination of entomophilous crops, particularly for hybrid seed productions at a time of climate change.

Contributions

S.C. and B.E.V. conceived the study, S.C., T.L. and M.R.C. performed field work, N.M. provided assistance and guidance for temperature recordings, S.C. analysed the data, S.C. and B.E.V. wrote the manuscript.

Funding

This work was supported by the Association Nationale de la Recherche et de la Technologie (ANRT) as part of the Conventions Industrielles de Formation par la REcherche (CIFRE) doctoral grants [convention number 2014/0070], allocated here to S.C. by the Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses (ANAMSO) through specific interprofessional actions with the Groupement National Interprofessionnel des Semences et plants (GNIS) and the Union Française des Semenciers (UFS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank all the members of the experimental unit of UMR EMMAH, INRA PACA, who grew the OSR crop under tunnels, Gilles Continsouzas, Véronique Desfonds, Serge Reno, Jérémy Theodore and Frédéric Venault. We are also grateful to Olivier Geist, Gaëtan Lecordier, Laëtitia Planque and Marine Willemin who helped in field work, as well as Arthur Cambert, Alexandra Drouet, Louna Fronteau, Tommy Gerez, Anne Laure Guirao, Estienne Liegeois, Nicolas Nalpowik and Cyril Scomparin who helped in preliminary works. We thank Violette Le Féon with whom we initiated our first reflections on the duration of pistil receptivity, as well as Laurent Guilbaud who improved the photographs quality, and Jean-Christophe Conjeaud of ANAMSO who, as a financial and technical partner, made this study possible. We also thank Monsanto® company for graciously providing us with the seeds to carry out this study and two anonymous referees for their constructive comments that enabled us to greatly improve the manuscript.

Appendix A. Supplementary data

Table A.1 Daily mean, minimum and maximum temperatures recorded for each day of measurements in 2017

Month	Day	Daily temperatures			Month	Day	Daily temperatures		
		Mean	Minimum	Maximum			Mean	Minimum	Maximum
April	7	15.8	7.7	25.8	May	1	12.9	5.3	21.0
	8	15.9	6.2	28.2		2	11.4	5.8	19.2
	9	15.6	5.5	27.8		3	13.6	5.5	22.3
	10	17.0	5.8	29.5		4	13.9	8.6	22.9
	11	16.9	10.5	23.0		5	15.4	7.6	26.3
	12	17.7	9.5	28.9		6	13.4	9.3	20.1
	13	17.8	7.3	30.3		7	16.0	7.7	23.6
	14	19.6	12.6	28.9		8	16.5	11.2	23.5
	15	17.5	11.1	26.7		9	16.5	9.6	25.5
	16	16.4	11.5	23.0		10	14.9	8.6	20.1
	17	16.5	9.6	25.1		11	15.6	9.9	22.9
	18	13.7	8.3	20.5		12	18.9	12.7	27.5
	19	11.6	5.2	20.0		13	19.7	10.1	30.7
	20	11.9	3.8	20.7		14	20.0	11.9	28.9
	21	14.2	4.9	25.9		15	22.2	14.1	31.2
	22	14.7	3.7	27.3		16	22.4	13.2	33.6
	23	16.6	7.8	25.6		17	22.4	12.8	33.8
	24	15.7	6.9	26.1		18	22.2	15.5	31.3
	25	14.5	7.4	24.3		19	18.3	11.3	27.4
	26	12.5	7.7	17.4		20	18.4	8.6	27.7
	27	9.3	5.8	15.4		21	20.6	11.8	31.3
	28	11.8	5.7	19.6		22	19.1	10.2	27.0
	29	14.8	3.9	26.7		23	23.4	11.5	33.6
	30	12.0	7.4	16.0		24	26.0	19.4	34.9
						25	25.6	17.4	36.1
						26	23.4	14.9	34.5
						27	23.6	13.4	35.4
						28	23.8	13.8	36.3
						29	24.0	12.1	36.4
						30	23.5	14.0	35.9

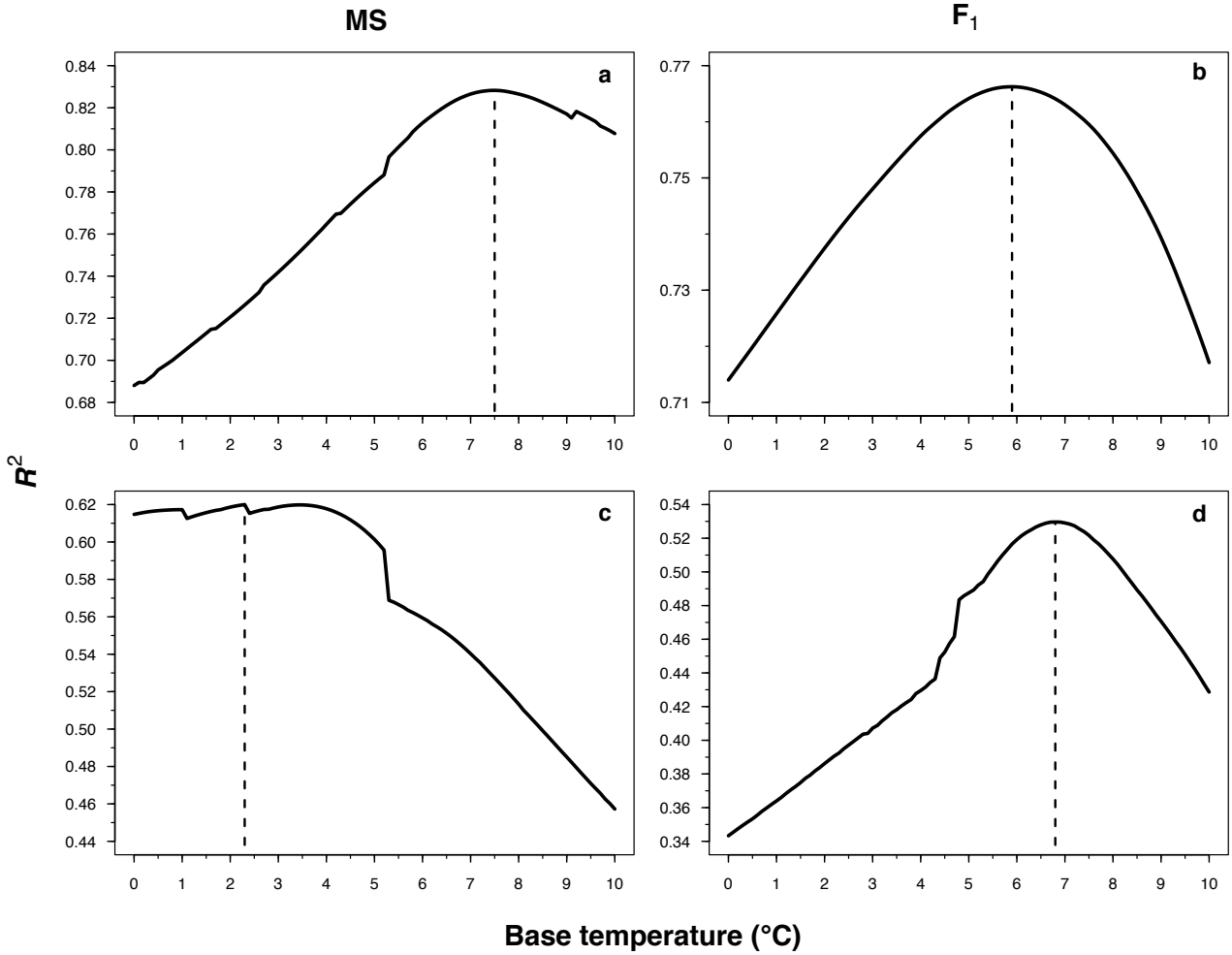


Fig. A.1. Coefficients of determination R^2 of the 101 piecewise thermal time polynomial functions (Eqs. (1) and (2)) computed each time with a different base temperature T_{base} value included from 0°C to 10°C with an increment of 0.1°C. (a, b) Pistil receptivity from Eq. (1); (c, d) nectar secretion from Eq. (2). (a, c) Male sterile winter oilseed rape parental line (MS); (b, d) male fertile winter oilseed rape F_1 line. Dashed lines represent the base temperature value for which the R^2 value was maximised.

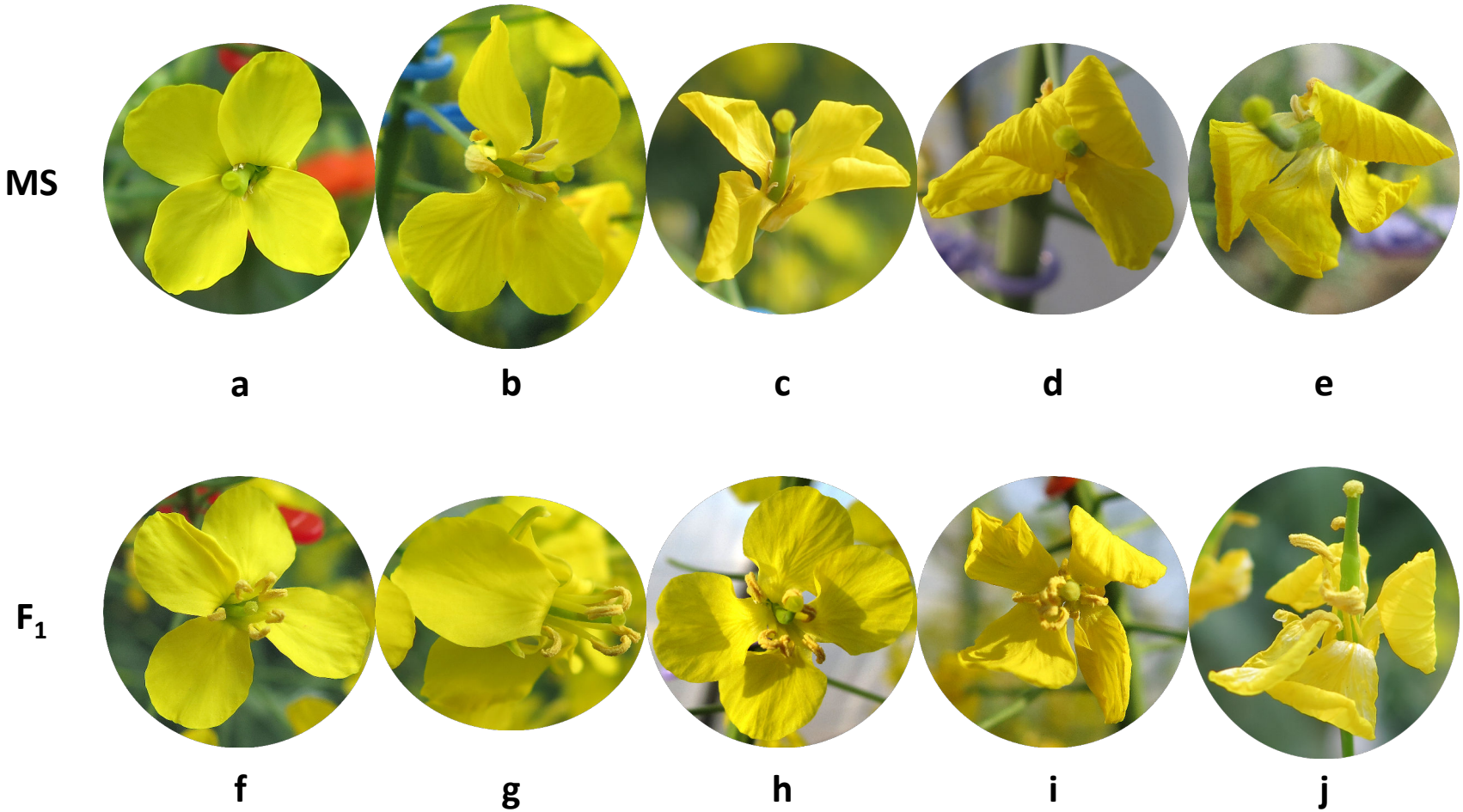


Fig. A.2. General appearance of winter oilseed rape flowers at different characteristic stages, in the male sterile parental line (MS; a-e), and the male fertile F_1 line (f-j). (a, f) Before the beginning of the decline of pistil receptivity and nectar reabsorption; (b) at the onset of the decline of pistil receptivity and at the onset of nectar reabsorption; (c) during the decline of pistil receptivity and the nectar reabsorption; (d) during the decline of pistil receptivity and the end of nectar reabsorption; (e) at the end of pistil receptivity; (g) at the onset of the decline of pistil receptivity and before the onset of nectar reabsorption; (h) during the decline of pistil receptivity and at the onset of nectar reabsorption; (i) during the decline of pistil receptivity and nectar reabsorption; (j) at the end of pistil receptivity and nectar reabsorption. © Stan Chabert and Taïna Lemoine / INRA

CHAPITRE II :

Évaluation du nombre minimum de grains de pollen viables devant être déposés par stigmate en fonction de la température pour que la pollinisation ne soit pas un facteur limitant pour la grenaison

The pollen dose-response relationship revisited: pollen limitation varies according to temperature and pollen population in oilseed rape (*Brassica napus* L.)

A soumettre à New Phytologist

Stan Chabert[1,2], Taïna Lemoine[1], Mathilde Raimond Cagnato[1], Marie-Josée Buffière[1], Olivier Geist[1], Nicolas Morison[1] and Bernard E. Vaissière[1]

[1]Institut National de la Recherche Agronomique (INRA), UR406 Abeilles et Environnement, site Agroparc, domaine Saint-Paul, CS 40509, F-84914 Avignon cedex 9, France

[2]Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses, Ferme expérimentale, 2485 route des Pécolets, F-26800 Étoile-sur-Rhône, France

Abstract

Integrated crop pollination requires that the level of pollination intensity beyond which pollination is no longer a limiting factor for zoophilous crop productivity be carefully assessed. We investigated the functional dose-response relationship between pollination intensity and seed set in oilseed rape (OSR) and integrated the temperature effect on pollen performance.

We mathematically formalised and compared models that took into account or not each of the following three biological processes: a temperature effect on pollen performance, a pollen population effect at low densities on pollen germination, and a bijective distribution of the pollen tubes among ovules in the ovary.

The most parsimonious model integrated, in order of importance, the temperature effect on pollen performance, the pollen population effect at low densities, and the bijective distribution of the pollen tubes among ovules in the ovary.

This new model enabled us to assess a threshold of pollination intensity beyond which pollination is no longer a limiting factor for seed set in relation with temperature at the time of pollen deposition on the stigma and according to the size of the successive pollen loads in the case of sequential pollen depositions.

Keywords: pollen limitation, pollination intensity, pollen dose-response relationship, temperature, pollen population effect, non-random fertilisation, seed set, oilseed rape (*Brassica napus*).

1. Introduction

Integrated crop pollination (ICP) is an emerging and developing concept, resting on a paradigm that emerged earlier (Kevan *et al.*, 1990; Corbet, 1991; Batra, 1995) and which consists in combining one or several managed pollinator species with native wild ones by adapting the farm management practices to help ensure stable and sustainable crop pollination (Isaacs & Kirk, 2010; Isaacs *et al.*, 2017; Klein *et al.*, 2018; Koh *et al.*, 2018; Pitts-Singer *et al.*, 2018). But, to be effective, the ICP concept requires in particular to define precisely the demand for animal pollination of a given zoophilous crop (Garratt *et al.*, 2018b) and so to start with the basic question of how much good quality pollen deposition is needed on the stigmas to reach full seed set (Ne'eman *et al.*, 2010; Isaacs *et al.*, 2017; Henselek *et al.*, 2018). Many studies have investigated the relationship between the stocking rate of managed honey bee colonies and entomophilous crop yields (review in Delaplane & Mayer, 2000; Rollin & Garibaldi, 2018; see also for instance Cunningham *et al.*, 2016, and Gaines-Day & Gratton, 2016), or between the abundance of insect foragers and entomophilous crop yields (e.g. Garibaldi *et al.*, 2013, 2016; review in Rollin & Garibaldi, 2018), but to understand the underlying mechanisms at work, the link remains to be made between seed set and pollination intensity and quality.

For instance, whether insect foragers contribute or not to pollination and seed yields of oilseed rape (OSR; *Brassica napus*; Brassicaceae) is still controversial. Some studies conclude that insect foragers increase OSR yields (e.g. Bommarco *et al.*, 2012; Stanley *et al.*, 2013; Perrot *et al.*, 2018), while others conclude on the opposite, depending on the cultivar tested (Hudewenz *et al.*, 2014; Marini *et al.*, 2015; Lindström *et al.*, 2016; Garratt *et al.*, 2018b), or on the nitrogen level input (Marini *et al.*, 2015; Garratt *et al.*, 2018a). OSR being fully self-compatible (Williams, 1978; Williams *et al.*, 1986; Ouvrard *et al.*, 2017), it would be necessary to know from which minimum amount of conspecific viable pollen deposited on the stigma pollination would no longer be a limiting factor, in relation to the number of ovules and the amount of maternal resources available (Ashman *et al.*, 2004; Harder & Routley, 2006; Knight *et al.*, 2006; Aizen & Harder, 2007; Harder & Aizen, 2010). A better assessment of the potential pollination deficit of this crop is of particular importance given its considerable areas grown around the world. Indeed, this could lead to a substantial re-evaluation of the world economic value of agricultural insect pollination assessed by Gallai *et al.* (2009) as pointed out by Melathopoulos *et al.* (2015). And this would also have a direct impact on the apparent deficit in honey bee colonies reported for some countries to match the overall reported entomophilous crop

pollination demand (Breeze *et al.*, 2014).

The functional dose-response relationship between pollination intensity, i.e. the number of conspecific viable pollen grains deposited on a receptive pistil, and seed set has been investigated during the last decades in diverse wild zoophilous and anemophilous plant species (e.g. Silander & Primack, 1978; Snow, 1982, 1986; Bertin, 1982; Field *et al.*, 2012; Labouche *et al.*, 2017), as well as in entomophilous crop species (Alkamine & Girolami, 1959; Falque *et al.*, 1995; Cane & Schiffhauer, 2003). The relationship between pollination intensity and the number of pollen tubes that grew into the style has also been investigated more recently (Alonso, 2005; Richards et al., 2009; Alonso et al., 2012; Harder *et al.*, 2016a,b). These relationships consisted, in most cases, in an increase of the seed set with pollination intensity, with an increase rate that gradually decreased as and when the seed set reached its maximum potential. Mathematically, Aizen & Harder (2007) formalised this relationship as a saturating negative-exponential function. But they explicitly formalised it on the assumption that pollen tubes reaching the ovary distributed themselves randomly among ovules. Yet pollen tube growth is guided in the ovary by attractant peptides emitted by the synergid cells (Higashiyama *et al.*, 2001; Okuda *et al.*, 2009; Higashiyama & Takeuchi, 2015), and it has been shown since, at least in *Arabidopsis thaliana*, a species also in the Brassicaceae, that the attraction of more than one pollen tube per ovule is prevented by the degeneration of the synergid cells following the double fertilisation, leading to a bijective distribution of pollen tubes among ovules in the ovary (Spielman & Scott, 2008; Beale *et al.*, 2012; Maruyama *et al.*, 2013).

Almost none of the above studies considered the pollen population effect on pollen germination at low densities in these relationships (except Harder *et al.*, 2016a,b). This phenomenon corresponds to an increase in the pollen germination rate, in the pollen tube growth rate, and in the proportion of the number of pollen tubes that reach the base of the style, when pollination intensity increases at low pollen loads (Brink, 1924; Brewbaker & Majumder, 1961; Brewbaker & Kwack, 1963; Cruzan, 1986; Niesenbaum, 1999; Zhang *et al.*, 2010; Harder *et al.*, 2016a,b). This facilitation between pollen grains is regulated by growth factors, probably such as peptide PSK-α, Ca^{2+} ion or flavonols (Brewbaker & Kwack, 1963; Taylor, 1997; Chen *et al.*, 2000), and it can be likened to an Allee effect (Harder *et al.*, 2016a,b). This effect has been highlighted in the genus *Brassica* (Brewbaker & Kwack, 1963), as well as in *A. thaliana* (Boavida & McCormick, 2007).

Finally, pollination intensity cannot always explain seed set alone. Some authors also found an effect of the genetic quality of pollen (Bertin, 1990; Waser & Price, 1991; Aizen & Harder, 2007), a maternal resource effect (Mitchell, 1997a), or an interference effect with heterospecific

pollen (Campbell, 1986), while some other authors could not conclude on the origin of the residual variability (McDade & Davidar, 1984; Shore & Barrett, 1984; Kohn & Waser, 1985; Aizen & Basilio, 1998; Fetscher & Kohn, 1999; Bosch & Waser, 2001). Aizen & Harder (2007) highlighted the importance to integrate genetic pollen quality into the pollen performance (*sensu* Williams & Mazer, 2016), but some other factors such as temperature can also greatly affect the reproductive ability of pollen (Rosbakh *et al.*, 2018).

Indeed, for instance pollen germination rate is driven by three cardinal temperatures: a minimal temperature below which there is no pollen germination, an optimum temperature at which the pollen germination rate is maximum, and a maximum temperature above which pollen germination is nil again (e.g. Kakani *et al.*, 2002, 2005; Acar & Kakani, 2010; Rosbakh & Poschlod, 2016; Hebbar *et al.*, 2018). This was especially showed in OSR (Singh *et al.*, 2008). The effect of temperature on the pollen germination rate has been investigated mainly *in vitro* (see studies previously cited), and sometimes *in vivo* directly on the stigma (Hedhly *et al.*, 2003, 2004, 2005), but, to our knowledge, never until fertilisation and seed production.

The aim of our study was to establish first the functional dose-response relationship between pollination intensity and seed set in OSR to assess in a second time the pollination intensity level beyond which pollination would no longer be a limiting factor for seed production. We compared four kinds of models of the pollen dose-response relationship: two models based on the hypothesis of a random distribution of pollen tubes among ovules in the ovary, one with a pollen population effect at low densities (Harder *et al.*, 2016a,b) and the other without it (Aizen & Harder, 2007), and two models based on the hypothesis of a bijective distribution of pollen tubes among ovules in the ovary, one with a pollen population effect at low densities and the other without it again. Two versions of each of these four models were compared: one version integrating a temperature effect on the pollen ability to germinate and develop into pollen tube that reach the ovary, and another version without a temperature effect, leading to the comparison of eight models in total. As *Brassica* pollen grains hydrate and germinate in less than one hour after reaching the stigma (Heslop-Harrison, 1992; Hiroi *et al.*, 2013), or they often lose their ability to germinate on stigma in less than three hours after being hydrated (Zuberi & Dickinson 1985), the temperature value we considered was that recorded at the time of pollen deposition. We hypothesised that the model that would be the most parsimonious would be the one that would take into account the three phenomena combined, that are a bijective relationship between pollen tubes and ovules in the ovary, a pollen population effect at low densities, and a temperature effect on the ability of pollen to grow into pollen tubes to reach the ovary, as it has been shown that all three occur in OSR or in closely

related Brassicaceae species.

From the most parsimonious pollen dose-response relationship previously found in OSR, we assessed the number of pollen grains needed to be deposited on the stigma to reach full seed set in one single deposition. As pollination may not to occur with a single deposition of pollen grains, and on the assumption of a pollen population effect at low densities and that *Brassica* pollen could lose its facilitation ability over time after being deposited on the stigma, we assessed also the minimum number of pollen grains needed to be deposited in total on the stigma to reach full OSR seed set in the case of a sequence of successive pollen depositions according to the size of the pollen deposition. And as the period of pistil receptivity can be predicted according to the ambient temperature (Chabert *et al.*, 2018), we assessed at last the minimal rate of stigmatic pollen deposition needed per day during the period of full pistil receptivity to reach full seed set according to the ambient temperature value.

To test these hypotheses and assess these levels of pollen limitation, we applied a range of pollination intensities through hand pollinations on stigmas of a winter OSR male sterile line (Pelletier & Budar, 2015) under a range of uncontrolled temperatures, we counted the number of pollen grains effectively deposited onto the stigmas by extracting pollen grains from dry stigmas by sonication, and we counted the number of seeds in the resulting pods that matured.

2. Materials and Methods

The general experimental setup that is detailed thereafter was the same as for the study of Chabert *et al.* (2018), but with two consecutive years of recordings instead of a single one. This study was carried out from Winter 2015 to Spring 2016 and from Winter 2016 to Spring 2017 under two tunnels located at the INRA centre of Avignon (France; 43°54'53.5"N, 4°52'39.2"E). The 22 m long x 8 m wide tunnels were covered with insectproof HD polyethylene crystal screen with 950 x 800 µm openings (Diatex, France; www.diatex.com) to prevent insect foraging and insect pollination. Seeds were sown in four rows 18 m long with two winter OSR lines (*Brassica napus* L.): the male fertile F_1 hybrid line 'Exocet' and its male sterile parent, both provided by Monsanto®.

The two tunnels were sown three weeks apart each year, the first one on 24 Nov. 2015 and on 21 Nov. 2016, and the second one on 11 Dec. 2015 and on 12 Dec. 2016, with the goal to have a difference in the flowering times of both tunnels and thus more extended flowering periods for recordings observations. The plants were watered daily with a drip system during the overall flowering periods. Ambient instant temperatures were recorded every 150 seconds

by a sensor HOBO® Pro v2 (Onset® Computer Corporation, USA; www.onsetcomp.com) placed in a meteorological shelter in the centre of each tunnel at average vegetation height. The average, minimum, and maximum daily temperatures recorded during the recordings are given in Table A.1.

2.1. Hand pollinations, pollen loads and seed set

Hand pollinations were made exclusively on flowers of the male sterile line to fully control pollination and on their first day of anthesis to be sure that pistil was fully receptive (Chabert *et al.*, 2018). Hand pollinations consisted of two treatments: hand pollinations made with an eyelash (HPE) glued to the end of a needle, itself attached to the end of a chuck, to control as accurately as possible the number of pollen grains deposited onto stigmas, and saturating hand pollinations (HPS) made with a brush to serve as an unrestricted positive control in stigmatic pollen load. The pollen used was harvested just before pollinations by brushing the anthers of flowers on their first day of anthesis of ten different plants of the male fertile line chosen at random and mixed into a metal cup from which HPS could then be applied directly. For HPE, the brush was then rubbed on the edge of the cup above a black metal plate to have scattered single pollen grains. Defined loads of 5, 10, 15, …, 45, 50, 60, …, 90 and 100 pollen grains, and more approximate loads of 150, 200 and 250 pollen grains in 2016 only, were then collected by the eyelash under a stereomicroscope and applied on the chosen stigmas to apply a range of pollination intensities. Pollen collection with the eyelash for HPE was carried out under a sunshade to prevent the loss of pollen viability due to the heating of the plate with solar radiation, and pollen loads were applied on stigmas by rubbing the eyelash repeatedly on the stigma to spread the pollen load.

To have flowers at their first day of anthesis, a first flower marker Cherry (Filpack® agricole, France; www.filpack-agricole.com) was placed under the lowest bud on a set number of racemes chosen at random among all available flowering plants of the male sterile line between 16:00 h and 18:00 h GMT the day before hand pollinations. Flowering occurs acropetally, i.e. from bottom to top, on OSR racemes, but not strictly so and one can find a few flowers at anthesis above some flower buds. The peduncles of all flowers at anthesis that were located above the marked bud were cut to retain only flowers in the bud stage above the marker. On the day of hand pollinations, a second flower marker was placed on these same racemes above the highest flower at anthesis at 08:00 h GMT. All flowers still at bud stage located below the second marker had their peduncle cut off in order to retain only flowers on their first day of anthesis between the two markers. Hand pollinations were then applied between 08:30 h and

15:30 h GMT on different days from 24 April to 18 May in 2016 and from 19 April to 2 June in 2017 (Tables A.2 and A.3). The number of sampled flowers per time and kind of hand pollination varied from 4 to 26 flowers (Tables A.2 and A.3). As only a very small fraction of flowers available per male sterile plant were pollinated and led to the production of a pod, we assumed that the produced pods were not limited in maternal resources.

Stigmas were harvested individually by cutting the style 2 mm under the stigmatic area with cutting tweezers (Dumont®, Switzerland; www.dumonttweezers.com) 8 days after flower anthesis to be sure that the period of pistil receptivity had ended (Chabert *et al.*, 2018) and not to interfere with the fertilisation process, and they were put in Eppendorf® tubes and dried for 24 hours at 30°C in a ventilated incubator. To assess the pollen loads effectively deposited on the stigmas, pollen grains were extracted from the stigmas by sonication in saline water solution containing basic fuchsine (Vaissière, 1991; Dafni, 1992; Vaissière & Froissart, 1996; Dafni *et al.*, 2005; see Appendix B for details) and by filtering the resulting pollen suspensions on white filter cloth (Fig A.1; see Appendix B for details). The pollen grains coloured with fuchsine were then counted either directly under a stereo microscope for the HPE stigmas, either for HPS stigmas by automated image analysis with the ImageJ software (Costa & Yang, 2009; Schneider *et al.*, 2012; see Appendix C and D for details). To make the connection with the seed set, the resulting pods were harvested forty days after flower anthesis, when seeds were developed enough to be accurately counted visually after pod dissection. The ambient temperature values at pollen deposition time were recovered from the temperature sensors. The ambient temperature values, and the minimum and maximum values of pollen loads and resulting seed set for each time and kind of hand pollination are given in Tables A.2 and A.3. The ambient temperature values at pollen deposition time were arbitrarily divided into four temperature groups for graphical representation: the values less than 15°C, those included between 15°C and 20°C, those included between 20°C and 28°C, and those greater than 28°C (see Fig 1).

The pollen used for hand pollinations of the male sterile flowers was the one of the male fertile F_1 hybrid line bred from the male sterile one. Therefore, pistils had in average half of the alleles in common with the pollen grains received. However, this should not have resulted in a bias for our study, as OSR is fully self-compatible (Williams, 1978; Williams *et al.*, 1986; Ouvrard *et al.*, 2017). And as the pollen was harvested only on flowers at their first day of anthesis, it was supposed to be fully viable.

To get an idea of the mean fraction of the fertilised ovules that matured into seeds when pollination was not limiting (Aizen & Harder, 2007), a sample of 20 male sterile ovaries were dissected and the ovules they contained were counted.

2.2. Data analysis

2.2.1. Mathematical formalisation of the dose-response relationship between pollination intensity and seed set

To establish the functional dose-response relationship between pollination intensity and seed set in OSR while considering the temperature effect on the pollen reproductive success rate, we compared 8 mechanistic models by using the nonlinear least squares as statistical inference method for estimating parameters (Bates and Watts, 1988; Bolker, 2008).

The first model considered was the one proposed by Aizen & Harder (2007) and the most commonly used so far (e.g. Kohn & Waser, 1985; Campbell, 1986; Waser & Price, 1991; Mitchell, 1997; Aizen & Basilio, 1998; Fetscher & Kohn, 1999; Bosch & Waser, 2001; Field *et al.*, 2012; Labouche *et al.*, 2017), that is a saturating negative-exponential function:

$$S(P) = d\bar{O}\,(1 - e^{-bP}), \qquad \text{Eqn 1a}$$

where S is the seed set per flower,
d is the fraction of fertilised ovules that mature into seeds,
$\bar{O}$ is the mean number of ovules per flower,
P is the number of pollen grains deposited on the stigma,
and b is the proportion of pollen grains P that germinate and develop into pollen tubes that reach the ovary.
This model is based on the assumption that pollen tubes that reach the ovary are distributed randomly among ovules.

To integrate the temperature effect on b, we used the equation proposed by Yin *et al.* (1995) and Yan & Hunt (1999) to model the temperature response of plants following a beta distribution from the cardinal temperatures (Eqn 8c without the shape parameter α in Yin *et al.*, 1995; Eqn 4 in Yan & Hunt, 1999):

$$\begin{cases} if\ T \le t_{max},\ b(T) = b_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}} \\ if\ T > t_{max},\ b(T) = 0 \end{cases}, \qquad \text{Eqn 2}$$

where T is the ambient temperature value at the time of pollen deposition on the stigma,

b_{max} is the maximum proportion of pollen grains P that germinate and develop into pollen tubes that reach the ovary at the temperature value t_{opt},
t_{min} is the minimal temperature value below which no pollen tube reaches the ovary,
and t_{max} is the maximum temperature value above which no pollen tube reaches the ovary again.
Eqns 1a and 2 combined together give:

$$\begin{cases} S(P,T) = d\bar{O}\left(1 - e^{-b(T)P}\right) \\ with \begin{cases} b(T) = b_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}} \; if\; T \le t_{max} \\ b(T) = 0 \; if \; T > t_{max} \end{cases} \end{cases}. \qquad \text{Eqn 1b}$$

Harder *et al.* (2016a,b) took Eqn 1a by integrating the pollen population effect with the magnitude parameter γ:

$$S(P) = d\bar{O}\left(1 - e^{-bP^{\gamma}}\right). \qquad \text{Eqn 3a}$$

If $\gamma \le 1$, this function describes a constantly decelerating relationship, whereas if $\gamma > 1$ the relationship is sigmoidal, indicating a pollen population effect. Combining Eqn 3a with Eqn 2 to integrate the temperature effect on b gives:

$$\begin{cases} S(P,T) = d\bar{O}\left(1 - e^{-b(T)P^{\gamma}}\right) \\ with \begin{cases} b(T) = b_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}} \; if\; T \le t_{max} \\ b(T) = 0 \; if \; T > t_{max} \end{cases} \end{cases}. \qquad \text{Eqn 3b}$$

But if pollen tubes that reach the ovary are not randomly distributed among ovules in the ovary, but rather follow a bijective distribution, and if there is no pollen population effect, Eqn 1a is then simply written:

$$\begin{cases} if\; P \le P_0,\; S(P) = bP \\ if\; P > P_0,\; S(P) = d\bar{O}, \\ \quad with\; P_0 = d\bar{O}/b \end{cases} \qquad \text{Eqn 4a}$$

where P_0 is the threshold of pollen grains deposited on the stigma beyond which all ovules are fertilized and seed set is full.

By combining Eqn 4a with Eqn 2 to integrate the temperature effect on *b*, this equation becomes:

$$\begin{cases} \begin{array}{c} if\ P \le P_0,\ S(P,T) = b(T)P \\ if\ P > P_0,\ S(P,T) = d\bar{O} \\ with\ P_0 = d\bar{O}/b(T) \end{array} \\ with \begin{cases} b(T) = b_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}}\ if\ T \le t_{max} \\ b(T) = 0\ if\ T > t_{max} \end{cases} \end{cases} . \qquad \text{Eqn 4b}$$

Now if pollen tubes that reach the ovary are not distributed randomly among ovules in the ovary because of a bijective distribution of pollen tubes among ovules, but if there is also a pollen population effect to consider at low pollen depositions on the stigma, we can assume that *b* follows itself a saturating negative-exponential function:

$$b(P) = b_{max}\,(1 - e^{-b'P}), \qquad \text{Eqn 5}$$

where *b'* is the rate of the proportion of pollen grains deposited on the stigma *P* that germinate and develop into pollen tubes that reach the ovary,
and b_{max} is the asymptotic value towards which converges *b'*.
This relationship was never mathematically formalised in that way, but see for instance Fig 1 in Brewbaker & Kwack (1963), Fig 2 in Cruzan (1986), Fig 1 in Holm (1994), or Fig 1 in Chen *et al.* (2000). By calculating the primitive of Eqn 5, the total expected seed set *S* per flower is written as (see Appendix E):

$$\begin{cases} \begin{array}{c} if\ P \le P_0,\ S(P) = b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right) \\ if\ P > P_0,\ S(P) = d\bar{O} \end{array} \\ with\ P_0 = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}}+1\right)}\right) + \frac{b'd\bar{O}}{b_{max}} + 1\right) \end{cases}, \qquad \text{Eqn 6a}$$

where P_0 is the threshold number of pollen grains *P* beyond which *S* reaches the maximum number of fertilised ovules that can mature into seeds $d\bar{O}$,
and W_0 is the principal branch of the Lambert *W* function (Corless *et al.*, 1996).
By combining Eqn 6a with Eqn 2 to integrate the temperature effect on b_{max}, this equation becomes:

$$\begin{cases} if\ P \le P_0(T),\ S(P,T) = b_{max}(T)\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right) \\ if\ P > P_0(T),\ S(P,T) = d\bar{O} \\ with\ P_0(T) = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}(T)}+1\right)}\right) + \frac{b'd\bar{O}}{b_{max}(T)} + 1\right) \\ with \begin{cases} b_{max}(T) = b'_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}}\ if\ T \le t_{max} \\ b_{max}(T) = 0\ if\ T > t_{max} \end{cases} \end{cases} .$$ Eqn 6b

The development of these last two models, Eqns 4a,b and 6a,b, that integrates a bijective distribution of pollen tubes among ovules, therefore enabled to predict the minimal stigmatic pollen load size P_0 needed to be reached in one single deposition, according to the temperature at pollen deposition for Eqns 4b and 6b, to reach the full seed set $d\bar{O}$.

These mechanistic models were compared with Akaike information criterion (AIC) values (Akaike, 1973; Burnham & Anderson, 2002). They were also compared with the null model for which seed set *S* did not vary with the number of pollen grains *P* deposited on the stigma. The coefficient of determination values of each of these models were calculated by the deviance ratio R^2_D (Nakagawa & Schielzeth, 2013).

For the model that was best supported by the AIC value, the estimated seed set *S* according to the number of pollen grains *P* deposited on the stigma was then divided by *P* to estimate the pollen reproductive success rate *R*, i.e. the proportion of *P* that fertilised an ovule and led to the production of a seed, according to *P*, as well as to *T* for Eqns 1b, 3b, 4b and 6b (see equations in Appendix F).

2.2.2. Predicted effect of a sequence of successive stigmatic pollen depositions on the threshold of pollen limitation

Pollination may not likely always to occur in one single pollen deposition on the stigma, but on the contrary may often occurs through a sequence of successive pollen depositions, for instance after a succession of pollinator visits. If there is no pollen population effect at low stigmatic pollen loads, this should not have any influence on the threshold of pollen limitation in Eqn 4a, nor in Eqn 4b if the temperature is constant, as successive pollen depositions have strictly additive effects on seed set *S* in that case. However, on the assumption that *Brassica* pollen could lose its facilitation ability over time after a few pollen depositions on the stigma under the pollen population effect hypothesis, the pollen load size deposited on the stigma at

each deposition can be of importance. In the case of a sequence of n successive pollen depositions on the stigma, the total expected pollen tube reaching the ovary B per flower is written as (see Appendix G):

$$B(P_n) = b_{max}\left(\sum_{i=1}^{n} P_i + \frac{1}{b\prime}\left(\sum_{i=1}^{n}(e^{-b\prime P_i}) - n\right)\right) \text{ for Eqn 6a,} \qquad \text{Eqn 7a}$$

$$\begin{cases} B(P_n, T_n) = \sum_{i=1}^{n} b_{max}(T_i)\left(P_i + \frac{1}{b\prime}(e^{-b\prime P_i} - 1)\right) \\ with \begin{cases} b_{max}(T_i) = b'_{max}\left(\frac{t_{max}-T_i}{t_{max}-t_{opt}}\right)\left(\frac{T_i-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}} \; if\; T_i \le t_{max} \\ b_{max}(T_i) = 0 \; if\; T_i > t_{max} \end{cases} \end{cases} \text{ for Eqn 6b.} \quad \text{Eqn 7b}$$

If the successive pollen depositions are of the same size P on the stigma and occur at the same ambient temperature T, the minimum number of pollen grains P_{tot} needed to be deposited in total on the stigma to reach full seed set $d\bar{O}$ can be written as (see Appendix G):

$$\begin{cases} if\; P \le P_0,\; P_{tot}(P) = \frac{d\bar{O}P}{b_{max}\left(P + \frac{1}{b\prime}\left(e^{-b\prime P} - 1\right)\right)} \\ if\; P > P_0,\; P_{tot}(P) = P_0 \\ with\; P_0 = \frac{1}{b\prime}\left(W_0\left(-e^{-\left(\frac{b\prime d\bar{O}}{b_{max}}+1\right)}\right) + \frac{b\prime d\bar{O}}{b_{max}} + 1\right) \end{cases} \text{ for Eqn 6a,} \qquad \text{Eqn 8a}$$

$$\begin{cases} if\; P \le P_0(T),\; P_{tot}(P,T) = \frac{d\bar{O}P}{b_{max}(T)\left(P + \frac{1}{b\prime}\left(e^{-b\prime P} - 1\right)\right)} \\ if\; P > P_0(T),\; P_{tot}(P,T) = P_0(T) \\ with\; P_0(T) = \frac{1}{b\prime}\left(W_0\left(-e^{-\left(\frac{b\prime d\bar{O}}{b_{max}(T)}+1\right)}\right) + \frac{b\prime d\bar{O}}{b_{max}(T)} + 1\right) \\ with \begin{cases} b_{max}(T) = b'_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}} \; if\; T \le t_{max} \\ b_{max}(T) = 0 \; if\; T > t_{max} \end{cases} \end{cases} \text{ for Eqn 6b.} \quad \text{Eqn 8b}$$

Yet, according to Eqn 3 in Chabert *et al.* (2018), the period of OSR pistil receptivity can be predicted according to temperature. Thus, if the temperature is constant, the average minimum number of pollen grains that have to be deposited in total on the stigma per day P_d, with the assumption of fixed pollen deposition size P, to reach a full seed set $d\bar{O}$ during full pistil receptivity can be written as (see Appendix G):

$$\begin{cases} if\ P \le P_0,\ P_d(P,T) = \frac{24d\bar{O}(T-t_{base})P}{b_{dh}b_{max}\left(P+\frac{1}{b'}\left(e^{-b'P}-1\right)\right)} \\ if\ P > P_0,\ P_d(P,T) = \frac{24(T-t_{base})P_0}{b_{dh}} \quad \text{for Eqn 6a,} \\ with\ P_0 = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}}+1\right)}\right)+\frac{b'd\bar{O}}{b_{max}}+1\right) \end{cases} \qquad \text{Eqn 9a}$$

$$\begin{cases} if\ P \le P_0(T),\ P_d(P,T) = \frac{24d\bar{O}(T-t_{base})P}{b_{dh}b_{max}(T)\left(P+\frac{1}{b'}\left(e^{-b'P}-1\right)\right)} \\ if\ P > P_0(T),\ P_d(P,T) = \frac{24(T-t_{base})P_0(T)}{b_{dh}} \\ with\ P_0(T) = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}(T)}+1\right)}\right)+\frac{b'd\bar{O}}{b_{max}(T)}+1\right) \quad \text{for Eqn 6b,} \\ with \begin{cases} b_{max}(T) = b'_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}}\ if\ T \le t_{max} \\ b_{max}(T) = 0\ if\ T > t_{max} \end{cases} \end{cases} \qquad \text{Eqn 9b}$$

where t_{base} is the base temperature below which there is no ovule ageing, and b_{dh} is the duration of the full pistil receptivity in °Ch. In the OSR male sterile parental line investigated in this study, t_{base} = 7.5°C and b_{dh} = 287 (± 25; 95% CI) (see Chabert *et al.*, 2018).

2.2.3. Software and packages

All the statistics were computed with the software R, version 3.2.0 (R Core Team, 2015). Asymptotic 95% confidence intervals (95% CI) of mechanistic model parameters were estimated with the package *nlstools*, version 1.0-2 (Baty *et al.*, 2015). The Lambert *W* function was computed with the package *lamW*, version 1.3.0 (Adler, 2017).

3. Results

We were able to cover a large array of temperatures during hand pollinations, between 10.4°C and 32.2°C for HPE, and between 10.6°C and 31.4°C for HPS (Tables A.2 and A.3). A total of 278 OSR flowers could be assayed over a relatively large range of low pollination intensities – between 1 and 256 pollen grains deposited per stigma – for HPE (Table A.2), and 101 flowers for HPS, with a mean pollinations intensity of 1,436 pollen grains, ranging from 177 to 3,457, deposited per stigma, and a mean seed set of 32.4 (± 0.7; 95% CI), ranging from 25 to 39 (Table A.3).

3.1. Dose-response relationship between pollination intensity and seed set

A descriptive graphic representation of the data between pollination intensity and seed set per OSR flower according to temperature groups is given in Fig 1, focusing on the very beginning of the pollen dose-response relationship (up to 100 pollen grains in stigmatic pollen loads) in Fig 1a, and on the overall range of pollination intensities applied in Fig 1b.

The model that was best supported by AIC was by far Eqn (6b), with a minimum ΔAIC of 34 compared to the other models and a R^2_D value of 0.91 (Table 1). The data set therefore best supported a pollen dose-response relationship with a pollen population effect at low stigmatic pollen loads, a temperature effect on the maximal proportion of pollen grains deposited on the stigma that develop into pollen tubes that reach the ovary, and a bijective distribution of pollen tubes among ovules for fertilisation (Fig 2a). The second model best supported was Eqn 3b integrating a pollen population and a temperature effects, but with a random distribution of pollen tubes among ovules for fertilisation (ΔAIC = 34 with Eqn 6b). The following model best supported was Eqn 1b integrating a temperature effect, but without pollen population effect and with a random distribution of pollen tubes among ovules for fertilisation (ΔAIC = 187 with Eqn 6b). The two following models best supported were Eqns 6a and 3a (ΔAIC = 324 and 328, respectively, with Eqn 6b), that were the equivalent models Eqns 6b and 3b, but without a temperature effect. And the last two models least supported were Eqns 4a and 1a (ΔAIC = 351 and 412, respectively, with Eqn 6b) without temperature effect nor pollen population effect for the both, but with a bijective distribution of pollen tubes among ovules for fertilisation for Eqn 4a, and a random distribution for Eqn 1a, respectively. In fact, Eqn 4b was less supported than the null model, mainly because the *nls* computer algorithm did not converge properly, as the estimated parameter value reached 3.10^5 for t_{min}, which is not biologically meaningful. From this hierarchy of models, we can conclude that the pollen dose-response relationship was most sensitive first to the temperature effect, then to the pollen population effect, and then to the kind of distribution considered for pollen tubes allocation among ovules before fertilisation (bijective *versus* random distribution).

The mean maximum seed set $d\bar{O}$ estimated by Eqn 6b was 32.5 (± 0.7; 95% CI; Table 1), that was very similar to the mean seed set of 32.4 (± 0.7; 95% CI) estimated for HPS flowers. As the mean number of ovules $\bar{O}$ counted in male sterile flowers was 35.6 (± 1.1; 95% CI; n = 20), we can calculate that the mean fraction of fertilised ovules that matured into seeds was equal to 91.3%. The mean maximal fraction of pollen grains deposited on the stigma that germinated and developped into pollen tubes that reach the ovary b'_{max} estimated by Eqn 6b

Table 4 Parameter values with the resulting statistics associated for each of the 8 models of the pollen dose-response relationship.

Model	k[a]	Mean parameter values (± 95% CI[b])									R^2_D[c]	AIC	Rank[d]	ΔAIC[e]
		$d\bar{O}$	b	b'	b_{max}	b'_{max}	γ	t_{min}	t_{opt}	t_{max}				
null	2											3,305	8	966
Eqn 1a	3	33.3 (± 1.4)	$1.55e^{-2}$ (± $1.8e^{-3}$)								0.746	2,751	7	412
Eqn 1b	6	33.9 (± 1.1)			$2.56e^{-2}$ (± $2.7e^{-3}$)			-5.3 (± 22.1)	23.5 (± 1.1)	31.7 (± 0.1)	0.856	2,526	3	187
Eqn 3a	4	32.5 (± 1.1)	$3.84e^{-4}$ (± $3.95e^{-4}$)				2.06 (± 0.29)				0.794	2,667	5	328
Eqn 3b	7	32.8 (± 0.8)			$5.88e^{-4}$ (± $4.43e^{-4}$)		2.13 (± 0.22)	9.1 (± 3.6)	21.8 (± 1.1)	31.4 (± 0.0)	0.902	2,373	2	34
Eqn 4a	3	32.4 (± 1.2)	0.394 (± 0.026)								0.781	2,690	6	351
Eqn 4b	6	33.6 (± 7.5)			$2.35e^{-2}$ (± $7.2e^{-3}$)			$3.0e^{5}$ (± $2.3e^{8}$)	24.6 (± 1.2)	30.0 (± 2.3)	-0.286	3,415	9	1,08
Eqn 6a	4	32.0 (± 1.1)		$4.13e^{-2}$ (± $3.30e^{-2}$)	0.832 (± 0.389)						0.796	2,663	4	324
Eqn 6b	**7**	**32.5 (± 0.7)**		**$5.41e^{-2}$ (± $2.65e^{-2}$)**		**1.20 (± 0.34)**		**0.5 (± 14.5)**	**22.5 (± 0.9)**	**31.5 (± 0.0)**	**0.910**	**2,339**	**1**	**0**

[a]k: number of estimated parameters per model. [b]CI: confidence interval. [c]Coefficient of determination calculated by the deviance ratio. [d]Model ranking by increasing AIC value. [e]AIC value minus the lowest AIC value. The parameter estimations are based on a sample size of 379 flowers.

$d\bar{O}$: mean maximum seed set per flower. b: proportion of pollen grains deposited on the stigma that develop into pollen tubes that reach the ovary; b': initial slope of the proportion of pollen grains deposited on the stigma that develop into pollen tubes that reach the ovary; b_{max}: maximum proportion of pollen grains deposited on the stigma that develop into pollen tubes that reach the ovary at the temperature t_{opt}; b'_{max}: asymptotic value of the proportion of pollen grains deposited on the stigma that develop into pollen tubes that reach the ovary at the temperature t_{opt}; γ: magnitude parameter of pollen population effect; t_{min}: minimum temperature below which no pollen tube reaches the ovary; t_{opt}: optimum temperature at which the proportion of pollen grains deposited on the stigma that develop into pollen tubes that reach the ovary is maximum; t_{max}: maximum temperature above which no pollen tube reaches the ovary.

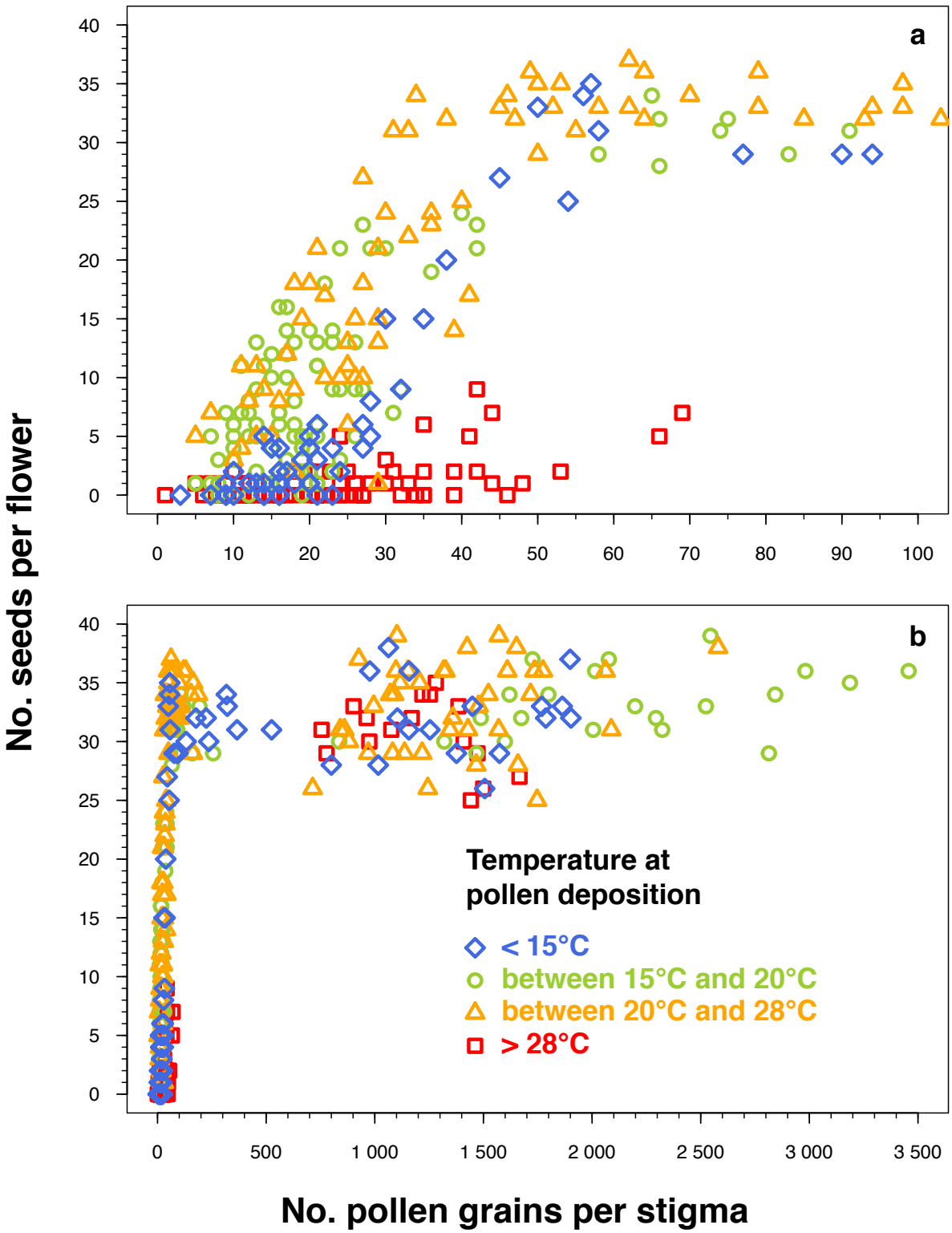


Fig. 1 Pollination intensity values and resulting number of seeds per male sterile OSR flower with the four temperature groups. (a) Focus on the very beginning of the pollen dose-response relationship. (b) Display on the overall range of pollination intensities applied.

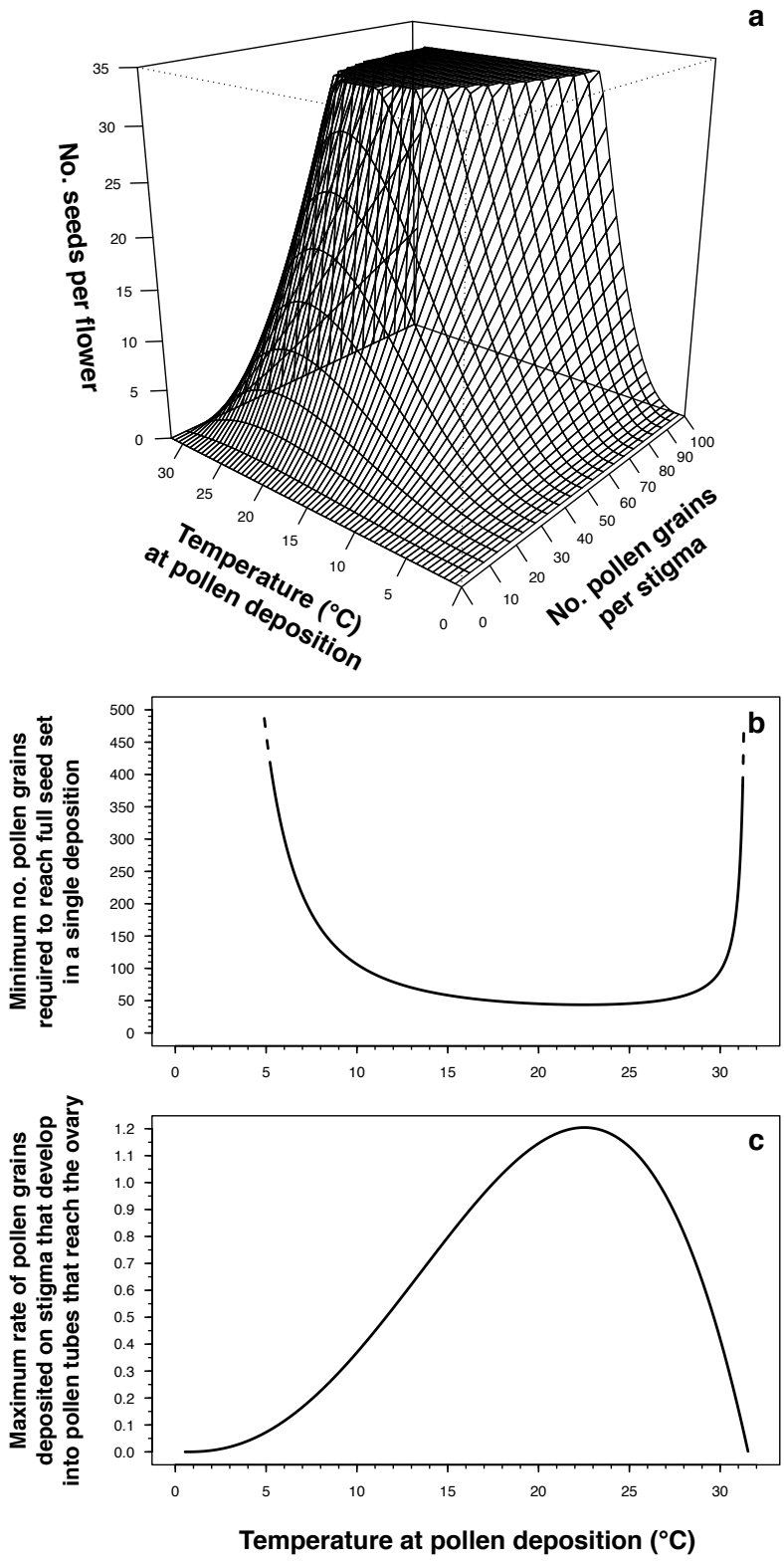


Fig. 2 (a) Functional dose-response relationship between pollination intensity and the resulting number of seeds per flower in relation with the temperature at pollen deposition on the stigma, based on the best supported model (Eqn 6b). (b) Predicted pollen limitation threshold according to temperature for a single pollen deposition (based on Eqn 6b). (c) Predicted maximum proportion of pollen grains that develop into pollen tubes that reach the ovary according to temperature (based on Eqn 6b).

was 1.20 (± 0.34; 95% CI), a value therefore similar to 1, at the estimated mean optimum temperature t_{opt} of 22.5°C (± 0.9; 95% CI). The estimated mean minimum temperature t_{min} below which there was no pollen tube reaching the ovary was 0.5°C with a very large 95% CI of ± 14.5°C, and the estimated mean maximum temperature t_{max} beyond which there was no pollen tube reaching the ovary again was 31.5°C (± 0.0; 95% CI).

From Eqn 6b, the predicted minimal stigmatic pollen load size P_0 needed to be reached in one single deposition varied with the ambient temperature value at pollen deposition time on the stigma according to the inverse of a beta distribution (Fig 2b). This predicted pollen load size had the minimum value of 44 pollen grains at t_{opt}, and it increased as ambient temperature increased or decreased from t_{opt} (Fig 2b). The predicted maximum proportion of pollen grains deposited on stigma that developped into pollen tubes that reach the ovary b_{max} had the maximum value b'_{max} at t_{opt} and decreased as ambient temperature increased or decreased from t_{opt} according to the beta distribution showed in Fig 2c. The predicted pollen reproductive success rate R had the maximum value of 0.74 at $P_0(t_{opt})$ (Eqn F1; Fig A2).

3.2. Predicted effect of successive stigmatic pollen depositions in sequence on the threshold of pollen limitation

If pollination occurs in a sequence of successive pollen deposition on a stigma, the predicted minimum number of pollen grains that has to be deposited in total on the stigma P_{tot} to reach the full seed set $d\bar{O}$ from Eqn 6b varies according to the pollen load size deposited at each successive deposition P and to the ambient temperature value at pollen deposition T (Eqn 8b; Figs 3a,b). This predicted minimum stigmatic pollen load size decreases as P increases up to the threshold P_0, and increases as T increases or decreases from t_{opt} (Figs 3a,b).

By considering the period of full receptivity of the OSR pistil that can be predicted according to the temperature, the predicted average minimum number of pollen grains that has to be deposited in total on the stigma per day P_d to reach the full seed set $d\bar{O}$ from Eqn 6b varies according to the pollen load size deposited at each successive deposition P and the temperature value T (Eqn 9b; Figs 4a,b). This predicted average minimum rate of pollen deposition on stigma per day decreases as P increases up to the threshold P_0, and increases as T increases (Figs 4a,b).

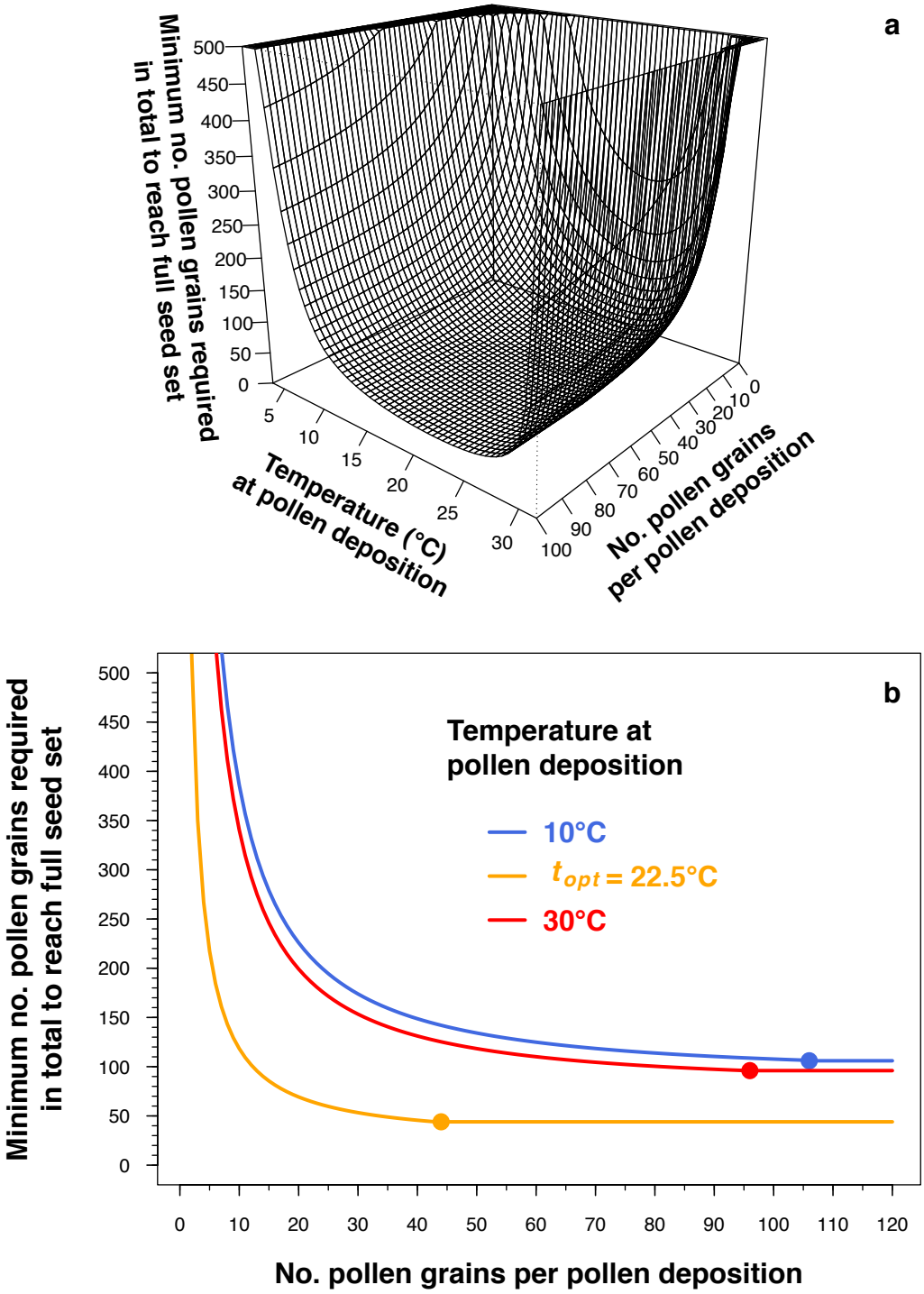


Fig. 3 Predicted pollen limitation threshold according to the number of pollen grains deposited at each successive deposition in relation to the temperature at pollen deposition in the case of successive pollen depositions of same size on the stigma (Eqn 8b) and calculated from the best supported model of pollen dose-response relationship (Eqn 6b): (a) three-dimensional representation; (b) two-dimensional representation with the number of pollen grains deposited at each successive deposition in the x-axis for three temperatures at pollen deposition. For each temperature, the point illustrates the minimum number of pollen grains required on the stigma to reach full seed set in the case of a single pollen deposition.

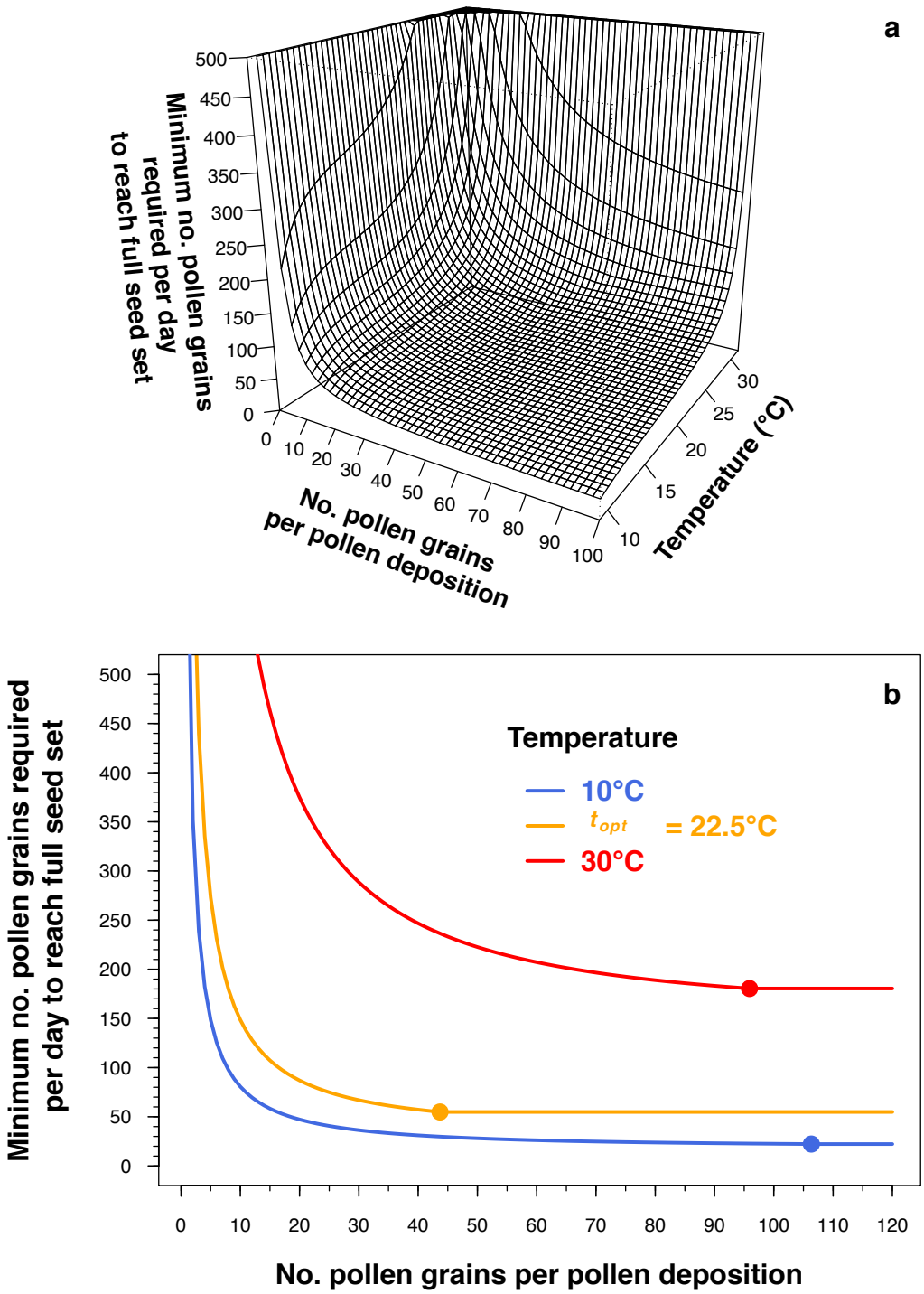


Fig. 4 Predicted average minimum rate of daily pollen deposition needed on the stigma to avoid pollen limitation according to the number of pollen grains deposited at each successive deposition and to the constant temperature in the case of a sequence of successive pollen deposition of same size on the stigma (Eqn 9b), estimated from the best supported model of pollen dose-response relationship (Eqn 6b): (a) three-dimensional representation; (b) two-dimensional representation with the number of pollen grains deposited at each successive deposition in x-axis for three constant temperatures. For each temperature, the point illustrates the minimum number of pollen grains required on the stigma during the duration of pistil receptivity to reach full seed set in the case of a single pollen deposition.

4. Discussion

As expected, the model of pollen dose-response relationship that was the most parsimonious among the 8 models compared for OSR was the one that integrated, in order of importance, a temperature effect on the ability of pollen grains to develop into pollen tubes that reach the ovary, a pollen population effect at low densities, and a bijective distribution of pollen tubes among ovules in the ovary (Table 1; Fig. 2a). These results therefore confirm phenomena already independently established and extend them together to the fertilisation process and resulting seed production, i.e. (*i*) that pollen performance depends on temperature (Rosbakh *et al.*, 2018), especially in OSR (Singh *et al.*, 2008), (*ii*) that there is facilitation between pollen grains to germinate (see references in the Introduction) and to develop into pollen tubes up to reach the ovary (Harder *et al.*, 2016a,b), especially in the *Brassica* genus (Brewbaker & Kwack, 1963), and (*iii*) confirm the bijective distribution of pollen tubes among ovules in the ovary found in *A. thaliana* (Beale *et al.*, 2012; Maruyama *et al.*, 2013; Higashiyama & Takeuchi, 2015) in another Brassicaceae species.

This is the second time to our knowledge that the beta distribution equation of Yin *et al.* (1995) and of Yan & Hunt (1999) is used to model the pollen performance according to temperature, after Rosbakh & Poschlod (2016). The optimal t_{opt} and maximal t_{max} temperature values, of 22.5°C and 31.5°C respectively (Table 1; Fig. 2c), found in our study for the ability of pollen grains to germinate and develop into pollen tubes that reach the ovary are very close to those reported by Singh *et al.* (2008) in 12 OSR cultivars for pollen germination *in vitro* (23.6°C for t_{opt} and 32.8°C for t_{max} in average) with a bilinear model. The minimal temperature value t_{min} of 0.5°C found in our study is, however, further from the average value of 7.7°C found by Singh *et al.* (2008). This is probably due to the very large size of the estimation error of t_{min} in our study, with a 95% CI of ± 14.5°C (Table 1). Indeed, there were very little measures at low temperature values in our data set (only three temperature values under 15°C for the HPE; Table A2), and so one cannot conclude from our study to assess the real t_{min} value for pollen performance of the OSR cultivar 'Exocet'. This kind of study should be repeated, for instance in a phytotron with controlled temperatures, under low temperature values. The maximal value of the proportion of pollen deposited on the stigma that developed into pollen tube that reach the ovary b'_{max} assessed by Eqn 6a at t_{opt} was of 1.20 (Table 1; Fig. 2c), a value therefore greater than the theoretical maximum of 1. But first the 95% CI value of ± 0.34 was large enough to encompass the value of 1, and also this large estimation error is probably due to the low pollination intensity level at which the ovule fertilisation and the seed set were

already full (44 pollen grains), while the pollen population effect on pollen germination and pollen tube growth was still probably not exhausted. We can see it in Fig. A2 where the maximum pollen reproductive success rate value obtanied at t_{opt} was 0.74, which is less than 1, and it is obtained while the curve is clearly still in an ascending phase, still far from the asymptotic value. But these results show that the b'_{max} value is at least greater than 0.74 and probably closer to 1, a value therefore quite high, especially compared to the average value of 0.43 found by Singh *et al.* (2008) for pollen germination *in vitro* of the 12 OSR cultivars studied, indicating that the pollen we used had a high viability rate and a high ability to develop into pollen tubes that reach the ovary at t_{opt}.

These results also reinforce the assumption that OSR pollen grains lose completely their germination ability after a certain period following their deposition and hydration on the stigma (Zuberi & Dickinson 1985). Indeed, if the pollen that did not germinate at the time of deposition could have germinated later over the duration of pistil receptivity, for instance if temperature got closer to t_{opt}, final seed set would not have been affected for a given stigmatic pollen load. Yet, the final seed set was affected by temperature. To further confirm this assumption, one would need to increase or decrease the temperature to t_{opt} for instance one hour after depositing the pollen on the stigma at low or high ambient temperature values, respectively.

Integrating a bijective distribution of pollen tubes among ovules in the pollen dose-response relationship enabled us to formalise a new threshold of pollination intensity beyond which pollination is no longer a limiting factor for seed production when this pollen load is deposited on the stigma in one single deposition (Eqn 6b; Fig. 2a,b), and the saturating negative-exponential function did not enable this. By integrating the bijective distribution of pollen tubes among ovules together with the temperature effect of pollen performance, this new model enabled us also to predict the threshold of pollen limitation according to the temperature at the time of pollen deposition on the stigma when pollen is deposited on the stigma in a single deposition (Fig. 2b). This threshold was minimum with a value of ca. 44 pollen grains at t_{opt}, and it increased as ambient temperature increased or decreased from t_{opt} according to the inverse of a beta distribution.

As there is a population effect at low levels of pollination intensities, the threshold of the total of pollen grains that have to be deposited on the stigma to reach full seed set may also depend on the size of the deposited pollen loads in the case of a sequence of successive pollen depositions (Eqn 8b; Figs 3a,b). While this phenomenon is a consequence of Eqn 6b on the assumption that OSR pollen loses its facilitation ability after being deposited and hydrated on the stigma. If this assumption is true, the time lapse after which pollen effectively loses its

facilitation ability after being deposited and hydrated on the stigma remains to be assessed. The predictions presented in Figs 3a,b may be true in the case where the elapsed time between the successive pollen depositions would exceed this time laps. Otherwise, the threshold of the total of pollen grains that have to be deposited on the stigma after a sequence of successive pollen depositions may be equal to the threshold of pollen limitation when pollen is deposited in one single deposition (Figs. 2a,b). This kind of study should therefore be repeated by adding successive small pollen loads of the same size with various time lapses between the depositions at a given temperature to test this assumption and to assess the potential duration after which OSR pollen loses its facilitation ability after being deposited and hydrated on the stigma. This loss of facilitation ability would correspond to the cessation of growth factor synthesis by the pollen (Brewbaker & Kwack, 1963; Taylor, 1997; Chen *et al.*, 2000).

As the period of pistil receptivity decreases as temperature increases (Chabert *et al.*, 2018), the available time to reach the threshold of pollen limitation decreases also as temperature increases. The minimum rate of pollen deposition per unit of time required to reach the threshold of pollen limitation during pistil receptivity therefore increases as temperature increases (Eqn 9b; Figs. 4a,b). The loss of pollen performance at low temperatures can therefore be counterbalanced by the lengthening of the period of pistil receptivity to reach the threshold of pollen limitation. Conversely, the negative effect of high temperature values on pollen performance is increased by the shortening of pistil receptivity period. This phenomenon is therefore important to consider for the seed production of zoophilous as well as anemophilous plant species with the advent of global warming (IPCC, 2013; Schmidhuber & Tubiello, 2007; Hedhly *et al.*, 2009; Zinn *et al.*, 2010; Wheeler & von Braun, 2013; Sage *et al.*, 2015).

The assessment of the pollination requirements in terms of pollination intensity according to temperature and pollen population of a given plant species so that it is not a limiting factor for fruit and seed set should enable to better understand the respective contributions of each pollination mode – self-, anemophilous or zoophilous pollination – to this fruit and seed set according to the ecological context, and especially to the climatic context. In the ICP framework, if we know the mean single-visit pollen deposition of a given pollinator species in a given ecological context (e.g. Rader *et al.*, 2009; Ne'eman *et al.*, 2010; King *et al.*, 2013; Willmer *et al.*, 2017), and if we know the mean flower visitation rate of this pollinator species according to the ambient temperature (Willmer & Stone, 2004; Couvillon *et al.*, 2015; Gunderson & Leal, 2016; Chambó *et al.*, 2017), then we could calculate the number of pollinators needed at a given mean temperature to complete the pollination made otherwise by autonomous self-pollination and the wind. Many other factors would have to be integrated, such

as the genetic quality of the pollen (Aizen & Harder, 2007), and the viability of the pollen carried by the pollinators (Vaissière *et al.*, 1996; Parker *et al.*, 2015). Furthermore, only the quantitative limitation on the progeny of a plant was considered here, without paying attention to gametophytic selection (Hormaza & Herrero, 1992). Another threshold of pollination intensity should therefore be investigated also to maximise progeny vigour too (Mitchell, 1997b).

Acknowledgements

We thank all the members of the experimental unit of UMR EMMAH, INRA PACA, who grew the OSR crop under tunnels, Gilles Continsouzas, Véronique Desfonds, Serge Reno, Jérémy Theodore and Frédéric Venault. We are also grateful to Gaëtan Lecordier, Laëtitia Planque, Céline Wenland and Marine Willemin who helped with field work or in the laboratory. We thank Jean-Christophe Conjeaud of ANAMSO who, as a financial and technical partner, made this study possible. We also thank Monsanto® company for graciously providing us with the seeds to carry out this study.

This work was supported by the European Agricultural Guarantee Fund (EAGF) as part of the French National Apiculture Programme [FranceAgriMer convention number 14-05 R], and by the Association Nationale de la Recherche et de la Technologie (ANRT) as part of the Conventions Industrielles de Formation par la REcherche (CIFRE) doctoral grants [convention number 2014/0070], allocated here to S.C. by the Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses (ANAMSO) through specific interprofessional actions with the Groupement National Interprofessionnel des Semences et plants (GNIS) and the Union Française des Semenciers (UFS).

Author contributions

S.C. and B.E.V. conceived the study, S.C., T.L. and M.R.C. performed field work, M.J.B., O.G., M.R.C. and T.L. performed laboratory analyses, N.M. provided assistance for temperature recordings, S.C. and N.M. implemented the automated image analysis, S.C. formalised the models and analysed the data, S.C. and B.E.V. wrote the manuscript.

Appendix A – Supplementary tables and figures

Table A1 Daily mean, minimum and maximum temperatures recorded for each day of hand pollinations.

Year	Month	Day	Daily temperatures			Year	Month	Day	Daily temperatures		
			Mean	Minimum	Maximum				Mean	Minimum	Maximum
2016	April	24	11.3	6.2	19.1	2017	April	29	14.8	3.9	26.7
		25	12.1	4.0	23.0			30	12.0	7.4	16.0
		26	12.8	2.2	23.9		May	1	12.9	5.3	21.0
		27	12.7	5.8	21.5			2	11.4	5.8	19.2
		28	13.5	5.6	23.2			3	13.6	5.5	22.3
		29	14.9	5.8	25.4			4	13.9	8.6	22.9
		30	11.9	7.2	19.0			5	15.4	7.6	26.3
	May	1	9.4	5.6	17.2			6	13.4	9.3	20.1
		2	16.5	8.1	26.0			7	16.0	7.7	23.6
		3	17.8	11.0	27.4			8	16.5	11.2	23.5
		4	17.7	8.8	29.5			9	16.5	9.6	25.5
		5	17.6	6.5	28.5			10	14.9	8.6	20.1
		6	17.1	7.8	27.5			11	15.6	9.9	22.9
		7	17.8	10.0	28.2			12	18.9	12.7	27.5
		8	16.4	8.5	24.1			13	19.7	10.1	30.7
		9	17.1	14.0	23.1			14	20.0	11.9	28.9
		10	19.9	14.4	28.1			15	22.2	14.1	31.2
		11	17.3	13.4	21.9			16	22.4	13.2	33.6
		12	17.2	10.6	26.2			17	22.4	12.8	33.8
		13	15.9	9.7	22.6			18	22.2	15.5	31.3
		14	18.9	12.4	27.9			19	18.3	11.3	27.4
		15	16.2	10.2	23.9			20	18.4	8.6	27.7
		16	17.0	8.3	27.0			21	20.6	11.8	31.3
		17	18.6	10.2	28.8			22	19.1	10.2	27.0
		18	18.2	9.1	28.5			23	23.4	11.5	33.6
2017	April	19	11.6	5.2	20.0			24	26.0	19.4	34.9
		20	11.9	3.8	20.7			25	25.6	17.4	36.1
		21	14.2	4.9	25.9			26	23.4	14.9	34.5
		22	14.7	3.7	27.3			27	23.6	13.4	35.4
		23	16.6	7.8	25.6			28	23.8	13.8	36.3
		24	15.7	6.9	26.1			29	24.0	12.1	36.4
		25	14.5	7.4	24.3			30	23.5	14.0	35.9
		26	12.5	7.7	17.4			31	22.4	15.4	32.3
		27	9.3	5.8	15.4		June	1	21.9	14.9	34.0
		28	11.8	5.7	19.6			2	24.2	17.0	34.4

Table A2 Sample size, temperature and extrema of pollen loads and seed set of each daily hand pollination made with an eyelash (HPE).

Year	Month	Day	Hour (GMT)	Temperature at pollen deposition (°C)	No. sampled flowers	Min - max[a] no. pollen grains deposited per stigma	Min - max[a] seed set per flower
2016	April	24	12:00	*14.8*	12	30 - 132	15 - 35
		25	12:00	22.4	11	46 - 186	29 - 36
		26	12:00	19.6	11	65 - 256	29 - 34
	May	7	12:00	26.5	12	20 - 164	18 - 37
		8	12:00	21.8	12	12 - 107	8 - 36
		18	12:00	27.9	8	22 - 70	17 - 34
2017	April	19	09:30	15.6	16	7 - 27	0 - 13
		20	08:30	*13.0*	10	3 - 35	0 - 15
		20	14:30	19.7	10	7 - 28	4 - 21
		21	09:30	20.0	9	10 - 103	3 - 33
		21	14:30	25.0	4	7 - 22	7 - 11
		22	14:30	25.9	4	11 - 18	2 - 8
		27	10:30	*10.4*	10	9 - 24	1 - 6
	May	1	15:30	19.6	6	10 - 58	1 - 29
		2	15:30	17.6	9	8 - 36	0 - 19
		8	15:00	21.7	15	5 - 41	1 - 25
		9	09:00	18.9	18	5 - 32	1 - 18
		10	09:30	19.3	15	7 - 23	0 - 14
		10	14:00	17.5	6	8 - 66	0 - 28
		16	14:00	**32.2**	8	16 - 39	0 - 1
		17	10:00	**29.7**	9	12 - 35	0
		31	14:30	**31.0**	25	6 - 48	0 - 7
	June	1	11:00	**30.9**	26	1 - 69	0 - 9
		2	14:30	**33.9**	12	8 - 39	0 - 5

[a]Min: minimum; max: maximum.

Table A3 Sample size, temperature and extrema of pollen loads and seed set of each daily hand pollination at saturation (HPS).

Year	Month	Day	Hour (GMT)	Temperature at pollen deposition (°C)	No. sampled flowers	Min - max[a] no. pollen grains deposited per stigma	Min - max[a] seed set per flower
2016	April	24	12:00	*14.8*	12	977 - 1,899	26 - 37
		25	12:00	22.4	10	714 - 1,735	26 - 39
		26	12:00	19.6	12	836 - 2,814	29 - 37
	May	7	12:00	26.5	8	1,071 - 2,580	31 - 38
		8	12:00	21.8	10	860 - 2,062	28 - 36
		18	12:00	27.9	11	841 - 1,774	25 - 38
2017	April	20	09:30	16.4	7	1,674 - 3,457	31 - 39
		27	12:00	*10.6*	12	177 - 1,964	28 - 38
	May	9	09:00	19.8	3	2,014 - 2,843	33 - 36
		16	15:00	**31.4**	16	755 - 1,666	25 - 35

[a]Min: minimum; max: maximum.

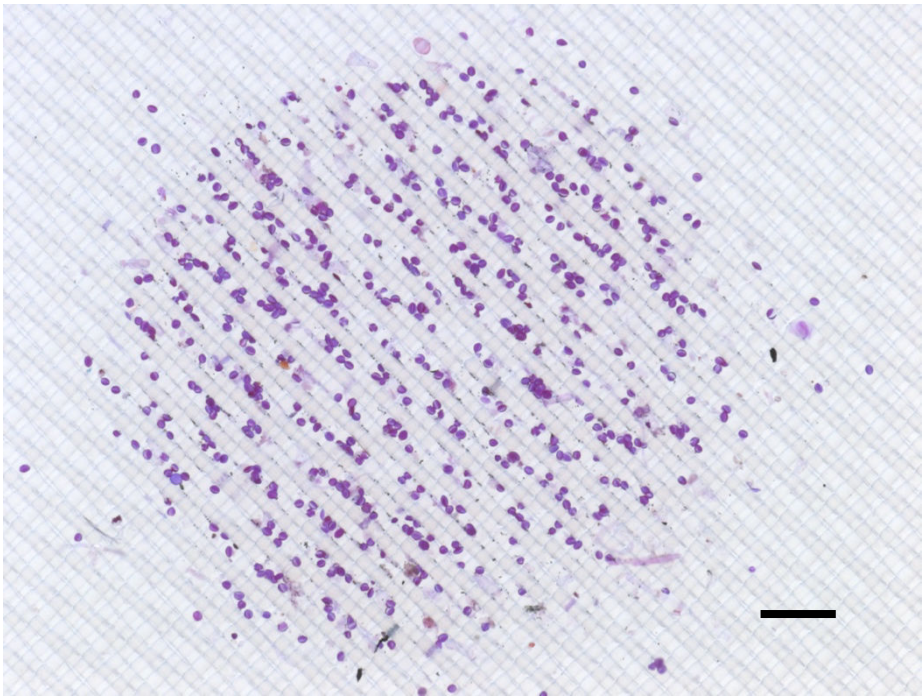


Fig. A1 Example of a photograph of a pollen deposition coloured with basic fuchsine get on a piece of 6 µm openings mesh polyester cloth. Black bar, 0.2 mm.

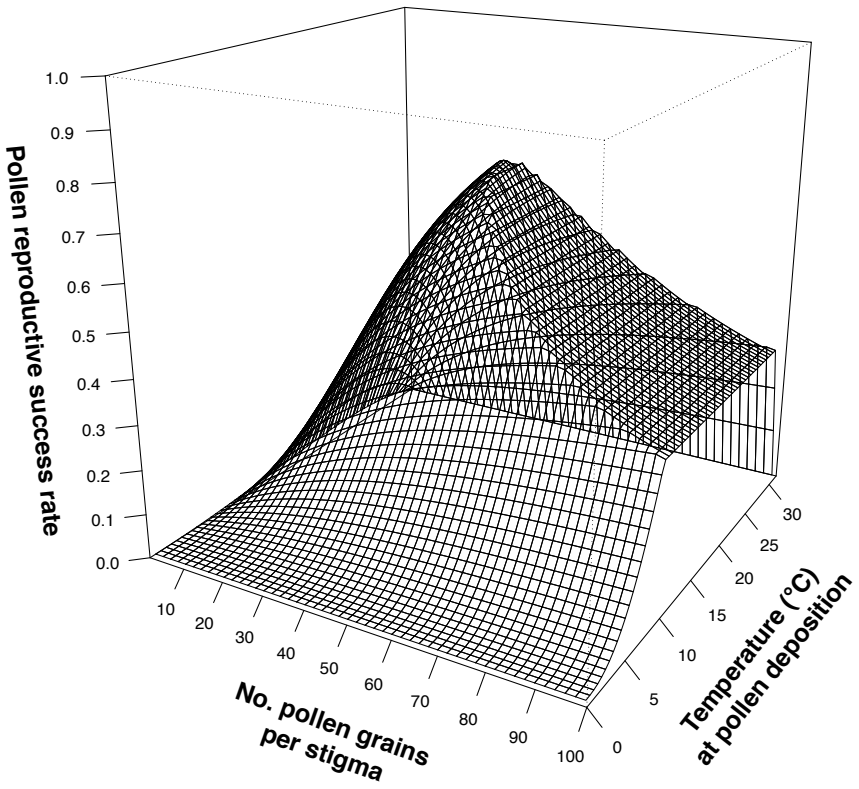


Fig. A2 Predicted pollen reproductive success rate according to the pollination intensity and to the ambient temperature value at the pollen deposition got from Eqn F1, calculated from the best supported model of pollen dose-response relationship Eqn 6b.

Appendix B – Methodology for extraction, staining and filtration of pollen loads from dry stigmas

The methodology which consists of removing pollen grains from dry stigmas by sonication was developed by Vaissière (1991) on *Gossypium hirsutum*. It allows to analyse stigmatic pollen loads quickly with a good reliability as it enables to remove all of the pollen grains from the stigmas without breaking them. This methodology was used then by Vaissière & Froissart (1996) on *Cucumis melo*, and reported also in Dafni (1992) and in Dafni *et al.* (2005). We took over this methodology in this study by adapting it on *Brassica napus*, which has smaller pollen grain size (20 µm x 40 µm; Chifflet, 2010) than the two previous species investigated.

The *Brassica napus* dry stigmas stocked in Eppendorf® tubes were first each transferred to a 15 mL glass shell vial (model 60965; Kimble®, USA; www.kimble-chase.com) and soaked for 1 h in 10 mL of 1% wt/vol saline solution previously filtered on a piece of 1 µm openings mesh nylon cloth (Nitex 03-1/1; Buisine®, France; www.buisine.fr) using a standard all-glass filtration system. The glass shell vials were a little vortexed to make the stigmas flow before adding 20 µL of 1% wt/vol basic fuchsine aqueous solution previously filtered on a piece of 1 µm openings mesh nylon cloth. They were then treated a first time with an ultrasonic probe (Q700 Sonicator; QSonica®, USA; www.sonicator.com) vibrating at 20 kHz with an amplitude of 24 µm for 90 s, the tip of the probe being located 5 mm above the bottom of the vial at rest, to extract pollen grains from the stigma. Stigmas were then examined crushed between two glass slides under a stereomicroscope to check that all pollen grains were removed. The pollen suspensions were treated a second time with the ultrasonic probe with an amplitude of 36 µm for 60 s to break the remaining pieces of stigmatic papillae. Pollen grains in suspension were recovered by filtration on a piece of 6 µm openings mesh polyester cloth (Petex 07-6/5; Buisine®, France) placed under a piece of 41 µm openings mesh nylon cloth (Nitex 03-41/31; Buisine®, France) to retain the largest stigmatic debris, using a standard all-glass filtration system with a 1.1 cm^2 filtering area. The two filter cloths were separated by a piece of rubber with four Ø1.5 mm holes to get four pollen depositions on the 6 µm openings mesh polyester cloth. The ultrasonic probe, the glass shell vials and the glass funnel were rinsed above the filtration system with a small jet of 1% saline solution to recover the potential remaining pollen grains, before cleaning them with the piece of rubber during 3 min in an ultrasonic cleaning bath (Bransonic® Ultrasonic M Cleaning Bath 5800; Branson®, USA; www.bransonic.com), the glass shell vials put upside down and the glass funnel standing up. These cleanings enabled to avoid any pollen contamination between samples. The two pieces of 6 µm and 41 µm openings mesh cloths were put on a glass slide with a drop of 50% vol/vol aqueous glycerol solution. The piece of 41 µm openings

mesh nylon cloth was examined under a stereomicroscope to check if there were remaining pollen grains. The piece of 6 μm openings mesh polyester cloth was either examined under a stereomicroscope for the stigmas hand pollinated with an eyelash to count the pollen grains stained with basic fuchsine directly with the human eye, either photographed under a microscope for the stigmas hand pollinated at saturation to count the pollen grains by image computational analysis (see Appendix C).

Appendix C – Photography and image computational analysis of stigmatic pollen loads recovered on filter cloths

To save the large time usually spent to visually count pollen grains deposited on filter cloths from stigmas pollinated at saturation, we developed a procedure of photography taking of the stigmatic pollen loads recovered on the filter cloths, and a procedure of automated image analysis with the ImageJ software (Schneider *et al.*, 2012) of these photographs, as it could already be used for counting pollen grains (Costa & Yang, 2009).

C.1. Photography taking

The four pollen depositions get on the piece of 6 µm openings mesh polyester cloth were photographed one after the other under the microscope Multizoom AZ100 (Nikon, Japan; www.nikon.com) at magnification 60x (eyepiece lenses = 10x; objective lens = 2x; zoom = 3x; the aperture stop adjusted to the maximum opening) with the camera ProgRes® CFscan (Jenoptik®, Germany; www.jenoptik.com) which has a magnification of 0.6x. The light was delivered by the VisiLED ACT Base (Schott®, Germany; www.schott.com) connected to the controller VisiLED MC 750 (Schott®, Germany), adjusted on a sixth of the opening, and to the controller VisiLED MC 1500 (Schott®, Germany), with the output B adjusted on one fourth of light intensity. The photographs were taken by the software ProgRes® Mac CapturePro, version 2.7.6 (Jenoptik®, Germany) with the following adjustments:

- Contrast = 1.00; Brightness = 1.00; Gamma = 0.50; Saturation = 0.00;
- Balance: Cyan/Red = 0.00; Magenta/Green = 0.00; Yellow/Blue = 0.00;
- Live = 1360 x 1024; Capture = 1360 x 1024;
- Exposure = 12 ms; Gain = 1.00; « Minimize gain for Capture » ticked.

A typical photograph is Fig A.1 (in TIF format).

C.2. Automated image analysis with ImageJ

To count the number of pollen grains on each photograph taken (Fig A.1), we used the macro given in Appendix D run with the software ImageJ (32 bits), version 1.49v. This macro uses the plugin *Threshold Colour* (www.mecourse.com/landinig/software/software) and the plugin *Nucleus Counter* implemented in the *Particle Analysis* plugin as part of the plugin *MBF "ImageJ for Microscopy" Collection* (http://imagej.net/plugins/mbf/; Collins, 2007). This macro involves putting all of the photographs taken in one single repertory file before running it. The macro starts by opening the repertory file containing the photographs and opens one photograph after the other with a *for* loop

to analyse each one independently from the file name. It selects then a minimum saturation threshold of 100 and hue values included between 180 and 215, to cover the range of saturation and of hue of the pollen grains staining with the basic fuchsine solution. It then counts the number of round shape particles with a range areas included between 19 and 300 pixels^2. It ends by displaying the number of pollen grains counted in a list next to another list of the file names of the photographs.

C.3. Selection of the ImageJ procedure parameter values, and assessment of the error made by this procedure

To select the appropriate set of parameter values of saturation, hue, and round shape particle areas given above, we simultaneously selected a sample of 92 photographs along a range of number of pollen grains to assess the parameter values that minimised the counting errors between those given by the ImageJ procedure and those given by the human eye, by taking the human eye as the reference. We could notice that the ImageJ procedure overestimated the number of pollen grains really present on the filter cloths for filter cloths containing less than about 250 pollen grains counted visually (Fig. C1a), corresponding about to when the ImageJ procedure counted less than 200 pollen grains (Fig. C1b). This overestimation probably comes from the "background noise" of the stigmatic debris which are negligible when the pollen grains are higher than 250.

We calculated the mean relative error (with ± 95% CI) of the counts given by the ImageJ procedure compared to those given by the human eye (see Eqn C1 below), as well as the 2.5% and 97.5% quantiles of the distribution of this relative error (see Eqns C2a,b below), according to the minimum threshold of pollen grains counted by the ImageJ procedure below which the sampled photographs were not considered (Fig. C2). We could notice that from 200 pollen grains counted by the ImageJ procedure, the counting error made by the ImageJ procedure had a 95% IC around the mean that overlapped 0, and that 95% of the probability of making an error for the ImageJ procedure was less than ± 20% (Fig. C2).

From these statistics, to set a maximum 95% error threshold of ± 20%, we concluded to recount visually the pollen grains of all of the photographs for which the ImageJ procedure counted less than 200 pollen grains, and to keep the ImageJ counts when they exceeded 200 pollen grains.

At last, we added the number of pollen grains counted for each of the four photographs taken per stigmatic pollen load, and added also with the potential pollen grains remaining on the stigma after sonication and on the piece of 41 µm openings mesh nylon cloth, to give the final counting of pollen grains that were deposited per stigma.

Eqns C1 and C2a,b are written as follows:

$$E(R_e) = E\left(\frac{P_J - P_e}{P_e}\right) \qquad \text{Eqn C1}$$

$$Q_{2.5\%} = E(R_e) - t_{97.5\%}^{n-1} SD(R_e)$$ Eqn C2a

$$Q_{97.5\%} = E(R_e) + t_{\gamma=97.5\%}^{k=n-1} SD(R_e)$$ Eqn C2b

where E is the expected value,

SD is the standard deviation,

R_e is the relative error of the ImageJ procedure counts compared to those of the human eye,

P_J is the number of pollen grains counted by the ImageJ procedure,

P_e is the number of pollen grains counted by the human eye,

$Q_{2.5\%}$ and $Q_{97.5\%}$ are the 2.5% and 97.5% quantiles of the distribution of R_e respectively,

and $t_{97.5\%}^{n-1}$ is the quantile of order 97.5% of the Student's law at $n-1$ degrees of freedom, with n the sample size.

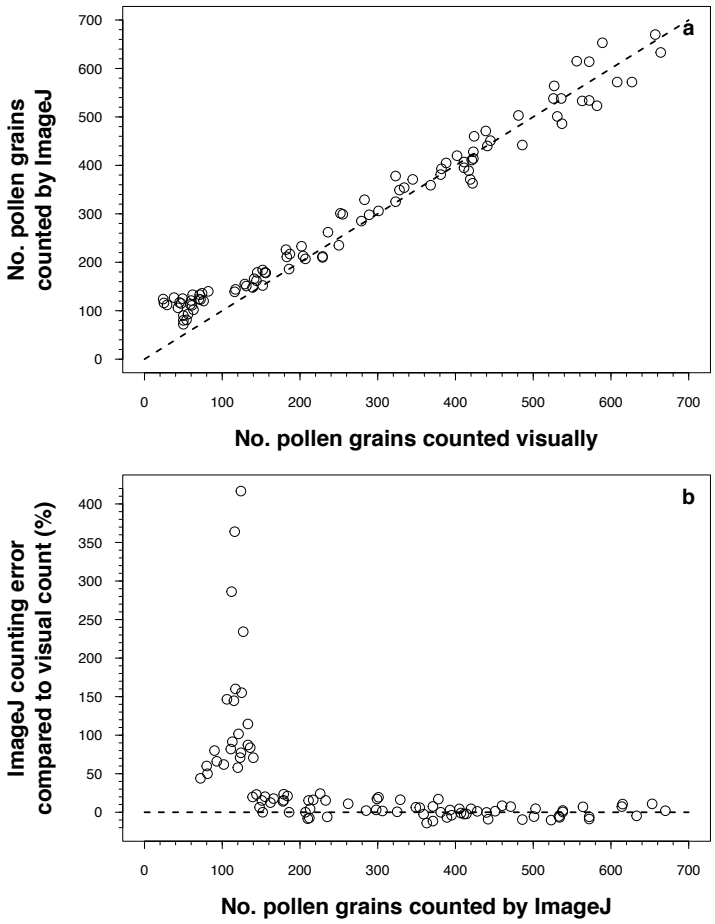


Fig. C1 (a) Number of pollen grains counted by the ImageJ procedure according to those counted by the human eye for each sampled photograph. The dashed line is the first bisector. (b) ImageJ relative counting error according to the number of pollen grains counted by the ImageJ procedure for each sampled photograph. The dashed line is the 0 value on the y-axis. The parameter values used for the ImageJ procedure are 100 for the minimum saturation threshold value, 180 and 215 for the minimum and maximum hue threshold values respectively, and 19 and 300 pixels^2 for the minimum and maximum threshold values of the area of round shape particles. The sample size is 92.

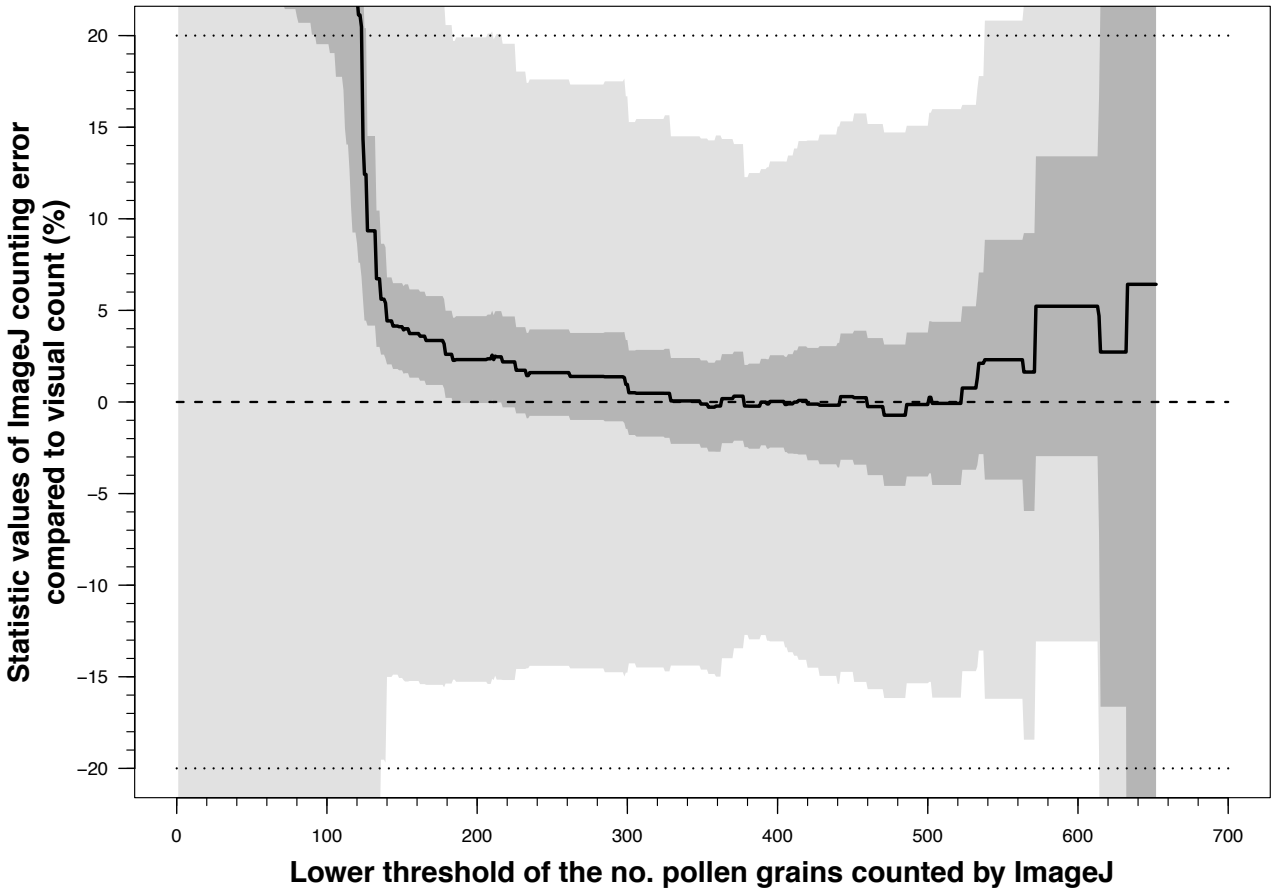


Fig. C2 Statistic values of the relative error made by the ImageJ procedure compared to the counts made by the human eye. Solid line: mean relative error $E(R_e)$ of the counts given by the ImageJ procedure compared to those given by the human eye (Eqn C1). Dark grey area: 95% CI of $E(R_e)$. Light grey area: interval included between $Q_{2.5\%}$ and $Q_{97.5\%}$, the 2.5% and 97.5% quantiles of the distribution of the relative error R_e respectively (Eqns C2a,b). The x-axis is the lower threshold of the number of pollen grains counted by the ImageJ procedure below which the sampled photographs are not considered. With pollen counted by ImageJ procedure > 200 pollen grains (n = 55, on a total of 92 sampled photographs): $E(R_e)$ = 2.31 % (± 2.37 %; 95% CI), $Q_{2.5\%}$ = -15.3%, and $Q_{97.5\%}$ = +19.9%.

Appendix D – Macro in Java language used by running the ImageJ software to count the number of pollen grains deposited on the filter cloths

```
// Write the real file path of the repertory file containing the photographs to analyse
// highlighted in yellow below

rep="File_path"
liste=getFileList(rep);

for(i=0;i<liste.length;i++){
open(rep+liste[i]);
run("Threshold Colour");
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
close();
selectWindow("Brightness");
close();
selectWindow("Saturation");
setThreshold(100, 255);
run("Convert to Mask");
run("Invert");
selectWindow("Threshold Colour");
run("Close");
open(rep+liste[i]);
imageCalculator("add create", liste[i], "Saturation");
selectWindow(liste[i]);
close();
selectWindow("Saturation");
close();
selectWindow("Result of "+liste[i]);
run("Threshold Colour");
run("HSB Stack");
```

```
run("Convert Stack to Images");
selectWindow("Saturation");
close();
selectWindow("Brightness");
close();
selectWindow("Hue");
setThreshold(180, 215);
run("Convert to Mask");
run("Invert");
selectWindow("Threshold Colour");
run("Close");
selectWindow("Hue");
run("Nucleus Counter", "smallest=19 largest=300 threshold=Current smooth=None subtract watershed show");
selectWindow("Hue");
close();
selectWindow("Result of Analysis");
close();
selectWindow("Results");
run("Close");
}

for(i=0;i<liste.length;i++){
print(liste[i]);
}
```

Appendix E – Calculations of the primitive function of Eqn 5 and of the associated threshold value P_0

We start from the assumption that the proportion *b* of pollen grain deposited on the stigma *P* that germinate and develop into pollen tubes that reach the ovary follows a saturating negative-exponential function according to *P*:

$b(P) = b_{max}\,(1 - e^{-b'P})$,

where *b'* is the rate of the proportion of pollen grains *P* that germinate and develop into pollen tubes that reach the ovary.

To get the number of pollen grains deposited on the stigma that germinate and develop into pollen tubes that reach the ovary according to *P*, we have to calculate the primitive function *B*:

$$
\begin{aligned}
B(P) &= \int b_{max}\left(1 - e^{-b'P}\right) dP \\
&= \int \left(b_{max} - b_{max}e^{-b'P}\right) dP \\
&= \int b_{max}\, dP + \int -b_{max}e^{-b'P} dP \\
&= b_{max} \int dP - b_{max} \int e^{-b'P} dP \\
&= b_{max}P - b_{max}\left(-\frac{1}{b'}e^{-b'P}\right) + C \\
&= b_{max}P + \frac{b_{max}}{b'}e^{-b'P} + C \\
&= b_{max}\left(P + \frac{1}{b'}e^{-b'P}\right) + C
\end{aligned}
$$

We now look for the constant value *C*, knowing that there is no pollen tube that reach the ovary when $P = 0$:

$$
\begin{aligned}
B(0) = 0 &\Leftrightarrow b_{max}\left(0 + \frac{1}{b'}e^{-b'.0}\right) + C = 0 \\
&\Leftrightarrow \frac{b_{max}}{b'}e^{0} + C = 0 \\
&\Leftrightarrow C = -\frac{b_{max}}{b'}
\end{aligned}
$$

The function B can therefore be written as:

$$\begin{aligned} B(P) &= b_{max}\left(P + \tfrac{1}{b'}e^{-b'P}\right) - \tfrac{b_{max}}{b'} \\ &= b_{max}\left(P + \frac{1}{b'}e^{-b'P} - \frac{1}{b'}\right) \\ &= b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right) \end{aligned}$$

On the assumption that pollen tubes that reach the ovary are not distributed randomly among ovules because of a bijective distribution of pollen tubes among ovules, the number of fertilised ovules that mature into seeds S according to P should be equal to B up to reach the maximum number of fertilised ovules that mature into seeds $d\bar{O}$ at a threshold P_0 of stigmatic pollen load beyond which S is equal to $d\bar{O}$:

$$\begin{cases} if\ P \leq P_0, \quad S(P) = b_{max}\left(P + \dfrac{1}{b'}(e^{-b'P} - 1)\right) \\ if\ P > P_0, \quad S(P) = d\bar{O} \end{cases}$$

To get the value P_0, we start from the equality $S(P_0) = d\bar{O}$:

$$\begin{aligned} S(P_0) = d\bar{O} &\Leftrightarrow b_{max}\left(P_0 + \frac{1}{b'}\left(e^{-b'P_0} - 1\right)\right) = d\bar{O} \\ &\Leftrightarrow P_0 + \frac{1}{b'}\left(e^{-b'P_0} - 1\right) = \frac{d\bar{O}}{b_{max}} \\ &\Leftrightarrow P_0 + \frac{1}{b'}e^{-b'P_0} - \frac{1}{b'} = \frac{d\bar{O}}{b_{max}} \\ &\Leftrightarrow P_0 + \frac{1}{b'}e^{-b'P_0} = \frac{d\bar{O}}{b_{max}} + \frac{1}{b'} \\ &\Leftrightarrow b'P_0 + e^{-b'P_0} = \frac{b'd\bar{O}}{b_{max}} + 1 \\ &\Leftrightarrow b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right) = -e^{-b'P_0} \\ &\Leftrightarrow \left(b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)\right)e^{b'P_0} = -1 \\ &\Leftrightarrow \left(b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)\right)e^{b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right) + \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)} = -1 \end{aligned}$$

$$\Leftrightarrow \left(b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)\right) e^{b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)} e^{\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)} = -1$$

$$\Leftrightarrow \left(b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)\right) e^{b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right)} = -e^{-\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)}$$

Yet according to Corless *et al.* (1996), we have:

$$xe^x = z \Leftrightarrow x = W_0(z)$$

with W_0 the principal branch of the Lambert W function. Hence we have:

$$S(P_0) = d\bar{O} \Leftrightarrow b'P_0 - \left(\frac{b'd\bar{O}}{b_{max}} + 1\right) = W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)}\right)$$

$$\Leftrightarrow b'P_0 = W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)}\right) + \frac{b'd\bar{O}}{b_{max}} + 1$$

$$\Leftrightarrow P_0 = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)}\right) + \frac{b'd\bar{O}}{b_{max}} + 1\right)$$

The expected seed set S can then be written as:

$$\begin{cases} if\ P \leq P_0,\ S(P) = b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right) \\ if\ P > P_0,\ S(P) = d\bar{O} \\ with\ P_0 = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)}\right) + \frac{b'd\bar{O}}{b_{max}} + 1\right) \end{cases} \quad \text{Eqn 6a}$$

Appendix F – Calculation of the pollen reproductive success rate *R* for each model

We calculated the pollen reproductive success rate *R*, i.e. the proportion of the number of pollen grains *P* deposited on the stigma that fertilised an ovule and led to the production of a seed, according to *P*, by dividing the estimated seed set *S* according to *P* by *P* for each model previously mathematically formalised.

For Eqn 1a:

$$R(P) = \frac{S(P)}{P} = \frac{d\bar{O}(1 - e^{-bP})}{P}$$

By combining with Eqn 2, it gives for Eqn 1b:

$$\begin{cases} R(P,T) = \dfrac{d\bar{O}\left(1 - e^{-b(T)P}\right)}{P} \\ with \begin{cases} b(T) = b_{max}\left(\dfrac{t_{max} - T}{t_{max} - t_{opt}}\right)\left(\dfrac{T - t_{min}}{t_{opt} - t_{min}}\right)^{\frac{t_{opt} - t_{min}}{t_{max} - t_{opt}}} \; if\; T \le t_{max} \\ b(T) = 0 \; if \; T > t_{max} \end{cases} \end{cases}$$

For Eqn 3a:

$$R(P) = \frac{S(P)}{P} = \frac{d\bar{O}\left(1 - e^{-bP^{\gamma}}\right)}{P}$$

By combining with Eqn 2, it gives for Eqn 3b:

$$\begin{cases} R(P,T) = \dfrac{d\bar{O}\left(1 - e^{-b(T)P^{\gamma}}\right)}{P} \\ with \begin{cases} b(T) = b_{max}\left(\dfrac{t_{max} - T}{t_{max} - t_{opt}}\right)\left(\dfrac{T - t_{min}}{t_{opt} - t_{min}}\right)^{\frac{t_{opt} - t_{min}}{t_{max} - t_{opt}}} & if\ T \le t_{max} \\ b(T) = 0\ if\ T > t_{max} \end{cases} \end{cases}$$

For Eqn 4a:

$$\begin{cases} if\ P \le P_0, \quad R(P) = \dfrac{S(P)}{P} \\ if\ P > P_0, \quad R(P) = \dfrac{S(P)}{P} \\ \quad with\ P_0 = d\bar{O}/b \end{cases} \Leftrightarrow \begin{cases} if\ P \le P_0, \quad R(P) = \dfrac{bP}{P} \\ if\ P > P_0, \quad R(P) = \dfrac{d\bar{O}}{P} \\ \quad with\ P_0 = d\bar{O}/b \end{cases}$$

$$\Leftrightarrow \begin{cases} if\ P \le P_0, \quad R(P) = b \\ if\ P > P_0, \quad R(P) = \dfrac{d\bar{O}}{P} \\ \quad with\ P_0 = d\bar{O}/b \end{cases}$$

By combining with Eqn 2, it gives for Eqn 4b:

$$\begin{cases} if\ P \le P_0, \quad R(P,T) = b(T) \\ if\ P > P_0, \quad R(P,T) = \dfrac{d\bar{O}}{P} \\ \quad with\ P_0 = d\bar{O}/b(T) \\ with \begin{cases} b(T) = b_{max}\left(\dfrac{t_{max} - T}{t_{max} - t_{opt}}\right)\left(\dfrac{T - t_{min}}{t_{opt} - t_{min}}\right)^{\frac{t_{opt} - t_{min}}{t_{max} - t_{opt}}} & if\ T \le t_{max} \\ b(T) = 0\ if\ T > t_{max} \end{cases} \end{cases}$$

For Eqn 6a:

$$\begin{cases} if\ P \le P_0, \quad R(P) = \dfrac{S(P)}{P} \\ if\ P > P_0, \quad R(P) = \dfrac{S(P)}{P} \\ with\ P_0 = \dfrac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b' d\bar{O}}{b_{max}} + 1\right)}\right) + \dfrac{b' d\bar{O}}{b_{max}} + 1\right) \end{cases}$$

$$\Leftrightarrow \begin{cases} if\ P \le P_0, \qquad R(P) = \dfrac{b_{max}\left(P + \dfrac{1}{b'}\left(e^{-b'P} - 1\right)\right)}{P} \\ if\ P > P_0, \qquad R(P) = \dfrac{d\bar{O}}{P} \\ with\ P_0 = \dfrac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}}+1\right)}\right) + \dfrac{b'd\bar{O}}{b_{max}} + 1\right) \end{cases}$$

$$\Leftrightarrow \begin{cases} if\ P \le P_0, \qquad R(P) = b_{max}\left(1 + \dfrac{1}{b'P}\left(e^{-b'P} - 1\right)\right) \\ if\ P > P_0, \qquad R(P) = \dfrac{d\bar{O}}{P} \\ with\ P_0 = \dfrac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}}+1\right)}\right) + \dfrac{b'd\bar{O}}{b_{max}} + 1\right) \end{cases}$$

We can note that: $\lim_{P,P_0 \to +\infty} R(P) = b_{max}$

By combining with Eqn 2, it gives for Eqn 6b:

$$\begin{cases} if\ P \le P_0(T),\ R(P,T) = b_{max}(T)\left(1 + \frac{1}{b'P}\left(e^{-b'P} - 1\right)\right) \\ if\ P > P_0(T),\ R(P,T) = \frac{d\bar{O}}{P} \\ with\ P_0(T) = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}(T)}+1\right)}\right) + \frac{b'd\bar{O}}{b_{max}(T)} + 1\right) \\ with \begin{cases} b_{max}(T) = b'_{max}\left(\frac{t_{max}-T}{t_{max}-t_{opt}}\right)\left(\frac{T-t_{min}}{t_{opt}-t_{min}}\right)^{\frac{t_{opt}-t_{min}}{t_{max}-t_{opt}}}\ if\ T \le t_{max} \\ b_{max}(T) = 0\ if\ T > t_{max} \end{cases} \end{cases} \qquad \text{Eqn F1}$$

We can note that: $\lim_{P,P_0 \to +\infty} R(P,T) = b_{max}(T)$

Appendix G – Calculations of the expected number of pollen tubes reaching the ovary *B* and of the threshold values P_{tot} and P_d for Eqns 6a and 6b in the case of a sequence of successive pollen depositions

In the case of a sequence of *n* successive pollen depositions on the stigma, we seek to calculate the total number of pollen grains deposited on the stigma that germinate and develop into pollen tubes that reach the ovary *B* according to the number of pollen P_i deposited on the stigma at each deposition.

For Eqn 6a, we have:

$$\begin{aligned} B(P_n) &= b_{max}\left(P_1 + \frac{1}{b'}\left(e^{-b'P_1} - 1\right)\right) + b_{max}\left(P_2 + \frac{1}{b'}\left(e^{-b'P_2} - 1\right)\right) + \cdots \\ &\quad + b_{max}\left(P_n + \frac{1}{b'}\left(e^{-b'P_n} - 1\right)\right) \\ &= \sum_{i=1}^{n} b_{max}\left(P_i + \frac{1}{b'}\left(e^{-b'P_i} - 1\right)\right) \\ &= b_{max}\sum_{i=1}^{n}\left(P_i + \frac{1}{b'}\left(e^{-b'P_i} - 1\right)\right) \\ &= b_{max}\left(\sum_{i=1}^{n} P_i + \frac{1}{b'}\left(\sum_{i=1}^{n}\left(e^{-b'P_i}\right) - n\right)\right) \end{aligned} \qquad \text{Eqn 7a}$$

By combining with Eqn 2 to integrate the temperature effect at pollen deposition, it gives for Eqn 6b:

$$\begin{cases} B(P_n, T_n) = \sum_{i=1}^{n} b_{max}(T_i)\left(P_i + \frac{1}{b'}\left(e^{-b'P_i} - 1\right)\right) \\ with \begin{cases} b_{max}(T_i) = b'_{max}\left(\frac{t_{max} - T_i}{t_{max} - t_{opt}}\right)\left(\frac{T_i - t_{min}}{t_{opt} - t_{min}}\right)^{\frac{t_{opt} - t_{min}}{t_{max} - t_{opt}}} \; if\; T_i \le t_{max} \\ b_{max}(T_i) = 0 \; if\; T_i > t_{max} \end{cases} \end{cases} \qquad \text{Eqn 7b}$$

In the case where the successive pollen depositions on the stigma are of the same size *P* less than P_0 and occur at the same ambient temperature *T*, we seek to calculate the minimal

number of pollen depositions N_{tot} of fixed size P needed to be deposited in total on the stigma to reach full seed set $d\bar{O}$, to be able to calculate next the minimal number of pollen grains P_{tot} needed to be deposited in total on the stigma to reach full seed set $d\bar{O}$.
For Eqn 6a, we have:

$$B\left(P_{N_{tot}}\right) = d\bar{O} \Leftrightarrow b_{max}\left(N_{tot}(P)P + \frac{1}{b'}\left(N_{tot}(P)e^{-b'P} - N_{tot}(P)\right)\right) = d\bar{O}$$

$$\Leftrightarrow N_{tot}(P)b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right) = d\bar{O}$$

$$\Leftrightarrow N_{tot}(P) = \frac{d\bar{O}}{b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right)}$$

$$\Leftrightarrow N_{tot}(P)P = \frac{d\bar{O}P}{b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right)}$$

$$\Leftrightarrow P_{tot}(P) = \frac{d\bar{O}P}{b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right)}$$

In combination with the fact that, if the size P of the successive pollen depositions is larger than P_0, then P_{tot} is equal to P_0, we have:

$$\begin{cases} if\ P \le P_0,\ P_{tot}(P) = \frac{d\bar{O}P}{b_{max}\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right)} \\ if\ P > P_0,\ P_{tot}(P) = P_0 \\ with\ P_0 = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}} + 1\right)}\right) + \frac{b'd\bar{O}}{b_{max}} + 1\right) \end{cases} \quad \text{Eqn 8a}$$

By combining with Eqn 2, it gives for Eqn 6b:

$$\begin{cases} if\ P \le P_0(T),\ P_{tot}(P,T) = \frac{d\bar{O}P}{b_{max}(T)\left(P + \frac{1}{b'}\left(e^{-b'P} - 1\right)\right)} \\ if\ P > P_0(T),\ P_{tot}(P,T) = P_0(T) \\ with\ P_0(T) = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}(T)} + 1\right)}\right) + \frac{b'd\bar{O}}{b_{max}(T)} + 1\right) \\ with \begin{cases} b_{max}(T) = b'_{max}\left(\frac{t_{max} - T}{t_{max} - t_{opt}}\right)\left(\frac{T - t_{min}}{t_{opt} - t_{min}}\right)^{\frac{t_{opt} - t_{min}}{t_{max} - t_{opt}}}\ if\ T \le t_{max} \\ b_{max}(T) = 0\ if\ T > t_{max} \end{cases} \end{cases} \quad \text{Eqn 8b}$$

According to Eqn 3 in Chabert *et al.* (2018) and if ambient temperature is constant, we seek to calculate the minimal number of pollen grains P_d needed to be deposited on the stigma per day, with fixed pollen deposition size P, to reach full seed set $d\bar{O}$ during full pistil receptivity, expressed in days, b_d.

For Eqn 6a, we have:

$$
\begin{aligned}
P_d(P,T) &= \frac{N_{tot}(P)P}{b_d(T)} \\
&= \frac{N_{tot}(P)P}{\frac{b_h(T)}{24}} \\
&= N_{tot}(P)P\frac{24}{\frac{b_{dh}}{T-t_{base}}} \\
&= \frac{d\bar{O}P}{b_{max}\left(P+\frac{1}{b'}\left(e^{-b'P}-1\right)\right)} \times \frac{24(T-t_{base})}{b_{dh}} \\
&= \frac{24d\bar{O}(T-t_{base})P}{b_{dh}b_{max}\left(P+\frac{1}{b'}\left(e^{-b'P}-1\right)\right)}
\end{aligned}
$$

where t_{base} is the base temperature below which there is no ovule ageing, b_h is the duration of the full pistil receptivity expressed in hours, and b_{dh} is that expressed in degree-hours (°Ch).

In combination with the fact that, if the size P of the successive pollen depositions is larger than P_0, then $N_{tot}(P)P$ is equal to P_0, we have:

$$
\begin{cases}
if\ P \le P_0,\ P_d(P,T) = \frac{24d\bar{O}(T-t_{base})P}{b_{dh}b_{max}\left(P+\frac{1}{b'}\left(e^{-b'P}-1\right)\right)} \\
if\ P > P_0,\ P_d(P,T) = \frac{24(T-t_{base})P_0}{b_{dh}} \\
with\ P_0 = \frac{1}{b'}\left(W_0\left(-e^{-\left(\frac{b'd\bar{O}}{b_{max}}+1\right)}\right)+\frac{b'd\bar{O}}{b_{max}}+1\right)
\end{cases}
\qquad \text{Eqn 9a}
$$

By combining with Eqn 2, it gives for Eqn 6b:

$$\begin{cases} if\ P \le P_0(T),\ P_d(P,T) = \frac{24 d \bar{O} (T - t_{base}) P}{b_{dh} b_{max}(T) \left(P + \frac{1}{b'} \left(e^{-b'P} - 1 \right) \right)} \\ if\ P > P_0(T),\ P_d(P,T) = \frac{24 (T - t_{base}) P_0(T)}{b_{dh}} \\ with\ P_0(T) = \frac{1}{b'} \left(W_0 \left(-e^{-\left(\frac{b' d \bar{O}}{b_{max}(T)} + 1 \right)} \right) + \frac{b' d \bar{O}}{b_{max}(T)} + 1 \right) \\ with \begin{cases} b_{max}(T) = b'_{max} \left(\frac{t_{max} - T}{t_{max} - t_{opt}} \right) \left(\frac{T - t_{min}}{t_{opt} - t_{min}} \right)^{\frac{t_{opt} - t_{min}}{t_{max} - t_{opt}}} \ if\ T \le t_{max} \\ b_{max}(T) = 0\ if\ T > t_{max} \end{cases} \end{cases}$$

Eqn 9b

CHAPITRE III :

Mesure de la vitesse de sécrétion nectarifère en fonction de la température

Mesurer la sécrétion nectarifère : exemple d'une lignée hybride F1 et de son parent mâle stérile chez le colza d'hiver (*Brassica napus* L.)

Stan Chabert[1,2,*], Taïna Lemoine[2], Louna Fronteau[2], Bernard E. Vaissière[2,3]

[1]Association nationale des agriculteurs multiplicateurs de semences oléagineuses, Ferme expérimentale, 2485 route des Pécolets, F-26800 Étoile-sur-Rhône, France

[2]Institut national de la recherche agronomique, UR406 Abeilles et Environnement, 228 route de l'aérodrome, site Agroparc, domaine Saint-Paul, CS 40509, F-84914 Avignon cedex 9, France

[3]UMT Protection des Abeilles dans l'Environnement, 228 route de l'aérodrome, site Agroparc, domaine Saint-Paul, CS 40509, F-84914 Avignon cedex 9, France

Oilseeds & fats, Crops and Lipids, 2017, 24(6): D602

Résumé

Au cours de l'histoire évolutive des plantes à fleurs, l'apparition des nectaires floraux a permis de substituer le pollen par du nectar pour attirer les animaux pollinisateurs, permettant de diminuer les coûts de la pollinisation animale liés à la consommation du pollen. Dans les productions de semence hybride des cultures entomophiles, connaître le niveau de sécrétion nectarifère des lignées en présence, mâle fertile (MF) et mâle stérile (MS), est important si l'on souhaite maximiser les transferts de pollen entre les deux.

Dans cet article, nous faisons tout d'abord une revue des méthodes qui existent pour mesurer la sécrétion nectarifère, puis retenons celle qui mesure un taux de sécrétion brut, qui permet d'exprimer une vitesse de sécrétion, pour l'utiliser sur deux lignées de colza d'hiver (*Brassica napus* L.), la variété hybride F1 'Exocet' MF, et son parent MS. Nous montrons que la sécrétion nectarifère du colza est constante sur un intervalle de temps de 6-8 heures durant les heures du jour, que cette sécrétion admet une température optimale se situant entre 20° et 30°C, et qu'elle est autour de deux fois moindre chez le parent MS par rapport à la lignée F1.

Ces résultats permettent de proposer une méthode de mesure rigoureuse pour comparer la sécrétion nectarifère entre lignées ou variétés. Nous concluons sur les principales autres variables dont il faudrait tenir compte pour notamment pouvoir estimer la quantité totale de nectar sécrétée par une surface donnée de culture.

Mots clés : méthode, sécrétion nectarifère, colza, semence hybride, température

Abstract

Measuring nectar secretion: the example of an F1 hybrid and its male sterile parent in winter oilseed rape (*Brassica napus* L.).

During the evolutionary history of flowering plants, the appearance of floral nectaries allowed the replacement of pollen by nectar to attract pollinators, allowing lower costs involved in animal pollination by reducing the consumption of pollen. In the hybrid seed productions of entomophilous crops, knowing the levels of nectar secretion of the different lines, the male fertile (MF) and the male sterile (MS) ones, is important to maximize pollen transfers between them.

In this study, we start with a review of current methods used to measure nectar secretion, and choose the one which provides a gross secretion rate in order to use it on two winter oilseed rape (*Brassica napus* L.) lines, the hybrid F1 'Exocet' and its MS parent. We show that oilseed rape has a gross nectar secretion rate that is constant over a period of 6-8 hours during daylight hours, that it has a thermal optimum included between 20° and 30°C, and that the parental MS line secretes about half as much as the hybrid F1 one.

These results enable us to propose a rigorous method to compare nectar secretions between lines and varieties. We conclude with the main other variables that should be taken into account to estimate the total amount of nectar produced by a given area of crop.

Keywords: method, nectar secretion, oilseed rape, hybrid seed, temperature

1. Introduction

1.1. Origine évolutive des nectaires floraux

Les premières plantes à fleurs dotées de nectaires floraux sont apparues au cours du Crétacé il y a environ 100 millions d'années (Friis *et al.*, 2011c), période qui correspond également à celle de l'apparition et de la diversification des abeilles (Poinar et Danforth, 2006 ; Michez *et al.*, 2012 ; Branstetter *et al.*, 2017), principal groupe d'animaux pollinisateurs de ces plantes, qui laisse supposer une co-radiation évolutive passée entre ces deux groupes (Michez *et al.*, 2012 ; Cappellari *et al.*, 2013).

Un grand nombre de plantes à fleurs actuelles sécrètent du nectar (Percival, 1961 ; Bernardello, 2007), mais ceci n'est pas un caractère propre à l'ensemble des Angiospermes : la très grande diversité de formes, structures et positions florales des nectaires laisse supposer de multiples événements d'apparitions indépendantes et de disparitions de ce caractère au cours de l'évolution au sein des différents lignées évolutives (Bernardello, 2007). Les nectaires ont par exemple disparu chez les fleurs du genre *Solanum* (exemple : la tomate), où seul le pollen attire les insectes qui les visitent, alors qu'ils sont présents dans la plupart des autres genres de la famille des Solanaceae. De même, les Poaceae ont perdu leurs nectaires ancestraux, parallèlement à un retour à une pollinisation abiotique stricte.

1.2. La sécrétion nectarifère florale est-elle forcément synonyme d'entomophilie ?

Le nectar a ainsi permis de se substituer au pollen dans l'attraction des insectes pollinisateurs chez un certain nombre de plantes à fleurs, permettant de diminuer les coûts de la pollinisation entomophile liée à la consommation de pollen par ces insectes (Takhtajan, 1980). La sécrétion de nectar est donc la plupart du temps associée à un mode de pollinisation entomophile, tout au moins partiel, mais ceci n'est pas systématique : un certain nombre de fleurs de l'archipel Juan Fernández au Chili sont par exemple exclusivement anémophiles, du fait de l'absence de visites d'insectes pollinisateurs, rares sur ces îles, alors qu'elles sécrètent pourtant du nectar (Anderson *et al.*, 2000a, 2000b, 2001 ; Bernardello *et al.*, 2000, 2001). Les auteurs font l'hypothèse que ces espèces ont perdu le caractère entomophile de leurs ancêtres continentaux, sans avoir encore perdu le caractère de sécrétion de nectar.

Ceci est d'autant plus vrai en agriculture, où la sélection humaine de certaines variétés a pu conduire à la perte du caractère entomophile, avec toutefois un maintien de la sécrétion de nectar (Vaissière, 1991). Ce n'est donc pas parce que les fleurs d'une culture produisent du nectar, que les insectes qui visitent cette culture améliorent forcément son niveau de pollinisation, et par extension les rendements, de cette culture.

1.3. La particularité des productions de semence hybride

Ce n'est en revanche pas le cas des cultures entomophiles en production de semence hybride qui alternent une lignée mâle fertile (MF) avec une lignée mâle stérile (MS), où la lignée MS est celle sur laquelle la semence est récoltée et qui ne produit pas de pollen : l'autopollinisation y est impossible, de même que l'entomophilie s'il n'y a pas de sécrétion nectarifère. Pierre et Renard (2010) ont évalué le niveau moyen de dépendance à l'entomophilie de la production de semence hybride chez le colza à 90%. La sécrétion nectarifère doit donc y être suffisamment importante pour attirer les insectes pollinisateurs, notamment par rapport à la production de nectar des autres plantes nectarifères présentes potentiellement dans l'environnement de la culture et qui peuvent entrainer une dilution importante des insectes pollinisateurs (Garibaldi *et al.*, 2017). Enfin, si l'on souhaite maximiser les transferts de pollen de la lignée MF vers la lignée MS, Pierre *et al.* (1999) ont suggéré que la production de nectar ne devait pas être trop disproportionnée entre les deux lignées. Dès lors, il est fondamental de savoir mesurer correctement la sécrétion nectarifère d'une espèce, d'une variété ou d'une lignée données.

1.4. Mesurer la sécrétion nectarifère

Il existe différentes méthodes pour mesurer la quantité de nectar disponible dans une fleur. Ces différences portent sur le matériel utilisé, ou sur le traitement de la fleur préalablement à la mesure. Concernant le matériel, la méthode la plus couramment choisie est celle utilisant des microcapillaires, étant simple, rapide et fiable (McKenna et Thomson, 1988 ; Mesquida *et al.*, 1988). Une méthode de récolte de nectar par centrifugation des fleurs a été mise au point pour obtenir rapidement des volumes de nectar suffisamment importants pour permettre l'analyse des constituants du nectar par chromatographie (Bosi, 1973). Mais cette méthode fournit un nectar artificiellement dilué et elle n'est pas exempte de modifier la composition chimique du nectar par lésion des tissus de la fleur (Mesquida *et al.*, 1988). Des méthodes de lavage et de rinçage des fleurs avec de l'eau distillée ont été proposées pour échantillonner le nectar des fleurs contenant des petits volumes de nectar inférieurs à 1 µL qui sont difficiles à échantillonner (Mallick, 2000 ; Morrant *et al.*, 2009). Ces méthodes permettent d'échantillonner plus de nectar que celle utilisant les microcapillaires dans ce type de fleur, mais la question de savoir laquelle de ces méthodes reflète le plus fidèlement la quantité de nectar pouvant être effectivement extraite par les insectes floricoles lors de leur visite reste posée (Petit *et al.*, 2011).

Il existe ensuite différents traitements de la fleur, préalablement à la mesure de la quantité de nectar, que nous examinons dans les paragraphes suivants.

1.4.1. Nectar résiduel

Une première méthode consiste à mesurer le « nectar résiduel » (*nectar standing crop*), c'est-à-dire la quantité de nectar disponible dans une fleur offerte au libre butinage des insectes (Kearns et Inouye, 1993 ; Corbet, 2003). Cette mesure, qui a l'intérêt de mettre en évidence la quantité moyenne de nectar réellement proposée par les fleurs aux insectes à un moment donné, souffre d'une très grande variabilité entre fleurs due au hasard de la durée écoulée entre la mesure et la dernière visite d'insecte, et ne permet pas de mettre en évidence les paramètres du processus de sécrétion en lui-même.

1.4.2. Taux de sécrétion apparent

Une deuxième méthode, la plus classiquement utilisée, consiste à mesurer le taux de sécrétion apparent. L'échantillonnage s'effectue sur des fleurs dont les hampes ont été ensachées sous tulle au moins 24 heures avant la mesure afin d'empêcher le butinage des fleurs par les insectes (Corbet, 2003). Cette méthode demeure pour le moment la méthode de référence pour estimer la quantité de nectar sécrétée par les plantes (exemple : Baude *et al.,* 2016). Mais cette méthode considère la sécrétion de nectar comme un processus statique. Or il a été montré que de nombreuses plantes, dont le colza, sécrètent du nectar en continu (Cruden *et al.*, 1983b ; Pacini et Nepi, 2007), et de surcroît pour certaines d'entre elles, dont le colza encore, un processus de réabsorption se produit également en continu, de façon concomitante au processus de sécrétion (synthèse dans Nepi et Stpiczyńska, 2008). Ce processus de réabsorption est inhibé chez un certain nombre d'espèces lorsque le nectar est régulièrement retiré de la fleur (Luo *et al.*, 2014), et ce notamment chez le colza (Burquez et Corbet, 1991). Une fleur régulièrement butinée par un insecte produit donc plus de nectar en cumulé qu'une fleur non butinée. Cette méthode peut donc conduire à des estimations erronées de la quantité totale de nectar sécrétée par une fleur accessible aux insectes.

1.4.3. Taux de sécrétion brut

Une troisième méthode, qui mesure le taux de sécrétion brut, consiste à retirer totalement le nectar d'une fleur à un instant donné, de l'ensacher sous tulle, puis de mesurer la quantité de nectar produite après un certain intervalle de temps, à déterminer (Corbet, 2003). Ceci permet d'exprimer une vitesse de sécrétion nectarifère tout en s'affranchissant des processus de

réabsorption, processus qui sont inhibés lorsque la fleur est régulièrement butinée. C'est donc cette méthode qui se révèle être la plus rigoureuse pour estimer des quantités de nectar sécrétées par des plantes butinées. Cette mesure est parfois appelée confusément taux de sécrétion net (exemple : Galetto et Bernardello, 2004).

1.4.4. Convertir le volume en masse de sucres

Enfin un certain nombre d'études ne mesurent que le volume de nectar sécrété. Or il a été montré que la concentration du nectar admet une très grande variabilité, due principalement aux variations d'humidité relative atmosphérique (Pacini & Nepi, 2007). Il est donc préférable, dans la mesure du possible, de mesurer simultanément le volume et la concentration du nectar sécrété par une fleur, puis d'exprimer la quantité de nectar sécrétée en masse de sucres totaux dissous (STD) par l'intermédiaire d'une formule combinant ces deux variables. Deux formules ont été proposées à cet effet, et elles donnent des résultats quasiment identiques (Cruden et Hermann, 1983a ; Prŷs-Jones et Corbet, 2011).

1.5. Les lignées MS sont-elles aussi nectarifères que les lignées MF ?

D'après le ressenti des apiculteurs, les rendements en miels des colonies d'abeilles mellifères sont moindres lorsqu'elles sont apportées sur des cultures de production de semence hybride. Une première étude a en effet pour l'instant montré que trois lignées MS de colza produisaient de 16 à 70% de nectar en volume de moins que leurs homologues isogéniques MF (Pierre *et al.,* 1999). Mais ces auteurs n'ont pas mesuré la concentration en STD de leurs échantillons de nectar pour connaître les quantités de STD effectivement produites. Ils ont par ailleurs mesuré le taux de sécrétion apparent, méthode qui souffre des biais évoqués précédemment. La température joue enfin un rôle majeur sur la sécrétion nectarifère, bien que ce rôle n'ait été encore que relativement peu étudié (Pacini & Nepi, 2007 ; Petanidou, 2007).

Nous avons donc mesuré le taux de sécrétion brut de nectar au premier jour d'anthèse de fleurs de colza d'hiver d'une lignée hybride F1, mâle fertile, et de son parent MS afin de tester s'il y a une différence de vitesse de sécrétion brute entre les deux types de lignées. Nous avons réalisé ces mesures durant les heures de jour sur une gamme d'intervalles de temps entre le prélèvement initial et la mesure s'échelonnant de 15 minutes à 8 heures, ceci pour tester si (i) la sécrétion nectarifère reprend rapidement après un premier prélèvement, et si (ii) cette sécrétion est constante tout au long des heures du jour après un premier prélèvement. Nous avons également testé l'effet de la température en répétant ces mesures sous tunnel dans différentes conditions de température ambiante durant les mois d'avril à mai. Enfin nous avons choisi la méthode utilisant des microcapillaires, adaptée pour le colza (Mesquida *et al.*, 1988).

2. Matériel et méthodes

Les relevés ont été réalisés au printemps 2017 sous deux tunnels *insectproof* localisés sur le centre INRA d'Avignon, ceci afin d'empêcher tout butinage des fleurs par les insectes (Fig. 1). Les tunnels, composés de 4 rangs de 18 m de long, ont été semés chacun avec deux lignées de colza d'hiver (*Brassica napus* L.) : la variété hybride F1 'Exocet' (mâle fertile ; Fig. 2A), obtention Monsanto®, et son parent femelle, mâle stérile (Fig. 2B). Les deux tunnels ont été semés avec trois semaines d'écart, le premier le 21 novembre 2016 et le second le 12 décembre 2016, dans le but d'avoir un décalage de floraison et de disposer ainsi d'une floraison plus étendue dans le temps pour nos mesures. Les plantes étaient arrosées quotidiennement par un système de goutte-à-goutte afin d'éviter qu'elles ne soient en déficit hydrique. La température instantanée était enregistrée toutes les 2 min 30 par un capteur HOBO® Pro v2 (Onset® Computer Corporation, USA).

2.1. Identification de cohortes de fleurs

Nous avons effectué les mesures de taux de sécrétion brut de nectar à cinq dates, échelonnées entre le 14 avril et le 29 mai 2017, afin de pouvoir échantillonner le nectar sous des conditions de températures les plus variées possible tout au long de la floraison du colza (Tab. 1). Ces mesures ont été réalisées sur des fleurs de même âge, au premier jour d'anthèse, en identifiant une cohorte de fleurs pour chaque date. Pour identifier une cohorte, nous déposions la veille des relevés entre 16h00 et 18h00 GMT (heure locale moins deux heures, proche de l'heure solaire) un premier marqueur de fleur (Cherry ; Filpack® agricole, France) sur 12 hampes florales choisies au hasard par lignée et par date sur l'ensemble des plantes, sous le bouton totalement fermé positionné le plus bas sur la hampe. La floraison du colza est acropétale, c'est-à-dire qu'elle se réalise de la base de la hampe vers le sommet, mais pas de façon stricte, de sorte que l'on peut trouver des fleurs épanouies au-dessus de fleurs encore au stade bouton (Fig. 3). Nous avons donc sectionné toutes les fleurs épanouies situées au-dessus du bouton marqué en haut du pédoncule (Fig. 3), pour ne disposer que de boutons fermés au-dessus de notre marqueur. Nous déposions ensuite le jour des relevés autour de 08h00 GMT un deuxième marqueur de fleur sur ces mêmes hampes au-dessus de la fleur épanouie en position la plus haute. Nous sectionnions alors tous les boutons non épanouis situés au-dessous de ce deuxième marqueur en haut du pédoncule, de façon à ne conserver que des fleurs épanouies du matin entre les deux marqueurs. Cette élimination de fleurs pouvait avoir pour effet potentiel d'augmenter les flux de sève vers les fleurs non retirées, mais nous

Fig. 1. Tunnel abritant les deux lignées de colza mâle stérile (les deux rangs de gauche) et F1 'Exocet' (les deux rangs de droite) en milieu de floraison. © Stan CHABERT / INRA

Fig. 2. (A) Fleurs mâle fertile F1 de colza d'hiver 'Exocet' au 1er jour d'anthèse (à droite) et 2ème jour d'anthèse (à gauche). (B) Fleur mâle stérile, lignée parentale d''Exocet', au 1er jour d'anthèse. © Taïna LEMOINE / INRA

Tableau 1. Températures d'échantillonnage mesurées pour chaque date de relevés.

Date	Température (°C)		
	moyenne	minimale	maximale
14.04.2017	24.98	22.07	28.66
25.04.2017	18.90	9.17	25.97
01.05.2017	17.11	12.13	22.12
23.05.2017	30.75	24.27	34.58
29.05.2017	33.43	27.71	35.74

Fig. 3. Hampe florale mâle fertile de colza dont une fleur épanouie (flèche blanche à gauche) se situe au-dessus d'un bouton encore fermé (flèche noire à droite). La ligne blanche en pointillés symbolise la zone où les fleurs étaient sectionnées, en haut du pédoncule, pour disposer de fleurs du même âge dans les cohortes sélectionnées. © Taïna LEMOINE / INRA

avons considéré ce potentiel effet comme négligeable. Nous disposions ainsi de 2 à 5 fleurs de même âge par hampe florale (Fig. 4). Pour faciliter l'organisation des mesures, les fleurs d'une même hampe recevaient le même traitement, à savoir, tout d'abord un prélèvement initial au même moment, puis une mesure de sécrétion nectarifère au même moment. L'heure de prélèvement initial était alors notée sur une étiquette à fil attachée autour de la hampe.

2.2. Mesure du taux de sécrétion brut de nectar

A chaque date de relevé, les prélèvements initiaux de nectar ont été réalisés successivement sur les 12 hampes de chacune des deux lignées entre 08h00 et 12h00 GMT. Chaque hampe florale servait ensuite pour une mesure du taux de sécrétion brut unique, avec un intervalle de temps différent testé pour chaque hampe. La gamme d'intervalles de temps testée s'échelonnait de la façon suivante : 15 min, 30 min, 45 min, 1h, 1h30, 2h, 3h, 4h, 5h, 6h, 7h et 8h – soit 12 intervalles de temps pour nos 12 hampes. Les hampes étant constituées de 2 à 5 fleurs marquées, nous disposions ainsi de 2 à 5 répétitions de mesure par intervalle de temps par lignée et par date. La première hampe florale recevant le traitement de prélèvement initial servait pour la mesure de taux de sécrétion brut avec le plus grand intervalle de temps (8 heures), puis la deuxième hampe recevant le traitement de prélèvement initial servait pour la mesure de taux de sécrétion brut avec l'intervalle de temps de 7 heures, et ainsi de suite jusqu'au plus petit intervalle de temps (15 minutes). Cette organisation permettait d'éviter un trop gros décalage de temps entre les mesures, avec notamment le midi solaire inclus dans tous les intervalles de temps.

Les récoltes de nectar des prélèvements initiaux ont été réalisées à l'aide de microcapillaires de 1 µL, puis de 0.5 µL pour retirer le nectar résiduel potentiel, cette étape ayant été réalisée avec précaution pour ne pas endommager les nectaires. Seul le nectar des deux nectaires latéraux était échantillonné dans chaque fleur (Fig. 5), ces nectaires produisant 95% du nectar sécrété par la fleur (Davis *et al.*, 1986, 1998). Lorsque le nectar était visqueux, c'est-à-dire dans les situations de fortes concentrations et de faibles températures (Nicolson et Thornburg, 2007), situations qui intervenaient systématiquement au moins en fin d'après-midi après 16h00 GMT, les microcapillaires étaient laissés en contact avec les nectaires durant 3 à 5 minutes (Fig. 6). Enfin, pour la valeur de température associée à chaque mesure de taux de sécrétion brut de nectar, nous avons calculé la moyenne de l'ensemble des températures instantanées enregistrées par le capteur durant la période comprise entre l'heure (à 5 minutes près) du prélèvement initial et l'heure de la mesure du taux de sécrétion brut.

Fig. 4. Hampe florale de colza avec deux marqueurs de fleur délimitant une cohorte de deux fleurs mâle fertile de même âge. © Stan CHABERT / INRA

Fig. 5. Fleur mâle fertile de colza d'hiver dont on a retiré les pétales et sur laquelle on distingue les deux gouttes de nectar situées au niveau des nectaires latéraux, à la base du pistil et des étamines courtes. © Nicolas CERRUTI / TERRES INOVIA

Fig. 6. Échantillonnage de nectar dans une fleur mâle fertile de colza à l'aide d'un microcapillaire.
© Stan CHABERT / INRA

La concentration du nectar en STD a été mesurée à l'aide de deux réfractomètres adaptés pour les petits volumes, permettant une lecture jusqu'à des volumes de 0,05 µL, pour des gammes de 0-50 et 45-80% Brix (Eclipse 45-03 et 45-05 ; Bellingham & Stanley Ltd, UK). La masse de STD était ensuite obtenue à l'aide de la formule de conversion de Cruden et Hermann (1983a) :

$$M = C \,.\, (4{,}6\ 10^{-5} \,.\, C + 9{,}946\ 10^{-3}) \,.\, V \,,$$

où M est la masse de STD en mg,
V le volume de nectar en µL,
et C la concentration du nectar en g de STD pour 100 g de solution (% Brix).

2.3. Analyses statistiques

Le nectar étant sécrété de façon continue (Pacini et Nepi, 2007), nous avons estimé les pentes, ainsi que les intervalles de confiance à 95% associés, de la masse de STD en fonction de la durée écoulée entre le prélèvement initial et la mesure effectuée à l'aide de modèles linéaires mécanistes de la forme $\hat{y} = \hat{a} \,.\, x$ (méthode d'inférence statistique dite des moindres carrés ; Bolker, 2008), pour chacune des deux lignées (F1 et MS) et pour chaque catégorie de température (< 20°C ; entre 20° et 30°C ; > 30°C). Deux moyennes dont les intervalles de confiance à 95% ne se recouvrent pas ne peuvent être considérées comme statistiquement identiques, avec une chance de 5% de se tromper (Nakagawa et Cuthill, 2007). Ces analyses ont été réalisées avec le logiciel R, version 3.2.0 (R Core Team, 2015).

3. Résultats

Les températures de relevés ont varié de 9 à 36°C sous les tunnels (Tab. 1). On peut tout d'abord constater que la masse de STD contenue dans les fleurs augmentait de façon constante au cours du temps après le prélèvement initial de nectar pour les deux lignées, F1 et MS, et ce dès 15 minutes et quelle que soit la température d'échantillonnage (Fig. 7). La vitesse de sécrétion, en masse de STD, est de deux à trois fois plus forte entre 20° et 30°C qu'en-dessous de 20°C et qu'au-dessus de 30°C pour les deux lignées (les intervalles de confiance à 95% ne se chevauchaient pas ; Tab. 2). D'autre part, cette vitesse de sécrétion était moindre pour la lignée MS par rapport à la lignée F1, et ce pour toutes les plages de température (Tab. 2). La vitesse de sécrétion de la lignée MS équivalait à 61% de celle de la lignée F1 entre 20° et 30°C. En-dessous de 20°C, cette vitesse équivalait à 35% de celle de la lignée F1, et au-dessus de 30°C à 55% de celle de la lignée F1.

Tableau 2. Vitesses de sécrétion nectarifère brutes estimées par catégorie de température pour chaque lignée.

Lignée	Catégorie de température (°C)	Nombre de fleurs échantillonnées	Vitesse de sécrétion (µg STD . h^{-1})	Intervalle de confiance à 95%	
				Borne inférieure	Borne supérieure
F1	< 20	73	45.18	40.60	49.76
	entre 20 et 30	111	83.48	76.53	90.44
	> 30	46	30.91	26.27	35.55
MS	< 20	102	15.66	13.00	18.32
	entre 20 et 30	73	50.81	44.94	56.68
	> 30	55	17.01	12.24	21.77

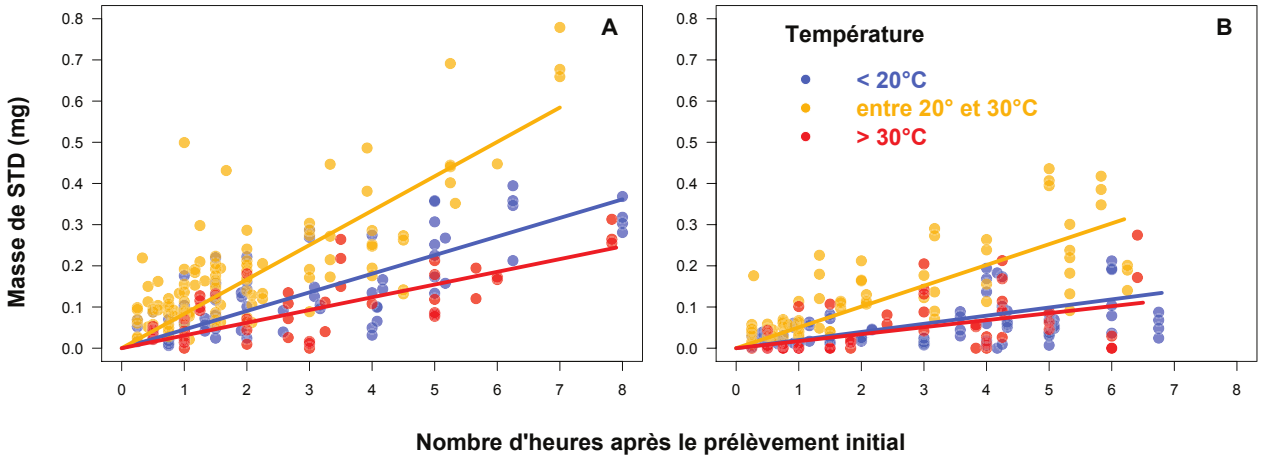


Fig. 7. Masse de sucres totaux dissous (STD) sécrétée par fleur pour (A) la lignée hybride F1 et (B) son parent femelle mâle stérile en fonction du nombre d'heures écoulées après le prélèvement initial et selon la gamme de température.

4. Discussion

4.1. L'effet unimodal de la température sur la sécrétion nectarifère

Pacini *et al.* (2003) distinguent deux types de nectaires floraux : ceux dont l'amidon, qui sert de réactif pour la production de sucres, provient directement de la photosynthèse et qui sécrètent par conséquent du nectar en continu et en petites quantités lorsque la photosynthèse est active, et ceux dont l'amidon est préalablement stocké dans leur parenchyme avant sécrétion, et qui peuvent sécréter de grandes quantités de nectar en très peu de temps indépendamment de la photosynthèse (ces nectaires peuvent donc par exemple sécréter la nuit dans le cas de pollinisateurs nocturnes). Le colza, comme l'ensemble des Brassicaceae, appartient au premier type de plante où il n'y a pas de stockage d'amidon (Davis *et al.*, 1986, 1998). Or l'activité photosynthétique des plantes admet un optimum de température, cet optimum étant lui-même dépendant de la température à laquelle les plantes se sont développées. Les plantes ont ainsi une capacité d'acclimatation qui leur permet d'adapter leur activité photosynthétique au climat rencontré (Berry et Björkman, 1980 ; Yamori *et al.*, 2014). Les variations de sécrétion nectarifère dues à la température s'expliqueraient donc directement par l'effet unimodal de la température sur la photosynthèse. De plus, l'optimum thermique observé pour la sécrétion de nectar pourrait également varier, à l'instar de la photosynthèse, suivant la température de développement des plantes : ceci a en effet déjà été observé par Huber (1956).

C'est à notre connaissance la troisième fois qu'un tel effet de la température a été mis en évidence sur une vitesse de sécrétion nectarifère (Fig. 7 et Tab. 2 ; Findley *et al.*, 1971 ; Nicolson, 1995), confirmant ce qui a déjà été observé chez plusieurs espèces sur le taux de sécrétion apparent (Huber, 1956 ; Villarreal et Freeman, 1990 ; Petanidou et Smets, 1996 ; Pacini et Nepi, 2007 ; Petanidou, 2007 ; Takkis *et al.*, 2015).

4.2. La lignée MS sécrète deux fois moins vite que la lignée F1

La lignée MS sécrètait du nectar en moyenne deux fois moins vite que la lignée F1 sur l'ensemble des températures (Fig. 7 et Tab. 2). Ces résultats rejoignent en partie les observations de Mesquida *et al.* (1991) et de Pierre *et al.* (1999) réalisées sur les volumes de nectar sécrétés par des lignées pures MF et MS avec la mesure du taux de sécrétion apparent. Cette différence de vitesse de sécrétion peut s'expliquer en partie par la mise en place du système de stérilité mâle cytoplasmique : lors de la conception de ce système, les premières lignées MS obtenues en combinant le génome nucléaire du colza avec le cytoplasme du radis *Raphanus sativus* ont eu pour effet d'altérer le développement des nectaires (Pelletier et Budar,

2015).

La différence de vitesse de sécrétion demeurant encore aujourd'hui entre les deux types de lignées peut également provenir de la différence de taille entre les fleurs, et donc de taille entre les nectaires. Il a en effet été montré chez plusieurs espèces que la quantité de nectar sécrétée est corrélée positivement avec la taille des nectaires (Teuber *et al.*, 1980 ; Dafni *et al.*, 1988 ; Petanidou *et al.*, 2000 ; Galetto et Bernardello, 2004). C'est l'idée qui a été proposée par Sammataro *et al.* (1985) pour expliquer les différences d'attractivité entre variétés chez le tournesol. Il serait donc intéressant de mesurer la taille des nectaires ou des fleurs pour tester si cette différence de vitesse de sécrétion observée entre les deux lignées provient effectivement de cette différence de taille ou non.

4.3. Une méthode rigoureuse pour comparer la sécrétion nectarifère de différentes lignées ou variétés

Des lignées MS plus productives en nectar pourraient être sélectionnées (Mesquida *et al.*, 1991 ; Pierre *et al.*, 1999), à l'instar des lignées MF conventionnelles (Kamler, 1984 ; Davis, 2001). Un certain niveau d'héritabilité pour le caractère de sécrétion de nectar a en effet été mis en évidence chez plusieurs espèces (Mitchell, 2004 ; Leiss et Klinkhamer, 2005 ; Kaczorowski *et al.*, 2008). Tout en sachant que la quantité de nectar sécrétée n'est pas le seul critère pour caractériser l'attractivité d'une espèce ou d'une lignée : l'accessibilité du nectar, comme la longueur des fleurons chez le tournesol (Mallinger et Prasifka, 2017), et la composition en sucres du nectar (Nicolson, 2007) y contribuent également.

Pour envisager un tel programme de sélection, la méthode que nous avons utilisée qui mesure le taux de sécrétion brut de nectar en STD sur des fleurs au 1er jour d'anthèse peut être retenue, en veillant à échantillonner le nectar des différentes lignées dans des conditions environnementales similaires, en particulier pour la température. L'intervalle de temps à retenir entre le prélèvement initial de nectar et la mesure peut être de 6 ou 8 heures, avec un prélèvement initial qui peut s'effectuer le matin entre 09h00 et 12h00, heure locale, et une mesure qui peut s'effectuer jusqu'à 18h00. Ensuite le nectar devient très visqueux et monte très lentement dans les microcapillaires. La sécrétion nectarifère est en effet bien constante sur un pas de temps de 6-8 heures quelle que soit la température (Fig. 7). Cette sécrétion étant directement dépendante de la photosynthèse, il est impératif que le prélèvement initial de nectar ait lieu lorsqu'il y a photosynthèse, c'est-à-dire durant le jour à partir du moment où le rayonnement est suffisant. Au-delà d'un intervalle de temps de 8 heures entre le prélèvement initial et la mesure, le processus de sécrétion devrait donc a priori continuer, jusqu'au moment

où le rayonnement devient limitant pour la photosynthèse. Enfin, le prélèvement initial de nectar peut être réalisé à l'aide de papier Whatman, à la place de microcapillaires, afin d'être certain de ne pas endommager les nectaires (McKenna et Thomson, 1988).

4.4. Perspectives : quels autres facteurs prendre en compte ?

4.4.1. Âge de la fleur et pollinisation

D'autres facteurs sont encore à prendre en compte pour expliquer la variabilité de la sécrétion nectarifère, avec en premier lieu l'âge de la fleur, la sécrétion se réalisant en continu jusqu'au moment de sa sénescence où le nectar est peu à peu réabsorbé (Nepi et Stpiczyńska, 2008). C'est un facteur particulièrement important pour les espèces protogynes ou protandres, comme le tournesol ou la carotte, où la sécrétion peut varier selon la phase sexuelle qui évolue avec l'âge (Langenberger et Davis, 2002 ; Wist et Davis, 2006, 2008 ; Varga *et al.*, 2013). Il faut également tenir compte de l'effet de la pollinisation, qui peut déclencher précocement le processus de sénescence de la fleur et du coup interrompre la sécrétion (Stead, 1992 ; van Doorn, 1997 ; Rogers, 2006). Avec ces connaissances, nous pourrions ainsi estimer de façon précise la quantité totale de sucres produite par un hectare de culture d'une espèce et d'une lignée donnée, disponible pour les abeilles. Et à terme identifier une charge de colonies d'abeilles mellifères par unité de surface à ne pas dépasser si l'on souhaite éviter que les colonies ne rentrent trop en compétition les unes avec les autres pour le nectar.

4.4.2. Humidité du sol

Enfin la teneur en eau du sol apparaît comme un dernier facteur important pour la sécrétion nectarifère. Différentes études sur espèces sauvages ont montré que la sécrétion de nectar augmente avec l'humidité du sol (Wyatt *et al.*, 1992 ; Villarreal et Freeman, 1990 ; Carroll *et al.*, 2001 ; Waser et Price, 2016 ; Gallagher et Campbell, 2017). Gillespie *et al.* (2015) ont observé une relation similaire en production de semence hybride d'oignon, mais avec un optimum d'humidité au-delà duquel la sécrétion diminue. Ceci rejoint le fait que l'activité photosynthétique des plantes diminue en situation de déficit hydrique (Chaves *et al.*, 2009), comme en situation d'excès hydrique (Balakhnina, 2015). Leiss et Klinkhamer (2005) ont quant à eux montré, à l'instar de Boose (1997), l'existence d'une interaction génotype-environnement sur la sécrétion nectarifère : l'effet positif de l'humidité du sol sur la sécrétion chez la vipérine commune (*Echium vulgare*) s'observe surtout pour des génotypes peu nectarifères avec un système racinaire peu développé. A l'inverse, des génotypes très nectarifères avec un système racinaire bien développé ont une forte capacité de sécrétion en condition de sol sec comme en

condition de sol humide. Il apparaît ainsi que des différences de sécrétion nectarifère entre lignées ou entre variétés peuvent ne se révéler que dans certains contextes environnementaux, par exemple lorsque les plantes sont en condition de stress. Lindström *et al.* (2018) viennent par exemple de montrer que l'irrigation permet de compenser l'effet négatif des méligèthes sur la sécrétion nectarifère du colza (Kirk *et al.*, 1995 ; Krupnick *et al.*, 1999).

Remerciements

Nous tenons à remercier l'ensemble des membres de l'Installation Expérimentale de l'unité EMMAH du centre INRA PACA qui nous ont permis de disposer d'une très belle culture de colza sous tunnel : Gilles CONTINSOUZAS, Véronique DESFONDS, Serge RENO, Jérémy THEODORE et Frédéric VENAULT. Nous remercions également la société Monsanto® qui nous a gracieusement mis ses semences à disposition pour réaliser cette étude. Nous remercions enfin Isabelle DAJOZ et une/un autre relectrice.teur anonyme pour leur relecture approfondie qui a permis d'améliorer grandement la qualité de cet article.

CHAPITRE IV :

Validation d'une méthode simple et rapide d'évaluation de la taille de population adulte des colonies d'abeilles mellifères

Towards a simple and robust estimate of adult honey bee population size to support colony survival and crop pollination service

En preparation, à soumettre à Ecological Applications

Stan Chabert[a,b,*], Fabrice Requier[a,c], Joël Chadoeuf[d],
Laurent Guilbaud[a], Bernard E. Vaissière[a]

[a]*Institut National de la Recherche Agronomique (INRA), UR406 Abeilles et Environnement, site Agroparc, domaine Saint-Paul, CS 40509, F-84914 Avignon cedex 9, France*
[b]*Association Nationale des Agriculteurs Multiplicateurs de Semences Oléagineuses (ANAMSO), Ferme expérimentale, 2485 route des Pécolets, F-26800 Étoile-sur-Rhône, France*
[c]*Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Am Hubland, DE-97074 Würzburg, Germany*
[d]*Institut National de la Recherche Agronomique (INRA), Statistics, UR1052, 67 allée des chênes, domaine Saint-Maurice, CS 60094, F-84143 Avignon cedex, France*

Abstract

The introduction of managed honey bee colonies, *Apis mellifera*, in croplands is a common worldwide practice to minimise pollination deficits of pollinator-dependent crops. However, the metric of a colony, or a hive, as a pollination supplement unit is controversy due to the wide range of adult population sizes in a colony over context variations in climate, season, and beekeeping management. Although various studies proposed estimates of the adult population size that can better indicates the colony efficiency for crop pollination, the complexity of these methods appeared limiting their direct application in the field.

In this study, we tested a simple, non-invasive and robust method to estimate the adult population size per colony, based on common beekeeping handlings. This method consisted in counting the number of inter-frames covered with adult honey bees (called IFB) on a top view of the hive body. First, we investigated the nature of the relationship between IFB and the adult bee population size, simultaneously measured, and its context-dependence to climate, and hive type. We further evaluated the robustness of the method and its potential improvement related to additional IFB counted in the super chambers and from a below view of the hive body. We also analysed the simplicity of the method comparing estimates between experimented observers and naive ones.

We revealed a clear-cut logarithmic relationship between the IFB and the adult population size, covering the effects of climate and hive type. The addition of an IFB counting through a below view improved consequently the estimates of the adult population sizes, even predicted better in the absence of top view, while the estimates were less sensitive to the additional IFB counted in the super chambers. Interestingly, the IFB counted from a below view were robust to climatic variations, contrary to those counted from a top view, and were therefore better indicators of the adult population size of honey bee colonies. No difference of estimate was detected between experimented observers and naive observers, while experimented observers maintained a slighlty lower estimation error.

The IFB counting method provides a simple, non-invasive and robust indicator of the adult population size of a managed honey bee colony. This method can be viewed as a standard for routine field monitoring in the current context of crop pollination deficits and honey bee colony losses, as two examples of field-realistic applications.

Keywords: Apis mellifera, adult bee population size, crop pollination, evaluation method, field monitoring

1. Introduction

Over the last century, changes in agricultural landscapes and practices have lead to pollination deficits in pollinator-dependant crops (Kremen *et al.*, 2002; Garibaldi *et al.*, 2016; Koh *et al.*, 2016), and to a growing interest in introducing managed pollinator species in these crops (Garibaldi *et al.*, 2017). This phenomenon started at the beginning of the 20th century in USA, where pome fruit farmers initiated to rent *Apis mellifera* (called honey bee hereafter) colonies from commercial beekeepers and to introduce them in their orchards as a full agronomical input as a result of four historical circumstances (Farrar, 1931; Crane, 1999; Kellar, 2018): (*i*) the growing scientific knowledge showing that quantitative and qualitative orchard yields could benefit from cross-pollination by insects and more particularly by bees and western honey bees (Darwin, 1876; Waite, 1898), (*ii*) the rise of the pollination demand from larger and more intensified orchards, (*iii*) the fall of the wild pollinating fauna consecutive to the rise of large mono-cropped areas and to the boom in use of insecticides, and (*iv*) the fall of small beekeepers present in rural areas and of growers keeping honey bee colonies on their own farms (Cook, 1897).

Since this time, farmers introduce diverse managed insect pollinator species in entomophilous crops, e.g. bumblebee colonies (genus *Bombus*; Hymenoptera: Apidae), solitary Mason bees (genus *Osmia*; Hymenoptera: Megachilidae) (Garibaldi et al. 2017), but the honey bee stays the most used species, especially in open fields (Farrar, 1931; Parker *et al.*, 1987; Garibaldi *et al.*, 2009, 2017). The current most common pollinator management practice to minimise crop pollination deficits consists in increasing stocking rate of honey bee colonies per unit of target crop area (Isaacs *et al.*, 2017). Yet some studies showed that this practice does not necessarily lead to a reduction in pollination deficit (Degrandi-Hoffman *et al.*, 1987; Viana *et al.*, 2014; Gaines-Day and Gratton, 2016; Garratt *et al.*, 2018), even it worsens it in some specific cases (Aizen *et al.*, 2014; Sáez *et al.*, 2014; Grass *et al.*, 2018; Sáez *et al.*, 2018). Delaplane and Mayer (2000) recommended a stocking rate value of honey bee colonies per hectare per each entomophilous crop, based on the mean values found in literature, whereas Farrar (1931) already questioned largely before the relevance of the unit of honey bee colony for stocking rate, raising “the lack of a uniform standard for measuring colony efficiency”. Indeed, adult population size of honey bee colonies can vary from 10,000 to 65,000 adult bees (Farrar, 1937). Estimating adult honey bee population size is therefore a first step needed for crop pollination improvement.

To date, three methods propose estimates of the adult population size of honey bee colony. The first method consists in weighing the overall adult honey bee population during the night with counting and weighing a sample of bees to get their individual mean weight (Farrar, 1937). The total population size is then get by a cross-multiplication. This method is accurate to estimate the adult population size, but very time-consuming and challenging to applied routinely in the field due to night inspections. The second method consists in sequentially removing the frames from the hive and measuring the frame areas covered with adult bees, either directly with human eye (Burgett and Burikam, 1985; Imdorf *et al.*, 1987; Imdorf and Gerig, 2001), either through a grid (Mattila and Seeley, 2007), either through picture taking and computer-assisted image analysis (Delaplane *et al.*, 2013), either by weighing the frame with and without bees (Odoux *et al.*, 2014; Requier *et al.*, 2017). The adult bee population size is then get by multiplying it with the bee density of fully covered frames, get either by bee weighing (Burgett and Burikam, 1985; Imdorf *et al.*, 1987; Imdorf and Gerig, 2001), either by bee counting in a sample area (Mattila and Seeley, 2007). This methods is less time-consuming and less restrictive than the first one, but it is also less accurate, e.g. the bee density of a fully covered frame can vary substantially (Imdorf and Gerig, 2001; see also the different values reported in Burgett and Burikam, 1985; Mattila and Seeley, 2007; Delaplane *et al.*, 2013), and it is often practiced by day, so the adult bee population sizes assessed can vary with the daily time, season, and climate. Indeed, the bee population volume in the colony can be affected by temperature (Szabo, 1980; Seeley, 1985; Southwick and Heldmaier, 1987; Corbet *et al.*, 1993; Sumpter and Broodhead, 2000; Abou-Shaara *et al.*, 2017; Nielsen *et al.*, 2017), solar radiation (Szabo, 1980; Vicens and Bosch, 2000; Clarke and Robert, 2018), wind (Pinzauti, 1986; Vicens and Bosch, 2000), and greatly varies over the season (Odoux *et al.*, 2014; Requier *et al.*, 2017). At last, as already highlighted by Dooremalen *et al.* (2018), this method is still too invasive for the colonies. The third method consists in counting, during the day, the number of inter-frames covered with adult honey bees (IFB) on the top of the hive body frames (and on the top or the bottom of the super chamber frames), without removing the frames from the hive (Nasr *et al.*, 1990; Dooremalen *et al.*, 2018). This method is the simplest and the fastest one, already commonly used to assess the *strength* of the colonies introduced for crop pollination service (McGregor, 1976; Delaplane and Mayer, 2000), but no equivalence with the adult bee population size is yet provided. Overall, no method provides a combination of simple measure and robust estimate (e.g. including effects of climate and hive type) of the adult population size of honey bee colonies.

The overarching objective of this study was to propose a simple and robust method, based on the former studies, to assess the adult honey bee (thereafter called simply bee) population size per colony and with a particular attention to its practicability in the field. For this purpose, we adapted the IFB method (Nasr *et al.*, 1990) by making the relationship with the bee population size, measured simultaneously using the night weighing method (Farrar, 1937). Given that bees are distributed in ellipses in the hive body, with a shift upwards (Owens, 1971; see also Fig. 8.2 in Seeley, 1985, p. 113), we first tested the assumption that the number of inter-frames covered with bees (IFB) increased logarithmically with bee population size. Indeed, the observation of IFB is realised in one spatial dimension, i.e. on the length of the inter-frames, whereas the bee population grows in two dimensions, on the length and the height of the inter-frames. Secondly, as the bee population does not have the same distribution behaviour in inter-frames between the top and the bottom of the hive (Owens, 1971; Seeley, 1985), we tested the assumption that adding an IFB counting with a below view of the hive body could lower the estimation error of the bee population size than consider only the IFB counted from above. We estimated also (*i*) the reliability of this estimation, (*ii*) its robustness against the effects of climate and hive type, and (*iii*) its objectivity regarding the observer experience (experienced versus naive observers). Given that such a simple and robust estimate of adult population size of honey bee colonies can help farmers and beekeepers as an indicator of colony efficiency for crop pollination (Geslin *et al.*, 2017), and the probability of seasonal and overwintering honey bee colony survival (Requier *et al.*, 2017), we further contextualised the use of this IFB method in the current context of crop pollination and honey bee colony losses, as two examples of field-realistic applications.

2. Materials and methods

2.1. Study site, biological model, and hive type

This study was carried out during four periods, in May 2014, July and October 2015, and March 2016, on a different apiary in each period located next to the INRA centre of Avignon (France; 43°54’53.5”N, 4°52’39.2”E). A total of 181 colonies were observed using the same protocol (see below). The number of inspected colonies per apiary is given in Table A.1.

The hives inspected in March, May and July were of Dadant type, whereas those inspected in October were of Langstroth type (i.e. the two common hive types used worldwide). The instruction given to beekeepers was to provide as many varied honey bee colonies as possible regarding the bee population sizes. The bee genetic origin was a mixed of various undefined breeds. Both Dadant and Langstroth hive types were composed of a 10 frames body, as well as

a 8 or 9 frames super chamber in May and July when the colony was sufficiently populous, excepted for hives inspected in March that never received any super chamber.

2.2. The IFB method – counting the number of inter-frames covered with adult honey bees

The observation of a colony consisted in smoking a little the nest entrance with a bee-smoker, and in lifting the roof and the covering frames with a hive tool one minute after. Then the number of IFB was counted to the nearest half on the top of the hive body by the observer (Fig. 1a,b), from the method of Nasr *et al.* (1990). The inter-frames located at the two external margins of the hive each equated to a half of inter-frame, so that a maximum of 10 IFB could be counted in total. Then the top of the hive body was a little smoked if necessary and the hive body was tipped on the back side on another hive located nearby (Fig. 1c) or directly on the floor of the inspected hive. To achieve that, the ties binding the floor and the hive body were previously removed. In cases where floors were attached with screws or nails, screws were removed with an electric screwdriver, and nails with a crowbar. The IFB on the bottom of the hive body were then counted in the same way as on the top of the hive body (Fig. 1d).

The hive body was then re-attached to the floor, and the hive closed with the covering frames. In the presence of a super chamber, the same kinds of counts were achieved above and below the super chamber, with a maximum of 9 IFB counted on each size, and the two counts were averaged.

One colony observation took about 3 minutes per hive without super chamber, and about 5 minutes per hive with one super chamber, when the floors were attached with hive bodies with simple ties. In cases where floors were attached with screws or nails, this time was naturally increased.

2.3. Measure of the bee population size

We used Farrar's method (1937) to measure the bee population size. This method implied to weigh the total number of adult honey bees contained in a given colony. At the end of each of the four periods of simple colony observations, frames, super chamber, hive body and floor of each hive were shaken and brushed above a box during the night to get all of the adult bees of the colony in the box. The box was then weighed and its weight subtracted to get the weight of the bee population. When the bees were re-paid in the hive, a sample of about 100 bees was taken from the bee population, weighed, and counted to obtain the average weight per bee (Fig. A.1). By a cross-multiplication, this weight was used to convert the bee population weight into the number of bees that it contained. Bee population weights data are summarized by period in Table A.1.

Fig. 1. Dadant hive bodies with about (a) 2 and (b) 7 inter-frames covered with adult bees (IFB) counted from above. (c) Dadant hive body tipped on the back side on another hive located nearby. (d) Dadant hive body with about 6 IFB counted from below.

2.4. Relationship between IFB and bee population size

To establish the relationship between the number of IFB and the bee population size, two situations were analysed separately, (*i*) hives for which there were no bees contained in the super chamber, called 'without super', either because the colony was too small, or simply because there was no super chamber, forcing bees to restrict their distribution in the hive body, and (*ii*) hives that were equipped with a super chamber, called 'with super' (colonies observed during the periods of May and July). Colonies insufficiently large for bees to be distributed in a super chamber but nevertheless equipped with a super chamber, in May and July, were therefore in both situations. To account for the presence of bees in the super chamber, the average IFB counted from above and from below one super chamber was divided by two before being added to the IFB counted in the hive body. This because the ratio of the area of one super chamber frame to that of one hive body frame is 0.55.

Four kinds of piecewise polynomial functions were also made (Bolker, 2008): two functions with two breakpoints for hives 'without super', one breakpoint b_1 from which there were enough bees in the bee population to start to observe IFB, and one breakpoint b_2 beyond which the ten inter-frames of the hive body were saturated with bees, and two functions with just the first breakpoint b_1 for hives 'with super'. Beyond the first breakpoint b_1, two kinds of relationships, linear and logarithmic, were compared in each situation.

Let y denote the number of IFB, and let x the bee population size. To express y according to x in hives 'without super' with a linear relationship:

$$\begin{aligned} &\text{if } x < b_1,\ y = 0 \\ &\text{if } b_1 < x < b_2,\ y = 10\,x / (b_2 - b_1) - 10\,b_1 / (b_2 - b_1) + \varepsilon \\ &\text{if } x > b_2,\ y = 10 \end{aligned} \qquad (1)$$

To express y according to x in hives 'without super' with a logarithmic relationship:

$$\begin{aligned} &\text{if } x < b_1,\ y = 0 \\ &\text{if } b_1 < x < b_2,\ y = 10 \ln x / \ln (b_2 / b_1) - 10 \ln b_1 / \ln (b_2 / b_1) + \varepsilon \\ &\text{if } x > b_2,\ y = 10 \end{aligned} \qquad (2)$$

where b_1 is the bee population size from which bees started to be visible in inter-frames, b_2 is the bee population size beyond which bees saturated the ten inter-frames of the hive body, and ε is the error parameter.

To express y according to x in hives 'with super' with a linear relationship:

$$\text{if } x < b_1,\ y = 0$$
$$\text{if } x > b_1,\ y = s\,(x - b_1) + \varepsilon \quad (3)$$

To express y according to x in hives 'with super' with a logarithmic relationship:

$$\text{if } x < b_1,\ y = 0$$
$$\text{if } x > b_1,\ y = s \ln (x / b_1) + \varepsilon \quad (4)$$

where b_1 is the bee population size from which bees started to be visible in inter-frames,
s is the slope of filling inter-frames by bees,
and ε is the error parameter.

The calculations made to obtain Eqs. (1), (2), (3) and (4) are given in Appendix B. Asymptotic 95% confidence intervals of piecewise polynomial functions parameters were estimated with the package *nlstools*, version 1.0-2 (Baty *et al.*, 2015).

As simple observations were repeated per colony (see section 2.6), the IFB counted during the various observations were averaged per colony. This analysis focused on the data collected by the experienced observer (see section 2.8) and on the IFB counted on the top of the hive body (+ in the super for hives 'with super') in a first analysis, on the bottom (+ in the super for hives 'with super') in a second one, and on the average of the two (+ in the super for hives 'with super') in a third one. Coefficients of determination R^2 were calculated for each relationship by the deviance ratio, written as R^2_D (Nakagawa and Schielzeth, 2013), as well as AIC values (Akaike, 1973; Burnham and Anderson, 2002), to compare linear and logarithmic relationships.

All the statistics were computed with the software R, version 3.2.0 (R Core Team, 2015).

2.5. Reliability of estimating the bee population size from IFB

To estimate a bee population size from a number of IFB, the converses of the best supported relationships previously found (linear or logarithmic) were estimated.

The converses of Eqs. (1) and (3) are of the form:

$$x = \alpha\, y + \beta + \varepsilon \text{ , with } \alpha, \beta \in \mathrm{R} \text{ and } \varepsilon \sim N(0,\sigma^2) \quad (5)$$

The converses of Eqs. (2) and (4) are of the form:

$x = \exp(\alpha y + \beta + \varepsilon)$, with $\alpha, \beta \in \mathrm{R}$ and $\varepsilon \sim N(0,\sigma^2)$

which can be also written as:

$$x = \exp(\alpha y + \beta) \,.\, \eta, \text{ with } \alpha, \beta \in \mathrm{R} \text{ and } \eta \sim \text{Log-}N(0,\exp(\sigma^2)) \quad (6)$$

In the case where the best supported relationships previously found were logarithmic, the dependant variable x of the converse relationship was transformed in logarithm to linearize Eq. (6) and enable the estimation of the α and β parameters with a linear model.

Six kinds of explanatory variables y were independently investigated: the IFB counted on the top of the hive body, on the bottom, the average of the both, and these three variables by adding the mean number of IFB on the top and the bottom of the super chamber divided by two when a super chamber was present and contained bees. In these three last cases, two converses were estimated: as before, a first one with hives for which there were no bees contained in the super chamber, called 'without super' (see section 2.4), and a second one with hives that were equipped with a super chamber, called 'with super' (colonies observed during the periods of May and July). Colonies insufficiently large for bees to be distributed in a super chamber but nevertheless equipped with a super chamber, in May and July, were therefore in both situations.

To compare the reliability of the six different explanatory variables investigated, some statistics were estimated for each converse relationship. As the residual error is constant in the linear Eq. (5) while it depends on the expected value in the exponential Eq. (6), these statistics were estimated differently between the two kinds of relationships.

In the case of the relationships of type Eq. (5), the three estimated statistics were: (*i*) the standard deviation σ of the residual error ε, (*ii*) the 97.5% quantile of the residual error ε distribution, called $Q_{97.5\%}$ and calculated by the product $t_{\gamma=97.5\%}^{k=n-1}.\sigma$, that express the absolute margin of error of estimating a bee population size from a number of IFB with a probability of 95%, and (*iii*) the minimum number of observations required to estimate the mean bee population size of a given apiary with a 95% confidence interval included in a given margin of error of 10 or 20%, called $N_{min\text{-}N}$ and calculated as follows:

$$N_{min\text{-}N}(x) = \left(t_{\gamma=97.5\%}^{k=n-1} \, \sigma \,/\, (\mu_{obs} \, M_e \,/\, 100) \right)^2 \quad (7)$$

where $t_{\gamma}^{k=n-1}$ is the quantile of γ order of the Student distribution with k degrees of freedom,
μ_{obs} is the mean of the bee population sizes observed,
and M_e is the given margin of error in % (usually 10 or 20%).

In the case of the relationships of type Eq. (6), the four estimated statistics were: (*i*) the standard deviation of the residual error η relative to the expected value, called $RSD\ (x)$, (*ii*) the 2.5% and 97.5% quantiles of the residual error η distribution relative to the expected value, called $RQ_{2.5\%}$ and $RQ_{97.5\%}$, that express the asymmetric relative margin of error of estimating a bee population size from a number of IFB with a probability of 95%, and (*iii*) the minimum number of observations required to estimate the mean bee population size of a given apiary with a 95% confidence interval included in a given margin of error of 10 or 20%, called $N_{min\text{-}LogN}$. These four statistics were calculated as follows:

$$RSD\ (x) = 100\ (\exp \sigma^2 - 1)^{1/2} \quad (8)$$

$$RQ_{2.5\%}\ (x) = 100 \exp t_{\gamma=2.5\%}^{k=n-1}\ (\exp \sigma^2 - 1)^{1/2} \quad (9)$$

$$RQ_{97.5\%}\ (x) = 100 \exp t_{\gamma=97.5\%}^{k=n-1}\ (\exp \sigma^2 - 1)^{1/2} \quad (10)$$

$$N_{min\text{-}LogN}\ (x) = (100 / 2\ M_e)^2\ (\exp t_{\gamma=97.5\%}^{k=n-1} + \exp t_{\gamma=2.5\%}^{k=n-1})^2\ 100^2\ (\exp \sigma^2 - 1) \quad (11)$$

where σ^2 is the variance of the residual error ε,
$t_{\gamma}^{k=n-1}$ is the quantile of γ order of the Student distribution with k degrees of freedom,
and M_e is the given margin of error in % (usually 10 or 20%).
Developments to get these equations are given in Appendix C.

2.6. Assessment of robustness – climate effect

Ambient instant temperature was recorded every 5 minutes throughout the overall duration of observations by a sensor HOBO® Pro v2 (Onset® Computer Corporation, USA) placed under shelter near the apiaries. Instant light intensity was recorded every minute in lux during the same period and at the same place by a sensor HOBO® Pendant (Onset® Computer Corporation, USA) placed horizontally in broad daylight to the South. Average wind speed was recorded from beginning to end of colony observations, for each half-day observation, by an anemometer SKYWATCH® Eole (JDC Electronic SA, Switzerland) placed two meters high, at the end of a telescopic tripod near the apiaries. These climatic data are summarized by period in Table A.1. Simple colony observations were repeated between four and seven times per colony, each time on a different but consecutive day (see Table A.1 for dates), to enable to test the climatic

variations effect on IFB on a given colony. Repeated colony observations alternated between morning and afternoon in a given period.

To test if climatic variations impacted the number of IFB, IFB counted from above and from below the hive body were modelled by a generalized linear mixed model (GLMM) approach using a binomial distribution. The fixed explanatory variables were, in order, the bee population size in interaction with the period to test if the occupancy rate of inter-frames by bees changed according to the period or the hive format (the ratio of the area of a Langstroth frame to that of a Dadant one is 0.79), ambient instant temperature (in °C), relative light intensity averaged on the 60 minutes preceding observation (in %), average wind speed of the half-day observation (in $km.h^{-1}$), the temporal shift between observation and bee population weighing (in days) in interaction with the period to take into account the potential evolution of the bee population size during this time, and so the period. Relative light intensity corresponds to instant light intensity divided by the maximum instant light intensity recorded during one given period. This helped to overcome strong differences in light intensity between periods. As colony observations were repeated by colony, the colony number was set as the random explanatory variable. Four GLMMs were generated to test the impact of climate on the IFB counted from above and from below the hive body by both experienced and naive observers. GLMMs were generated with package *lme4*, version 1.1-14 (Bates *et al.*, 2015b).

To check for collinearity between fixed explanatory quantitative variables, two variables were incorporated together in GLMMs only if absolute value of correlation coefficient was less than 0.7 (Dormann *et al.*, 2013). Fig. A.2 displaying correlation coefficients values was generated with package *Rarity*, version 1.3-6 (Leroy, 2016). To standardize effects sizes, fixed explanatory variables were centered and standardized (Schielzeth, 2010).

As the null hypothesis significance testing approach is discussed (e.g. Stephens *et al.*, 2007; Stanton-Geddes *et al.*, 2014; McShane *et al.*, 2017), we set the *p* value threshold to 0.001 in order to be more conservative (Johnson, 2013). Effect size and 95% confidence intervals were also reported (Nakagawa and Cuthill, 2007). 95% confidence intervals were estimated with Wald method (Bates *et al.*, 2015a). Marginal R^2 values were also calculated, written as $R^2_{GLMM(m)}$ (Nakagawa and Schielzeth, 2013).

2.7. Assessment of robustness – effect of hive type

To test whether the hive type affected the occupancy rate of inter-frames by bees regarding the bee population size and therefore the parameter values of the relationship between IFB and bee population size, the colony observations and bee population weighing were made on two

hive types, Dadant and Langstroth. Dadant and Langstroth frames have indeed different areas that can affect the occupancy rate: the ratio of the area of a Langstroth frame to that of a Dadant one is 0.79, so a Langstroth should fill up in bees 1 / 0.79 = 1.3 times faster with bee population size than a Dadant frame. To compare the occupancy of inter-frames by bees regarding the bee population size between the Dadant and Langstroth types, the relationship between the number of IFB and the bee population size found for the Langstroth type was divided by the one found for the Dadant type on the range of bee population sizes sampled in common for both types (see Table A.1). The Dadant type was investigated during the periods of Mars, May and July, while the Langstroth format was investigated in October. The period of October was therefore mixed up with the hive type.

2.8. Assessment of objectivity – experienced versus naive observers

To test if estimating a bee population size from IFB was objective and if the robustness regarding the climatic variations was an objective pattern, the colony observations were performed each time by two kinds of observer: someone who had already experience in beekeeping or had already applied this method, called experienced observer, and a naive one in both. Both types of observers counted independently. The statistics presented in section 2.5 were compared between the two kinds of observer to assess if the estimation error made by the naïve observer was similar or not to that of the experienced observer.

2.9. Application for ecological issues of colony loss and crop pollination

We used the BEEHAVE model (Becher *et al.*, 2014) to illustrate the interest of the inter-frame estimate as an ecological indicator of colony survival and crop pollination performance. A total of 200 simulations of honey bee colony dynamics were computed during a complete year, with a Dadant hive type and *ad libitum* addition of super chambers. We first calibrated the model with Becher *et al.* (2014) initial colony settings, i.e. European conditions of climate and landscape composition, associated with a random parameterisation of the initial population size for model stochasticity improvement. To do so, we randomly attributed an initial adult population size ranging from 9,000 to 13,000 bees at January 1st. In order to consider beekeeping management conditions, we also enabled the *ad-hoc* options such as the *Varroa destructor* mites treatment and the honey harvests.

Then, we distinguished the simulations in two lots, the healthy colonies (n = 100 simulations) and the disturbed colonies (n = 100 simulations), by a change in demographic and health parameters on the first day of simulation (and then applied during the complete run). Healthy colonies consisted in simulations with random-boost of colony demographic rate and

individual survivorship, and the absence of *Varroa destructor* mite infestation. The boost of the colony demographic rate was computed with an increase of the maximal egg-laying capacity of the queen between 1,600 and 1,800 eggs per day (default value of 1,600 eggs per day). The boost of the survivorship of bees was computed with a decrease of the probability of worker larvae mortality between $3/10^3$ and $1/10^2$ per day of larvae development (default value of $1/10^2$ per day of larvae development), and with a decrease of the probability of forager mortality between $7/10^6$ and $1/10^5$ per second of flight (default value of $1/10^5$ per second of flight). The disturbed colonies consisted in simulations with random-weakening of colony demographic rate (between 1,400 and 1,600 eggs per day) and individual survivorship (between $1/10^2$ and $3/10^2$ per day of larvae development and between $1/10^5$ and $3/10^5$ per second of flight), and an infestation with 2 to 10 *Varroa destructor* mites/100 bees from which the prevalence virus-infected mites range from 30 to 50%.

The colony dynamic of the total number of bees was then converted into a number of IFB inferred with Eq. (4), while the simulations included super chambers and the below view turns out to be a more robust method (see results). The BEEHAVE model estimates the number of forager bees (an indicator of crop pollination performance) that was also transformed into a number of IFB. Simulation endpoints that were deemed insufficient for colony survival, i.e. the risk of colony collapse, was estimated through two following thresholds according to Becher *et al.* (2014): (*i*) simulations that reach population size smaller than 4,000 adult bees during the winter, and (*ii*) simulations that reach a null amount of honey stock during the winter season.

3. Results

A total of 181 colonies were observed during the four periods, with bee population sizes varying from 2,893 to 53,546 adult bees, and a smaller variability in October (Table A.1). As colonies had time to grow during the temporal shift between the first colony observations and the bee population weighing in the period of March, the observations made with a temporal shift strictly greater than four days were removed in the analyses of the sections 3.1 and 3.2 (see section 3.3).

3.1. Relationship between IFB and bee population size

The logarithmic relationships between number of IFB and bee population size were systematically better supported than the linear ones by R^2_D and AIC values (all ΔAIC values > 9) in the Dadant hives observed by the experienced observer, regardless of the observation considered, IFB counted from above the body hive, from below, the both averaged, or when

considering the bees contained in the super chamber (Table A.2; Fig. 2). Conversely, the linear relationships were better supported, but with much less evidence, than the logarithmic ones (all ΔAIC values < 2) in the Langstroth hives (Table A.2).

Bees started to be visible with a smaller bee population size by observing the Dadant hive body from above than observing from below, according to the logarithmic relationships in hives with or without super chamber (Table A.2). The ten inter-frames were however saturated with the same bee population size on the top or the bottom of the hive body in Dadant hives without super chamber. The occupancy rate of inter-frames by bees with bee population size was also higher when counting the IFB from below the hive body (plus in the super chamber) than counting from above (plus in the super chamber) in Dadant hives with a super chamber.

The occupancy rate of inter-frames by bees regarding the bee population size was nearly the same in Langstroth and Dadant hives when counting the IFB from above the hive body on the range of bee population sizes included between 6,000 and 15,000 bees (Figs. 2a and A.2a). This rate was, however, between 2.1 and 1.2 times higher in Langstroth hives compared to Dadant hives when counting the IFB from below the hive body, with a ratio approaching 1.3 between bee population sizes of 8,000 and 12,000 bees (Figs. 2b and A.2b).

In Dadant hives with or without super chamber, the models with the best R^2_D values were those where the IFB considered were those counted only from below the hive body (Table A.2). In Langstroth hives, the models with the best R^2_D values were however those where the IFB counted from above and below the hive body were averaged.

3.2. Reliability of estimating the bee population size from IFB

Only the converses of the best supported kinds of relationships found in section 3.1 were investigated in the following analyses, i.e. the exponential relationship for Dadant hives, and the linear relationship for Langstroth hives.

When disregarding the bees contained in the super chamber, the kind of observation with the smallest *RSD*, *RQs* or $N_{min\text{-}LogN}$ values in Dadant hives observed by the experienced observer was the one with the IFB considered from below only (Table A.3). Considering the averaged numbers of IFB counted from above and below increased the estimation error. And considering only the IFB counted from above increased even more strongly this error. When considering bees contained in the super chamber, still in Dadant hives observed by the experienced observer, the same pattern was observed. There was in that case a little reduction of the error of estimation compared to when bees contained in the super chamber were disregarded, considering the IFB counted from above only or from below only (Table A.3).

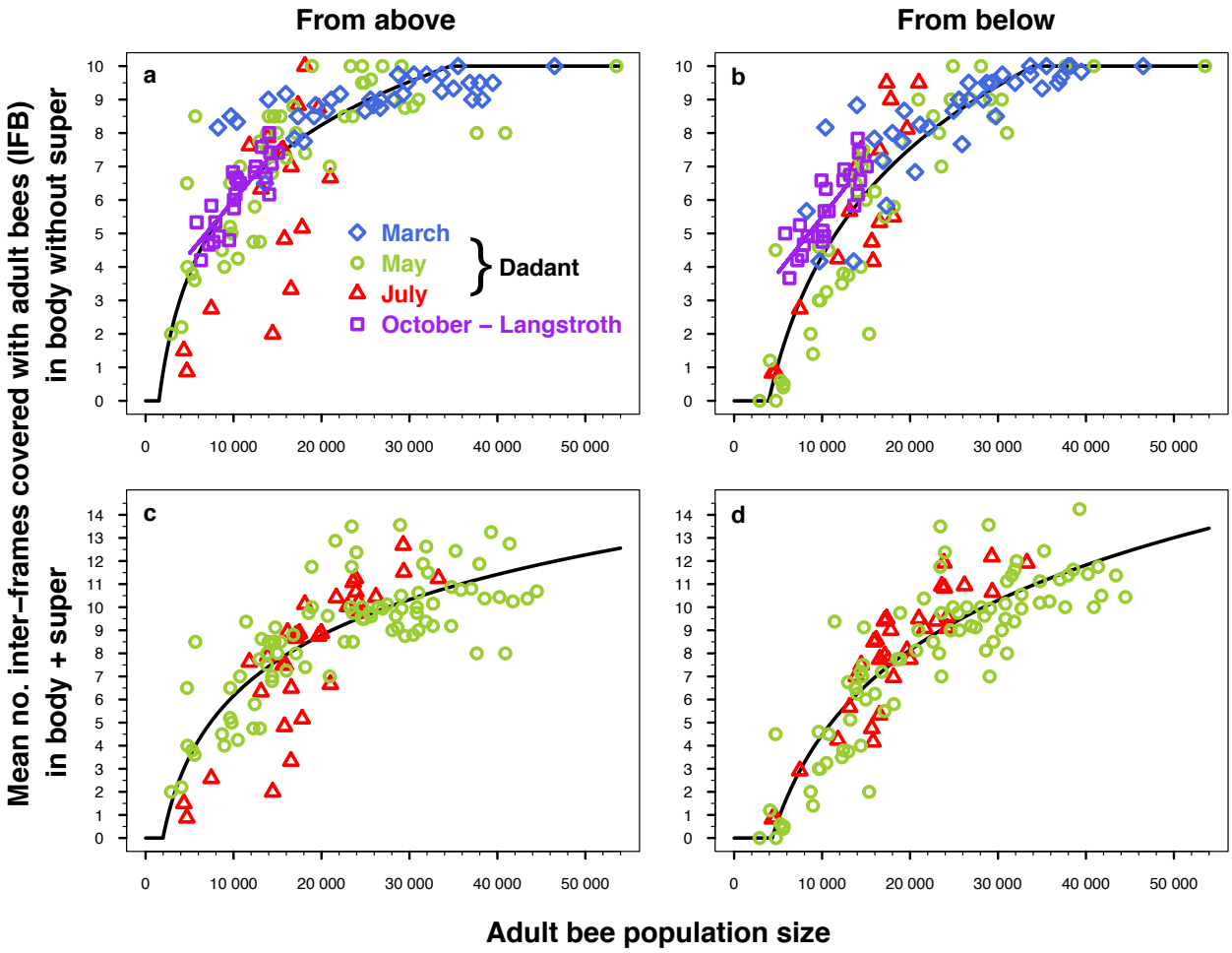


Fig. 2. Relationships between the mean number of inter-frames covered with adult bees (IFB) counted by the experienced observer and the bee population size, given for (a, b) colonies without super chamber or without adult bees contained in the super chamber when present, and for (c, d) colonies equipped with a super chamber. The IFB were counted from (a, c) above or from (b, d) below the hive body. Solid lines represent the mean predictions of piecewise polynomial functions (Table A.2; see Eqs. (2) and (4) for black lines, and Eq. (1) for purple line). Dadant and Langstroth are the two hive formats tested.

The same pattern as before was observed for Dadant hives inspected by the naive observer, with higher *RSD*, *RQs* or $N_{min\text{-}LogN}$ values compared to when colonies were observed by the experienced observer (Table A.3).

As for Dadant hives, the kind of observation with the smallest σ, $Q_{97.5\%}$ or $N_{min\text{-}N}$ values in Langstroth hives observed by the experienced observer was the one with the IFB considered from below only (Table A.4). And still as for Dadant hives, considering the averaged numbers of IFB counted from above and below increased the estimation error, and considering only the IFB counted from above increased even more strongly this error. On the other hand, in Langstroth hives observed by the naive observer, σ, $Q_{97.5\%}$ or $N_{min\text{-}N}$ values were the smallest when the IFB counted from above and below were considered both and averaged. The estimation error was slightly increased when the IFB was considered only from below, and it was even more increased when the IFB was considered only from above (Table A.4). Finally, σ, $Q_{97.5\%}$ or $N_{min\text{-}N}$ values were higher for the Langstroth hives observed by the naive observer than for those observed by the experienced observer (Table A.4).

3.3. Assessment of robustness

We were able to cover a relatively large array of climatic variations, from 5.1 to 35.6°C for temperature, from 257 to 211,812 Lux for light intensity, and from 0 to 11.5 $km.h^{-1}$ for wind speed.

All the fixed explanatory quantitative variables investigated could be integrated together in models because of values of correlation coefficients less than 0.7 (Fig. A.3). The number of IFB counted from above and below the hive body was dependant in the first place on the bee population size, during the four periods and for the two kinds of observer (Table 1 and Fig. A.4). Colony observation from below was however more robust to climate than from above.
The IFB counted from above increased with temperature, and decreased with relative light intensity for the two kinds of observer, with a more markedly slope for temperature, while the IFB counted from below did not (Table 1). The IFB counted from above increased moreover with the temporal shift between observation and bee population weighing during the period of July in the two kinds of observer, as well as the IFB counted from below during the period of March (Fig. A.4). The marginal $R^2_{GLMM(m)}$ values were higher in models with the IFB counted from below than from above, for the two kinds of observer (Table 1).

Table 5 Statistic values of the GLMMs computed to test the climate effect on the number of inter-frames covered with adult bees counted from above and below the hive body by the two kinds of observer

View	Predictor	Modality	Experienced observer			Naive observer		
			Estimated parameter (± 95% CI)	z statistic	*p*	Estimated parameter (± 95% CI)	z statistic	*p*
Above	Intercept	March	2.266 (± 0.322)	13.80	< 0.001	2.277 (± 0.290)	15.50	< 0.001
	Bee population size		0.708 (± 0.307)	4.52	< 0.001	0.795 (± 0.281)	5.59	< 0.001
	Temperature		0.547 (± 0.134)	8.00	< 0.001	0.535 (± 0.136)	7.74	< 0.001
	Relative light intensity		-0.213 (± 0.077)	-5.41	< 0.001	-0.204 (± 0.076)	-5.27	< 0.001
	Average wind speed		-0.041 (± 0.083)	-0.96	0.335	-0.078 (± 0.081)	-1.89	0.0588
	Temporal shift		-0.065 (± 0.162)	-0.79	0.432	-0.144 (± 0.168)	-1.69	0.0919
	Period	May	-1.021 (± 0.522)	-3.83	< 0.001	-1.457 (± 0.470)	-6.08	< 0.001
		July	-2.255 (± 0.675)	-6.55	< 0.001	-2.503 (± 0.628)	-7.79	< 0.001
		October	-0.577 (± 1.223)	-0.93	0.355	-0.483 (± 1.050)	-0.89	0.372
	Bee population size × Period	May	0.369 (± 0.377)	1.92	0.0555	0.076 (± 0.340)	0.44	0.660
		July	1.033 (± 0.590)	3.43	< 0.001	1.188 (± 0.510)	4.34	< 0.001
		October	0.759 (± 1.238)	1.20	0.230	0.645 (± 1.087)	1.17	0.241
	Temporal shift × Period	May	0.346 (± 0.379)	1.79	0.0733	0.719 (± 0.369)	3.81	< 0.001
		July	1.346 (± 0.783)	3.37	< 0.001	1.550 (± 0.767)	3.94	< 0.001
		October	-0.029 (± 0.214)	-0.27	0.789	0.198 (± 0.220)	1.76	0.0781
			$R^2_{GLMM(m)} = 0.250$			$R^2_{GLMM(m)} = 0.260$		
Below	Intercept	March	1.599 (± 0.252)	12.45	< 0.001	1.702 (± 0.276)	12.19	< 0.001
	Bee population size		1.307 (± 0.252)	10.17	< 0.001	1.363 (± 0.284)	9.51	< 0.001
	Temperature		0.114 (± 0.128)	1.74	0.0821	0.105 (± 0.131)	1.57	0.117
	Relative light intensity		-0.057 (± 0.074)	-1.50	0.134	-0.077 (± 0.074)	-2.03	0.0421
	Average wind speed		0.069 (± 0.080)	1.68	0.0928	0.088 (± 0.081)	2.12	0.0342
	Temporal shift		0.327 (± 0.150)	4.27	< 0.001	0.254 (± 0.155)	3.21	0.00134
	Period	May	-0.470 (± 0.473)	-1.95	0.0513	-0.703 (± 0.489)	-2.82	0.00476
		July	-0.236 (± 0.597)	-0.78	0.439	-0.906 (± 0.595)	-2.98	0.00289
		October	0.049 (± 0.943)	0.10	0.918	-0.286 (± 1.018)	-0.55	0.583
	Bee population size × Period	May	0.829 (± 0.344)	4.72	< 0.001	0.618 (± 0.373)	3.29	0.00101
		July	0.303 (± 0.476)	1.25	0.212	0.289 (± 0.495)	1.09	0.274
		October	0.166 (± 0.950)	0.34	0.731	0.004 (± 1.060)	0.01	0.993

Temporal shift × Period	May	-0.296 (± 0.365)	-1.59	0.112	-0.104 (± 0.374)	-0.54	0.587
	July	-0.674 (± 0.733)	-1.80	0.0715	-0.130 (± 0.715)	-0.35	0.723
	October	-0.380 (± 0.201)	-3.71	< 0.001	-0.250 (± 0.204)	-2.40	0.0166
		$R^2_{GLMM(m)}$ = 0.449			$R^2_{GLMM(m)}$ = 0.448		

All of the quantitative fixed explanatory variables were centered and standardized. $R^2_{GLMM(m)}$ is the marginal R^2 value.

According to these results, the observations made with a temporal shift between observation and bee population weighing strictly greater than four days during the period of March were removed in the analyses of the previous sections 3.1 and 3.2, to free themselves from an unwanted effect observed on the IFB counted from below. No observations were removed for the period of July, despite a similar effect on the IFB counted from above, because the temporal shifts between observation and bee population weighing were all less than four days.

3.4. Application for ecological issues of colony loss and crop pollination

The performed simulations showed the capability of the inter-frame estimate to discriminate the population dynamic of healthy *vs.* disturbed honey bee colonies (Fig. 3a). While healthy and disturbed colonies initiated the simulations with the same bee population size of 4.90 ± 0.52 (mean ± sd) inter-frames (representing 10,920 ± 1,058 adult bees), these two lots of simulations (n = 100 for each) fitted different temporal patterns. The healthy colonies increased the population size during the year, reaching to a bee population size of 10.44 ± 0.79 inter-frames (31,294 ± 4,611 adult bees) at the peak period of colony growth (from July 15th to September 15th), an finishing the year with an annual increase of 0.47 ± 1.26 inter-frames (1,235 ± 2,811 adult bees). On the other hand, the disturbed colonies showed a weakened temporal pattern with 3.18 ± 2.74 inter-frames (9,138 ± 5,183 adult bees) at the peak period of colony growth, and an annual decrease of 4.62 ± 0.87 inter-frames (16,834 ± 6,971 adult bees). Interestingly, the BEEHAVE model allows to estimate the forager force of the honey bee colony at a given date. Beside the estimation of the bee population size, the inter-frame estimate could inform on the forager force of the honey bee colonies, this later varying between healthy colonies (10,878 ± 2,650 forager bees) and disturbed colonies (2,163 ± 1,381 forager bees) at the peak period of colony growth (Fig. 3b). While the simulated healthy colonies showed 100% of survival over the year instead of 27% for the disturbed colonies (Fig. 3c), the inter-frame estimates measured at the peak period of colony growth was positively correlated to the survival of the colonies (GLM with a binomial error distribution, n = 200, z = 3.364, p = 0.00073). Thus, the measure of the number of inter-frames at a given date can be viewed as an ecological indicator the colony survival probability.

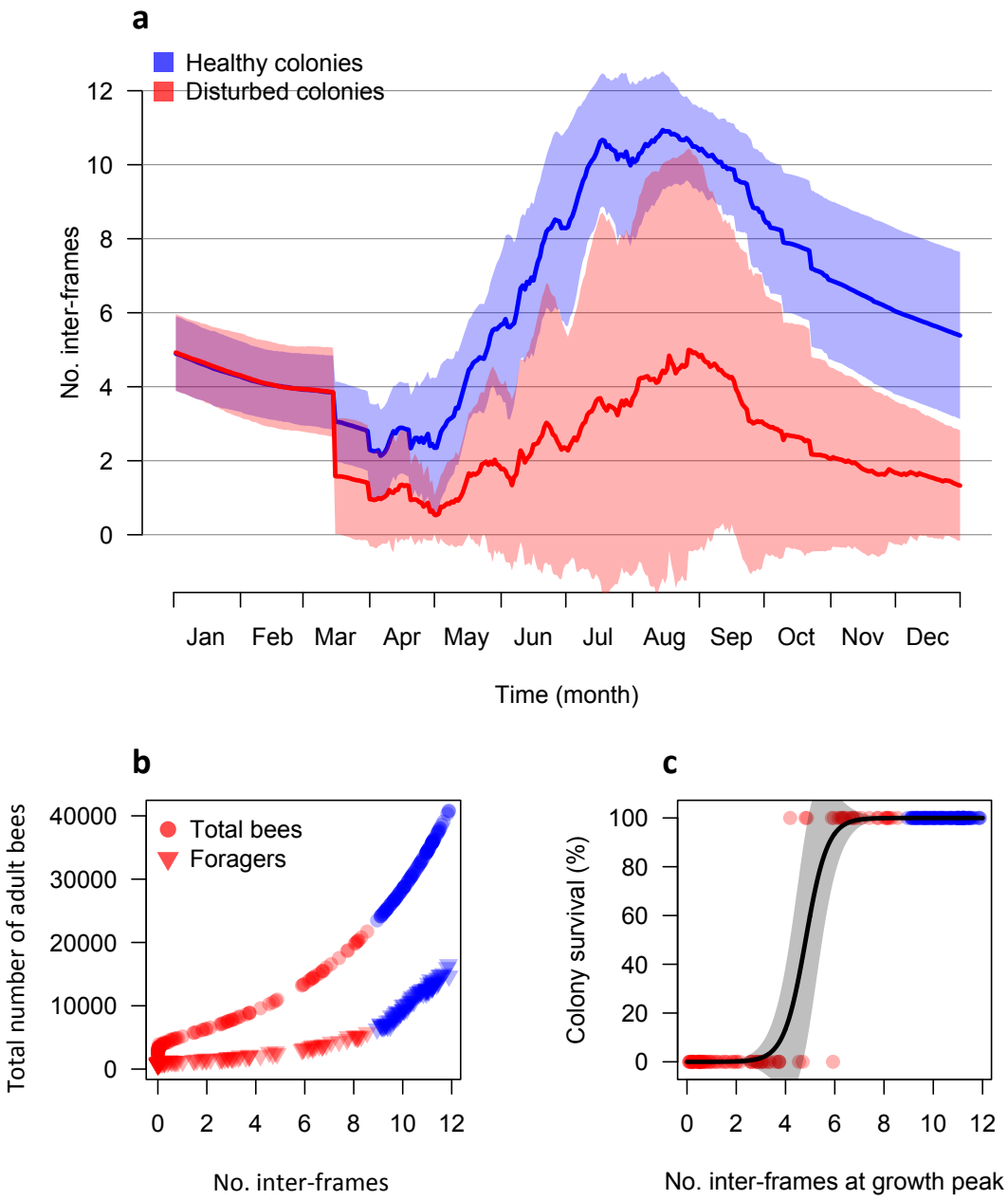


Fig. 3. Integrating the inter-frame estimate in ecological issues of honey bee colony survival and crop pollination. (a) The simulated yearly population size dynamic of healthy and disturbed honey bee colonies expressed as number of inter-frames covered with adult bees (IFB) inferred with a below view (Eq. (4)) and a Dadant hive type. Thick lines show the average value of the simulations (n = 100 healthy colonies and n = 100 disturbed colonies) at day *d* with shaded areas indicating the 95 % confidence interval. (b) The number of inter-frames indicates the adult bee population size (i.e. the total number of adult bees) and the foraging force of the colony (i.e. the number of forager bees). We show the averaged value per colony at the peak period of colony growth (from July 15th to September 15th). (c) The number of inter-frames at the peak period of colony growth increases survival of simulated bee colonies (GLM with Binomial error distribution: $p < 0.001$). Line represents model fit while polygons show 95 % confidence intervals.

4. Discussion

We revealed a clear-cut logarithmic relationship between the IFB and the adult population size, covering the effects of climate and hive type, and whatever the presence or not of a super chamber. This result is consistent with our assumption: the more the colony and the bee population grow, the more the bees cover the entire surface of the frames they occupy, and the less they occupy new inter-frames. However, the best supported relationship in Langstroth hives was the linear one but with little evidence compared to the logarithmic relationship. This can be easily explained by the fact that the colonies inspected to test this hive format were inspected in October, so with too small bee population sizes, ranging from 6,000 to 15,000 bees.

The estimates of adult population size of honey bee, derived from the IFB method, are consistent with previous data. The inspected colonies were of very different sizes during the three periods March, May and July, from near 3,000 workers for the smallest one, to near 53,500 bees for the biggest one. These values are consistent with the extrema described in the Schmickl and Crailsheim's honey bee population dynamics model (2007), that were of about 5,500 and 50,000 bees. They are nearly consistent too with the higher values described in Farrar's study (1937), that were of about 10,000 and 65,000 bees. The proposed method allows to count some IFB in 10 frames Dadant hive body from near 1,500 bees contained in the colony when counting from above, and from near 4,000 bees when counting from below. Bees occupied the bottom of inter-frames of 10 frames Dadant hive body as bee population size grew faster than the top, leading to a saturation of the top and the bottom of inter-frames around the same bee population size of 34,000 bees without super chamber. This is in agreement with Owens (1971) who found that bees distributed in the hive with a shift upwards using the measure of isothermal curves.

The occupancy of inter-frames by bees regarding the bee population size seemed nearly the same in Langstroth and Dadant hives when counting the IFB from above the hive body on the range of bee population sizes encountered in Langstroth type, while it was more variable when counting the IFB from below the hive body, between 2.1 and 1.2 times higher in Langstroth hives compared to Dadant hives. This ratio seemed to approach the 1.3 value between bee population sizes of 8,000 and 12,000 bees, corresponding to the ratio of the Dadant frame area to the Langstroth frame one. The study should be nevertheless extended beyond the range of population sizes tested in Langstroth format to be able to infer a generality of these ratio values.

Moreover, the occupation of inter-frames by bees as bee population grew was completely robust to climate on the bottom of the hive body, whereas it largely depended on temperature

and relative light intensity on the top, whether the kind of observer. Indeed, the higher the temperature was, more the bees dispersed in the inter-frames on the top of the hive body. This is in agreement with the dispersion of the bee population when temperature rises above 15°-18°C (Seeley, 1985; Southwick and Heldmaier, 1987; Sumpter and Broodhead, 2000). The effect of the decreasing in bees contained in the nest by the increase of the honey bee foraging activity with temperature (Szabo, 1980; Corbet *et al.*, 1993; review in Abou-Shaara *et al.*, 2017; Nielsen *et al.*, 2017) is therefore negligible in front of the effect of the bee population dispersion. To a lesser extent, the higher the relative light intensity was, the fewer bees were observed in the inter-frames on the top of the hive body. This is in agreement with the increase of the honey bee foraging activity when solar radiation rises above 300 $W.m^{-2}$ (Vicens and Bosch, 2000; Clarke and Robert, 2018). Although honey bee foraging activity decreases sharply beyond 10 $km.h^{-1}$ of wind speed (Pinzauti, 1986; Vicens and Bosch, 2000), the wind speeds measured during the colony observations were relatively small, this not permitting to conclude to a wind speed effect on the number of IFB counted from above or below the hive body.

Interestingly, the assessment of the robustness of the method showed that using a below view improves the estimate of bee population size from a number IFB, in comparation to using a top view, nor using both observations. Considering the number of IFB in the super chamber when present allows to improve a little more this estimation. The most relevant and the most effective method consists therefore in choosing either to count, ideally, the IFB from the single bottom of the hive body when it is possible and by adding the possible counting ones in the super chamber when present, either to count those from the single top of the hive body when counting from the bottom is too challenging (e.g. when the floor is attached to the hive body with screws or nails), with adding also the possible counting IFB in the super chamber when present.

This estimation was quite objective, as the estimation error was similar between an experimented observer and a naive one. The estimation error was nevertheless slightly lower for the experienced observer than for the naive one. The study should be continued for the Langstroth hive format and extended beyond the range of population sizes encountered. Moreover, as the naive observer counted the IFB in a little more variable manner than the experienced one, it is recommended for a naive observer to practice the method a little before using it in a real manner.

The reliability estimates in Langstroth hive type gave the same results, with the slight difference that the estimation was better when averaging the IFB counted from above and below the hive body for the observations of the naive observer. But the estimation errors between the

IFB counted from above and below averaged and the IFB counted from below only were quite similar. This therefore does not really call into question the previous conclusions. However, a conversion is necessary to cross estimate the bee population size from the counting of IFB through Dadant and Langstroth hive types. As bees occupied super chamber when present only when they occupied at least six inter-frames on the top or on the bottom of the hive body, the physical coercion (i.e. the spatial limit) of the hive body of limited volume should apply on the relationship between IFB and bee population size only from six inter-frames occupied on the top or the bottom of the hive body. This is therefore solely from this threshold of IFB that the relationship between the number of IFB and bee population size should be different between hives equipped with super chamber and hives non equipped with. This is the reason why the conversion between the number of IFB and bee population size in hives without super chamber is given only from six IFB. Below this threshold, one can refer to the conversion given for hives equipped with a super chamber. It is also worth to note that the estimated bee population size given for hives containing more than 13 IFB counted from below the hive body, or 12.5 from above, are quite approximate, as the relationship is exponential, and as a colony of more than 45,000 bees is quite exceptional. The maximum estimated bee population size proposed is 65,000 bees, as it is the maximum bee population size observed by Farrar (1937).

This method may help to better manage crop pollination service by introducing the appropriate amount of adult honey bees in a given crop area to minimise pollination deficits (Garibaldi *et al.*, 2016), in complementarity with the wild pollinators still present in the environment of the target crop, as recommended by the Integrated Crop Pollination concept (Isaacs *et al.*, 2017). Indeed, Geslin *et al.* (2017) showed that honey bee colonies with higher number of frames covered with bees on the top of the hive body increased apple flower-visitation rates by honey bees, and subsequently fruit set, seed set, fruit sugar content and farmers' profits. This would lead to redefine the currently used unit of managed honey bee colonies introduced per unit of target crop area for stocking rate into the direct required number of adult honey bees per crop area. This would enable beekeepers to manage their beekeeping at their best, for instance in introducing more small colonies to make them grow during the crop flowering.

As a perspective, it would be relevant to investigate the forager population of a honey bee colony according to its size. Indeed, the adult population size includes various casts of bees that provide different work tasks from in-nest work (e.g. nest cleaning, brood rearing) to outside tasks such as the flight learning and patrol flights, and foraging flight that are the most probable indicator of colony efficiency for crop pollination issues. Using bee colony simulator, such as

the BEEHAVE model (Becher *et al.*, 2014), it is now possible to predict the number of forager bees in the adult population size of honey bee colonies, and therefore go further in the precision of colony efficiency for pollination service. Moreover, combining the IFB method with such simulations provides a tool for beekeepers to anticipate, and mitigate colony mortality, a current issue worldwide (Potts *et al.*, 2010; Goulson *et al.*, 2015). It is well-established that the adult population size of honey bee colonies indicates the health status of a colony, and also can be used as an early-warning signal of the probability of seasonal and overwintering honey bee colony mortality (Requier *et al.*, 2017). Thus, the IFB counting method provides a simple and robust indicator of the adult population size of a managed honey bee colony with perspectives of field-realistic applications in the current context of crop pollination deficit and honey bee colony losses.

5. Conclusion

Counting the number of IFB constitutes a simple, fast, non-invasive and quite robust method to assess routinely the adult population size of a honey bee colony in the field. This method can be viewed as a standard for routine field monitoring in the current context of crop pollination deficit and honey bee colony losses, as two examples of field-realistic applications. It is recommended to favour the IFB counted from a below view the hive body, when it is possible, against those counted from above, and to add the IFB counted in the super chamber when it is present. It is also recommended for a naive observer to practice the method a little before using it in a real manner, in order to reduce the risk of the estimation error. The number of managed adult honey bees introduced per unit of target crop area should therefore be used as a more relevant variable than the mere stocking rate of honey bee colonies. This unit will enable to better coincide the overall supply of insect pollinators, including managed and wild insect pollinators, with the pollination requirements of a given target entomophilous crop.

Author contributions

B.E.V., S.C., and F.R. conceived the idea and designed the methodology, S.C., L.G., and B.E.V. collected the data, S.C., J.C., and F.R. analysed the data, F.R. performed the simulations, S.C., F.R., and B.E.V. wrote the manuscript, all authors gave final approval for publication.

Funding

This work was supported by the European Agricultural Guarantee Fund (EAGF) as part of

the French National Apiculture Programme [FranceAgriMer convention number 14-05 R], and by the Association Nationale de la Recherche et de la Technologie (ANRT) as part of the Conventions Industrielles de Formation par la REcherche (CIFRE) doctoral grants [convention number 2014/0070], allocated here to S.C. by the Association Nationale des Agriculteurs Multiplicateurs de Semences Olégineuses (ANAMSO) through the specific interprofessional actions with the Groupement National Interprofessionnel des Semences et plants (GNIS) and the Union Française des Semenciers (UFS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank all the beekeepers for providing us with the colonies to carry out this study or preliminary works, Simon Bellot, Raymond Bernadet, Philippe Dauzet, Cyril Folton, Alexandre Gorit, Eric Lelong, Sonia Martaresche, John Rigon, Jacques Sénéchal, Bertrand Stiers and Paul Thirion. We are also grateful to Cécilia Alin, Cécile Antoine, Jérémy Bellanger, Arthur Cambert, Elisa De Santis, Julie Dietrich, Alexandra Drouet, Louna Fronteau, Olivier Geist, Tommy Gerez, Coralie Guerry, Anne Laure Guirao, Zhor Lebbar, Pierre Le Bivic, Estienne Liegeois, Solène Marion, Nicolas Nalpowik, Cyril Scomparin, Julie Valognes and Marine Willemin who helped in field work. We thank Jean-Christophe Conjeaud of ANAMSO who, as a financial and technical partner, made this study possible.

Appendix A. Tables and figures

Table A.1 Mean (minimum – maximum) of the swarm sizes of the observed colonies and of the climate encountered during each period of observations

Period	Hive format	No. colonies	Dates	Population size (no. adult bees)	Temperature (°C)	Light intensity (Lux)	Average wind speed ($km.h^{-1}$)
March	Dadant	35	7 - 17 March 2016	26,588 (8,236 - 46,504)	12.3 (5.1 - 19.3)	5,936 (257 - 14,741)	1.9 (0.0 - 6.4)
May	Dadant	89	29 Apr - 4 May 2014	22,182 (2,893 - 53,546)	16.6 (12.0 - 24.3)	86,094 (2,861 - 211,812)	4.0 (0.9 - 11.5)
July	Dadant	33	27 - 29 July 2015	18,556 (4,351 - 33,293)	26.6 (20.5 - 35.6)	91,941 (12,486 - 204,831)	1.5 (0.0 - 9.4)
October	Langstroth	24	28 Sept - 5 Oct 2015	10,642 (5,792 - 15,078)	19.2 (11.8 - 25.0)	5,496 (368 - 11,460)	1.9 (0.0 - 4.3)

Table A.2 Statistic values of the two kinds of mechanistic relationships investigated between the number of inter-frames covered with adult bees counted by the experienced observer and the adult bee population size, in each case of observations considered

Hive format	Super chamber	Considered observations	Kind of mechanistic relationship	Estimated parameter values (± 95% CI)			R^2_D	AIC	ΔAIC
				b_1	b_2	s			
Dadant	Without super	Above	linear	-11,058 (± 5,704)	26,069 (± 2,706)		0.565	367.01	9.18
			logarithmic	1,524 (± 619)	34,696 (± 5,770)		0.603	357.83	0
		Below	linear	-89 (± 2,202)	26,085 (± 1,725)		0.787	346.65	12.24
			logarithmic	3,934 (± 646)	34,050 (± 3,505)		0.811	334.41	0
		Above + Below	linear	-4,624 (± 2,811)	26,079 (± 1,740)		0.774	316.61	16.04
			logarithmic	2,611 (± 515)	34,558 (± 3,555)		0.807	300.57	0
	With super	Above + Super	linear	-21,219 (± 7,428)		$2.000e^{-4}$ ($\pm 3.37e^{-5}$)	0.537	498.52	27.28
			logarithmic	1,975 (± 640)		3.795 (± 0.528)	0.630	471.24	0
		Below + Super	linear	-6,854 (± 3,697)		$2.764e^{-4}$ ($\pm 3.38e^{-5}$)	0.688	498.94	43.31
			logarithmic	4,313 (± 698)		5.308 (± 0.537)	0.782	455.63	0
		Above + Below + Super	linear	-12,886 (± 4,639)		$2.382e^{-4}$ ($\pm 3.07e^{-5}$)	0.665	475.81	42.82
			logarithmic	3,089 (± 615)		4.534 (± 0.472)	0.765	432.99	0
Langstroth	Without super	Above	linear	-8,370 (± 5,459)	22,582 (± 3,482)		0.708	47.01	0
			logarithmic	1,536 (± 853)	33,876 (± 12,003)		0.700	47.62	0.61
		Below	linear	-6,148 (± 4,801)	23,336 (± 3,665)		0.711	49.00	0
			logarithmic	1,888 (± 960)	36,963 (± 14,341)		0.690	50.64	1.64
		Above + Below	linear	-7,232 (± 4,750)	22,968 (± 3,317)		0.739	44.40	0
			logarithmic	1,706 (± 845)	35,416 (± 12,244)		0.726	45.73	1.33

b_1: minimum number of bees contained in the colony from which bees started to be visible in inter-frames; b_2: minimum number of bees beyond which bees saturated the ten inter-frames of the hive body; s: slope of filling inter-frames by bees. R^2_D is the coefficient of determination R^2 value calculated by the deviance ratio. ΔAIC is the AIC gap value between the linear and the logarithmic models in each case of observations considered. Parameter values estimated from Eqs. (1), (2), (3) and (4).

Table A.3 Reliability of the conversion relationships between the number of inter-frames covered with adult bees counted in Dadant hives and the adult bee population size, for each case of observations considered

Observer	Model	Submodel	Estimated parameter values (± 95% CI)		*RSD* (%)	$RQ_{2.5\%}$ (%)	$RQ_{97.5\%}$ (%)	$N_{min\text{-}LogN}$ in a margin of error of	
			intercept	slope				10%	20%
Experienced	Above		8.366 (± 0.127)	0.1899 (± 0.0156)	40.23				
	Below		8.534 (± 0.0697)	0.1833 (± 0.0091)	27.8				
	Above + Below		8.262 (± 0.085)	0.2119 (± 0.0108)	28.64				
	Above + Super	without super	8.390 (± 0.141)	0.1742 (± 0.0184)	42.52				
		with super	8.496 (± 0.113)	0.1591 (± 0.0130)	38.59				
	Below + Super	without super	8.549 (± 0.077)	0.1754 (± 0.0110)	29.21				
		with super	8.638 (± 0.068)	0.1541 (± 0.0082)	27.74				
	Above + Below + Super	without super	8.297 (± 0.095)	0.2004 (± 0.0131)	30.13				
		with super	8.456 (± 0.081)	0.1710 (± 0.0096)	29.02				
Naive	Above		8.506 (± 0.137)	0.1741 (± 0.0171)	44.31				
	Below		8.587 (± 0.075)	0.1783 (± 0.0099)	30.14				
	Above + Below		8.309 (± 0.093)	0.2084 (± 0.0120)	31.14				
	Above + Super	without super	8.475 (± 0.150)	0.1640 (± 0.0197)	45.49				
		with super	8.601 (± 0.124)	0.1487 (± 0.015)	43.06				
	Below + Super	without super	8.555 (± 0.080)	0.1735 (± 0.0114)	30.08				
		with super	8.675 (± 0.071)	0.1521 (± 0.0088)	29.40				
	Above + Below + Super	without super	8.316 (± 0.101)	0.1981 (± 0.0140)	31.89				
		with super	8.488 (± 0.087)	0.1700 (± 0.0106)	31.23				

The inferred relationships here are exponential (i.e. the converse of logarithmic relationships). *RSD*: relative standard deviation of estimation (Eq. (8)); $RQ_{2.5\%}$ and $RQ_{97.5\%}$: respectively 2.5% and 97.5% quantiles of the residual error distribution relative to the expected value, that express the asymmetric relative margin of error of estimating a bee population size from a number of IFB with a probability of 95% (Eqs. (9) and (10)); $N_{min\text{-}LogN}$: minimum number of observations required to estimate the mean bee population size of a given apiary with a 95% confidence interval included in a given margin of error of 10 or 20% (Eq. 11). Parameter values estimated from Eq. (6).

Table A.4 Reliability of the conversion relationships between the number of inter-frames covered with adult bees counted in Langstroth hives and the adult bee population size, for each case of observations considered

Observer	Model	Estimated parameter values (± 95% CI)		σ	$Q_{97.5\%}$	$N_{min\text{-}N}$ in a margin of error of	
		intercept	slope			10%	20%
Experienced	Above	2,794 (± 1671)	1,270 (± 263)	2,136	4,187	18	5
	Below	452 (± 1444)	1,786 (± 247)	1,753	3,466	12	3
	Above + Below	832 (± 1507)	1,657 (± 249)	1,845	3,617	13	4
Naive	Above	3,250 (± 1901)	1,179 (± 292)	2,252	4,415	20	5
	Below	2,981 (± 1434)	1,451 (± 264)	2,031	3,980	16	4
	Above + Below	2,028 (± 1550)	1,503 (± 264)	1,998	3,917	16	4

The inferred relationships here are linear. σ: standard deviation of estimation; $Q_{97.5\%}$: 97.5% quantile of the residual error distribution, that express the absolute margin of error of estimating a bee population size from a number of IFB with a probability of 95%; $N_{min\text{-}N}$: minimum number of observations required to estimate the mean bee population size of a given apiary with a 95% confidence interval included in a given margin of error of 10 or 20% (Eq. (7)). Parameter values estimated from Eq. (5).

Table A.5 Conversion grid between the number of inter-frames covered with adult bees counted and the estimated adult bee population size

No. inter-frames recovered with adult bees counted on the bottom or the top of the body hive, plus in the super(s) if present	Estimated no. adult bees contained in the colony					
	Dadant format				Langstroth format	
	Inter-frames counted on the **bottom** of the hive body, plus in the super(s) if present		Inter-frames counted on the **top** of the hive body, plus in the super(s) if present		Inter-frames counted on the **bottom** of the hive body	Inter-frames counted on the **top** of the hive body
	Hive with adult bees in at least one super, or without any super but with less than 6 inter-frames recovered with adult bees on the bottom of the hive	Hive without any super and with more than 6 inter-frames recovered with adult bees on the bottom of the hive	Hive with adult bees in at least one super, or without any super but with less than 6 inter-frames recovered with adult bees on the top of the hive body	Hive without any super and with more than 6 inter-frames recovered with adult bees on the top of the hive body	Hives without any super in the study (month of October)	
0	Between 0 and 4,313		Between 0 and 1,975			
0.5	4,739		2,253			
1	5,207		2,570			
1.5	5,721		2,932			
2	6,286		3,345			
2.5	6,907		3,816			
3	7,589		4,353			
3.5	8,339		4,966			
4	9,162		5,665		5,494	
4.5	10,067		6,463		6,999	5,293
5	11,061		7,373		8,505	6,877
5.5	12,154		8,412		10,010	8,461
6	13,354	14,361	9,596	9,939	11,516	10,040
6.5	14,673	15,998	10,947	11,620	13,021	11,630
7	16,123	17,820	12,489	13,585	14,527	13,214
7.5	17,715	19,851	14,248	15,883	16,032	14,798
8	19,465	22,113	16,254	18,569		
8.5	21,387	24,633	18,543	21,711		
9	23,500	27,440	21,154	25,383		
9.5	25,821	30,567	24,133	29,676		
10	28,371	34,050 and more	27,531	34,696 and more		
10.5	31,173		31,408			
11	34,252		35,831			
11.5	37,635		40,876			
12	41,352		46,633			
12.5	45,437		53,199			
13	49,925		60,691			
13.5	54,856		65,000			
14	60,274		65,000			
14.5	65,000		65,000			

The estimated numbers of adult bees in light grey are quite approximative, as the relationship is exponential, and as a colony of more than 45,000 adult bees is quite exceptional. The maximum estimated number of adult bees proposed is 65,000 (in dark grey), as it is the maximum colony size observed by Farrar (1937).

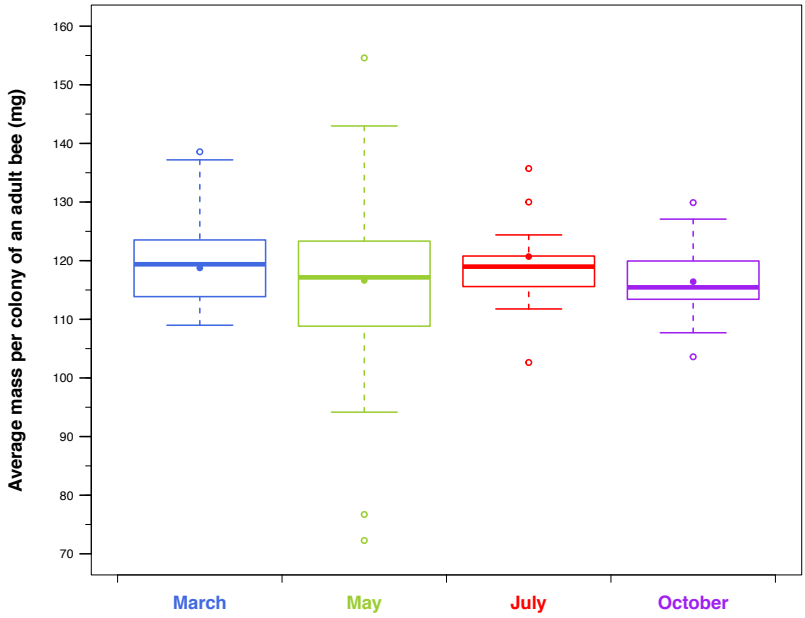


Fig. A.1. Box plot of average mass of adult bees per colony, for each period. Each box plot represent, from bottom to top, the minimum, the first quartile, the median, the third quartile and the maximum of the data. Circles represent outliers, and full points represent the mean of the data.

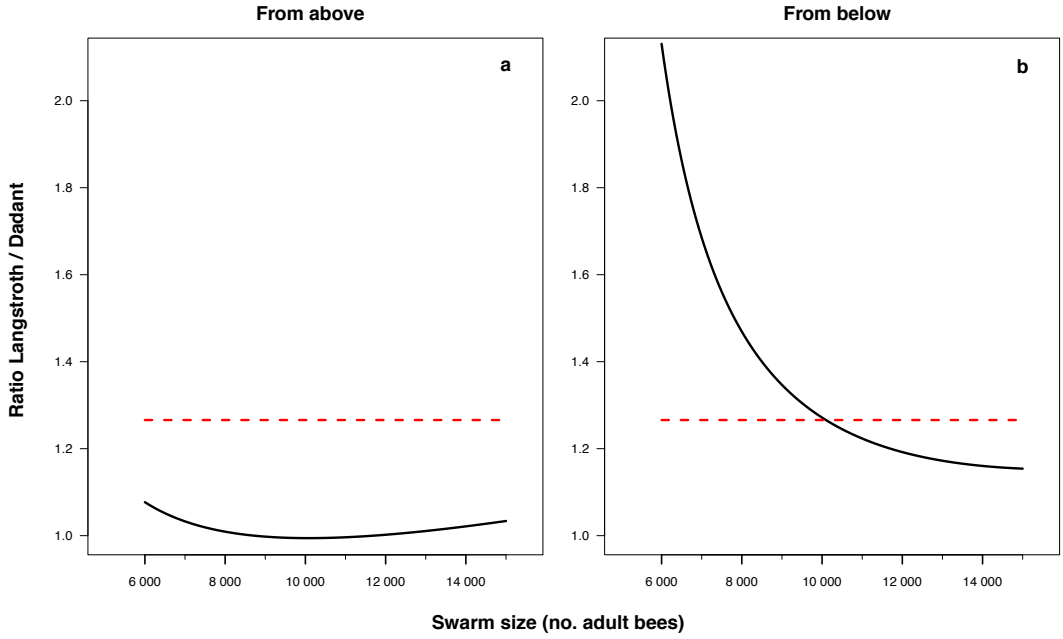


Fig A.2. Ratio of the number of inter-frames covered with adult bees (IFB) counted in Langstroth hives on the number of IFB counted in Dadant hives regarding the adult bee population size (solid black line). IFB counted from (a) above and (b) below the hive body. The range of adult bee population sizes used in abscissa corresponds to the range sampled in common for both hive formats (see Table A.1). The ratio of the area of a Langstroth frame to that of a Dadant one is 0.79, so a Langstroth should fill up in bees $1/0.79 = 1.3$ times faster with adult bee population size than a Dadant frame (value depicted by the red dashed line).

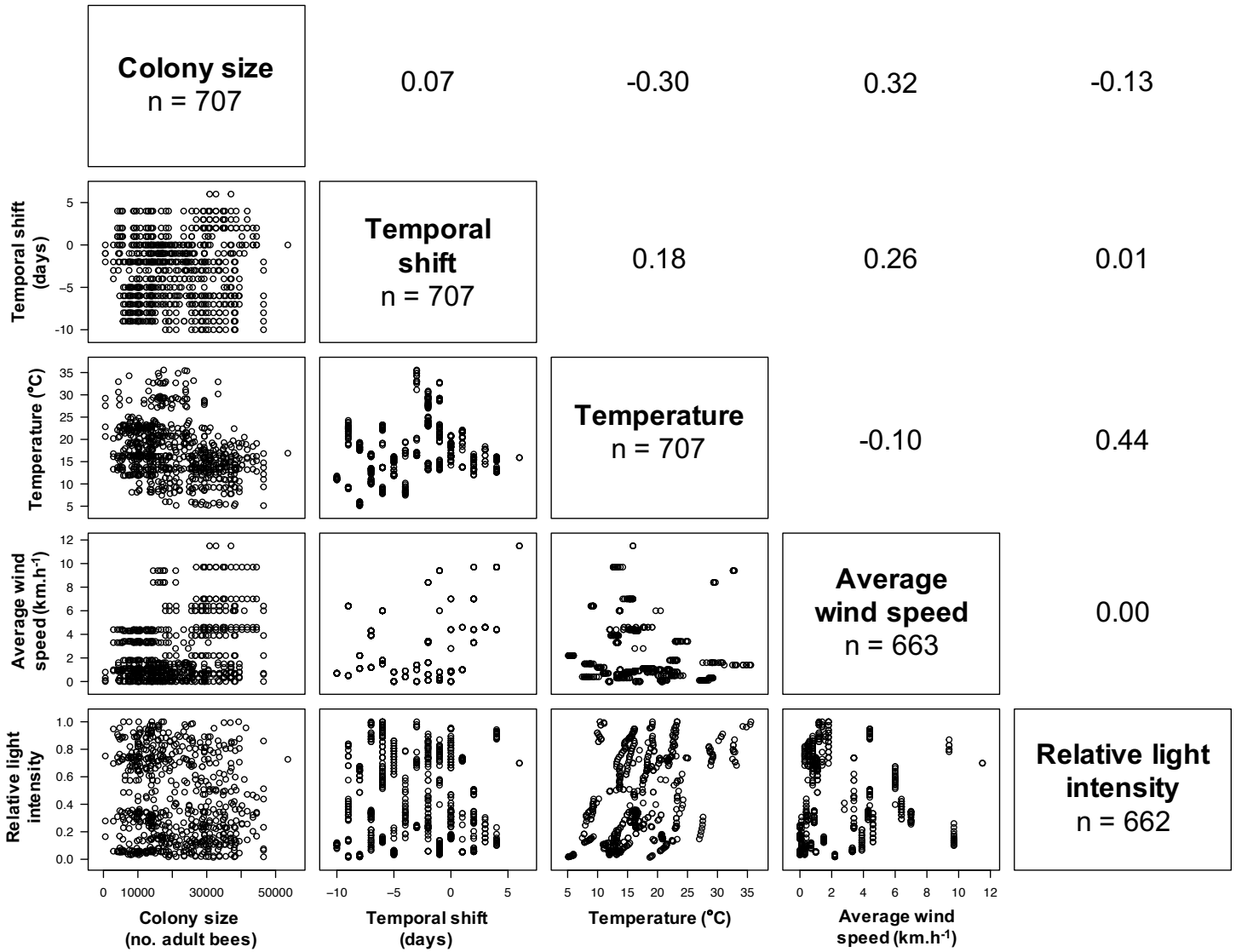


Fig. A.3. Correlation coefficient values between each quantitative fixed explanatory variables taken two by two for the GLMMs (Table 1). Figure generated with the package *Rarity* (Leroy, 2016).

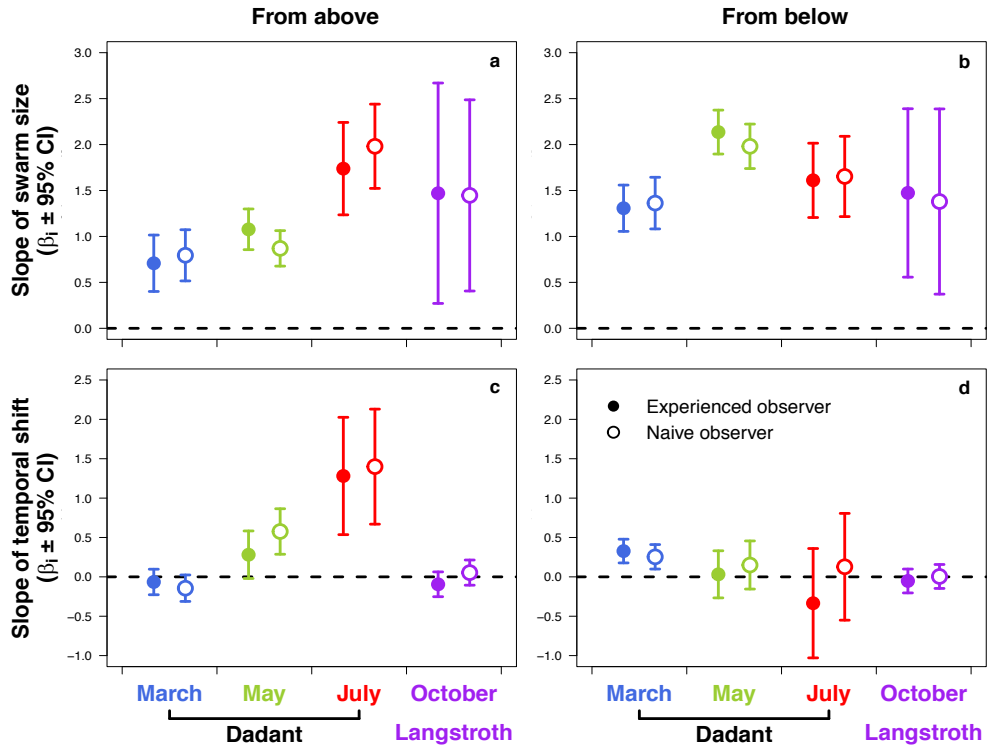


Fig. A.4. Slope coefficient values β_i of the scaled fixed explanatory variables, (a, b) adult bee population size and (c, d) temporal shift between colony observation and adult bee population weighing, given for each period, each kind of observer, and for the number of inter-frames covered with adult bees counted from (a, c) above and from (b, d) below the hive body. Values are extracted from GLMMs (Table 1).

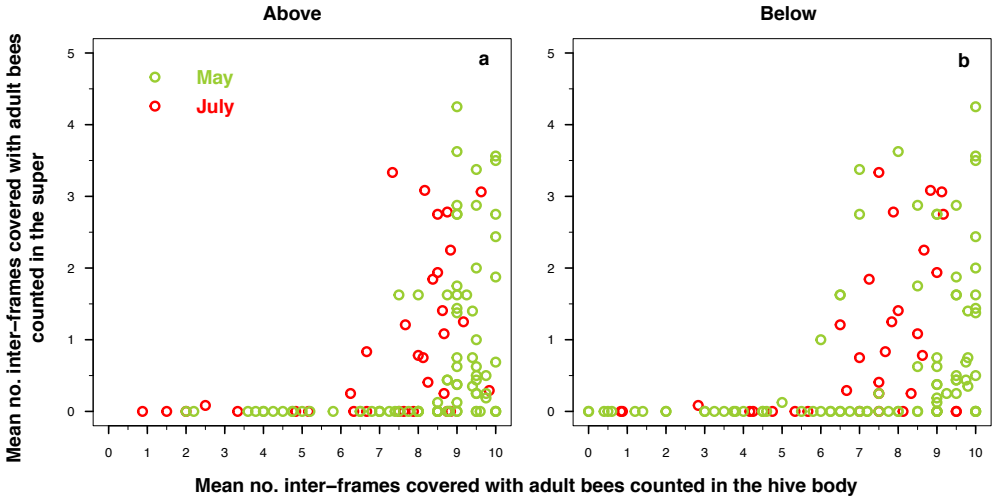


Fig. A.5. Mean number of inter-fames covered with adult bees (IFB) counted by the experienced observer in the super chamber per colony, in Dadant hives equipped with a super chamber, in relation to the mean number of IFB counted by the experienced observer per colony from (a) above and from (b) below the Dadant hive body. Note that IFB are counted in the super chamber mainly when a minimum of 6 IFB are counted from above or below the hive body.

Appendix B. Calculations to get Eqs. (1), (2), (3) and (4)

To get the linear relationship in Eq. (1), we start from a simple linear relationship *y(x)=ax+c* with the two parameters *a* and *c* to be determined with the two following conditions:

$$\begin{cases} y(b_1) = 0 \\ y(b_2) = 10 \end{cases} \Leftrightarrow \begin{cases} ab_1 + c = 0 \\ ab_2 + c = 10 \end{cases}$$

$$\Leftrightarrow \begin{cases} c = -ab_1 \\ ab_2 - ab_1 = 10 \end{cases}$$

$$\Leftrightarrow \begin{cases} c = -ab_1 \\ a = \dfrac{10}{b_2 - b_1} \end{cases}$$

$$\Leftrightarrow \begin{cases} c = -\dfrac{10b_1}{b_2 - b_1} \\ a = \dfrac{10}{b_2 - b_1} \end{cases}$$

Then we have:

$$y(x) = ax + c \Leftrightarrow y(x) = \frac{10}{b_2 - b_1}x - \frac{10\,b_1}{b_2 - b_1} \quad (1)$$

To get the logarithmic relationship in Eq. (2), we start from a simple linear relationship *y(x)=aln(x)+c* with the two parameters *a* and *c* to be determined with the two following conditions:

$$\begin{cases} y(b_1) = 0 \\ y(b_2) = 10 \end{cases} \Leftrightarrow \begin{cases} a\,ln(b_1) + c = 0 \\ a\,ln(b_2) + c = 10 \end{cases}$$

$$\Leftrightarrow \begin{cases} c = -a\,ln(b_1) \\ a\,ln(b_2) - a\,ln(b_1) = 10 \end{cases}$$

$$\Leftrightarrow \begin{cases} c = -a\,ln(b_1) \\ a = \dfrac{10}{ln\left(\frac{b_2}{b_1}\right)} \end{cases}$$

$$\Leftrightarrow \begin{cases} c = -\dfrac{10\,ln(b_1)}{ln\left(\frac{b_2}{b_1}\right)} \\ a = \dfrac{10}{ln\left(\frac{b_2}{b_1}\right)} \end{cases}$$

Then we have:

$$y(x) = a\ ln(x) + c \Leftrightarrow y(x) = \frac{10}{ln\left(\frac{b_2}{b_1}\right)} ln(x) - \frac{10\ ln(b_1)}{ln\left(\frac{b_2}{b_1}\right)} \quad (2)$$

To get the linear relationship in Eq. (3), we start from a simple linear relationship $y(x)=sx+c$ with the parameter c to be determined with the following condition:

$$y(b_1) = 0 \Leftrightarrow sb_1 + c = 0$$

$$\Leftrightarrow c = -sb_1$$

Then we have:

$$y(x) = sx + c \Leftrightarrow y(x) = sx - sb_1$$

$$\Leftrightarrow y(x) = s(x - b_1) \quad (3)$$

To get the logarithmic relationship in Eq. (4), we start from a simple linear relationship $y(x)=sln(x)+c$ with the parameter c to be determined with the following condition:

$$y(b_1) = 0 \Leftrightarrow s\ ln(b_1) + c = 0$$

$$\Leftrightarrow c = -s\ ln(b_1)$$

Then we have:

$$y(x) = s\ ln(x) + c \Leftrightarrow y(x) = s\ ln(x) - s\ ln(b_1)$$

$$\Leftrightarrow y(x) = s\ ln\left(\frac{x}{b_1}\right) \quad (4)$$

Appendix C. Calculations to get Eqs. (8), (9), (10) and (11)

Let *x* population size of a colony in number of adult bees, and *y* the IFB. If the relationship between *y* and *x* is logarithmic, then the converse is:

$$f^{-1}(y_i;\alpha,\beta) = e^{\alpha y_i+\beta}, \text{ for each } i^{th} \text{ colony, with } \alpha,\beta \in \mathbb{R}.$$

If we seek to estimate *x* from *y*, we have:

$$\hat{x}_i = e^{\alpha y_i+\beta}\,.\eta_i, \text{ with } \eta_i \sim Log-\mathcal{N}\left(0, e^{\sigma^2}\right).$$

The expected value and the variance of *x* are written as follows:

$$E(x_i) = f^{-1}(y_i;\alpha,\beta).E(\eta_i), \text{ with } E(\eta_i) = e^{\frac{\sigma^2}{2}}$$

$$Var(x_i) = f^{-2}(y_i;\alpha,\beta).Var(\eta_i), \text{ with } Var(\eta_i) = \left(e^{\sigma^2}-1\right).e^{\sigma^2}$$

To get the relative standard deviation *RSD* of estimation (%) of *x*, we calculate:

$$RSD(x_i) = 100\,\frac{SD(x_i)}{E(x_i)}$$

$$= 100\,\frac{\sqrt{f^{-2}(y_i;\alpha,\beta)\,.\,Var(\eta_i)}}{f^{-1}(y_i;\alpha,\beta).E(\eta_i)}$$

$$= 100\,\frac{f^{-1}(y_i;\alpha,\beta).\sqrt{Var(\eta_i)}}{f^{-1}(y_i;\alpha,\beta).E(\eta_i)}$$

$$= 100\,\frac{\sqrt{Var(\eta_i)}}{E(\eta_i)}$$

$$= 100\,\frac{\sqrt{\left(e^{\sigma^2}-1\right).e^{\sigma^2}}}{e^{\frac{\sigma^2}{2}}}$$

$$= 100\sqrt{\left(e^{\sigma^2}-1\right)} \qquad (8)$$

To get the relative 2.5% and 97.5% quantiles (%) of the error distribution η, called $RQ_{2.5\%}$ and $RQ_{97.5\%}$, we calculate:

$$RQ_{2.5\%}(x_i) = 100\,\frac{e^{t_{\gamma=2.5\%}^{k=n-1}}.SD(x_i)}{E(x_i)}$$

$$= e^{t_{\gamma=2.5\%}^{k=n-1}}.RSD(x_i)$$

$$= 100\,e^{t_{\gamma=2.5\%}^{k=n-1}}\sqrt{\left(e^{\sigma^2}-1\right)} \qquad (9)$$

The calculation is the same for Eq. (10) with $e^{t_{\gamma=97.5\%}^{k=n-1}}$.

To calculate the minimum number of inspections of a given apiary required to estimate its mean colony size with a 95% confidence interval included in a given margin of error M_e (in %), called $N_{min\text{-}LogN}$, we use the gap Δ between the relative 97.5% and 2.5% quantiles of the error distribution η, as they are asymmetric around the estimated mean:

$$\frac{\Delta(x_i)}{\sqrt{N_{min-LogN}(x_i)}} = \frac{2M_e}{100} \Leftrightarrow N_{min-LogN} = \left(\frac{100}{2M_e}\right)^2 \Delta(x_i)^2$$

$$\Leftrightarrow N_{min-LogN}(x_i) = \left(\frac{100}{2M_e}\right)^2 \left(RQ_{97.5\%}(x_i) + RQ_{2.5\%}(x_i)\right)^2$$

$$\Leftrightarrow N_{min-LogN}(x_i) = \left(\frac{100}{2M_e}\right)^2 \left(\frac{\left(e^{t_{\gamma=97.5\%}^{k=n-1}} + e^{t_{\gamma=2.5\%}^{k=n-1}}\right).100.SD(x_i)}{E(x_i)}\right)^2$$

$$\Leftrightarrow N_{min-LogN}(x_i) = \left(\frac{100}{2M_e}\right)^2 \left(e^{t_{\gamma=97.5\%}^{k=n-1}} + e^{t_{\gamma=2.5\%}^{k=n-1}}\right)^2 100^2 \left(e^{\sigma^2} - 1\right) \quad (11)$$

At last, the variance value σ^2 was obtained with a linear model by transforming x in logarithm: $ln(\hat{x}_i) = \alpha y_i + \beta + \varepsilon_i$, with $\alpha, \beta \in \mathbb{R}$ and $\varepsilon \sim \mathcal{N}(0, \sigma^2)$.

DISCUSSION GENERALE

Ce travail de thèse a donc permis (*i*) de déterminer la quantité minimale de grains de pollen devant être déposés par stigmate de colza en une fois en fonction de la température pour que la pollinisation ne soit pas un facteur limitant pour la grenaison, (*ii*) de prédire la quantité minimale de grains de pollen devant être déposée au total par stigmate de colza dans le cas de dépôts successifs en fonction de la température, (*iii*) de déterminer la période effective de pollinisation – ou durée de réceptivité du pistil – et la période de sécrétion nectarifère du colza en fonction de la température, (*iv*) de déterminer la vitesse de sécrétion nectarifère du colza en fonction de la température, et (*v*) d'établir l'équivalent du nombre d'inter-cadres recouverts d'abeilles mellifères adultes dans une ruche en nombre d'abeilles mellifères adultes contenues au total dans la colonie. Les quatre premiers éléments caractérisent des processus de biologie florale uniquement, mesurés indépendamment du butinage des insectes, tandis que le dernier caractérise la quantité d'insectes introduits dans la culture. C'est dans le sens de cette dichotomie que je propose d'introduire les concepts de *demande* et d'*offre* en pollinisation d'une culture cible, introduits dans la partie 4 de l'Introduction Générale.

1. Proposition d'un nouveau concept : l'offre et la demande en pollinisation

1.1. La demande en pollinisation

Je propose de définir la *demande* en pollinisation d'une culture comme : *le nombre minimum de grains de pollen de qualité, c'est-à-dire conspécifiques, viables, compatibles et de qualité génétique satisfaisante, devant être déposés sur le stigmate d'un carpelle* (ou les stigmates dans le cas de gynécées syncarpes à stigmates libres) *durant la période de réceptivité de ce carpelle, divisé par le nombre d'ovules contenus dans l'ovaire, pour que l'ensemble des ovules puissent être fécondés et potentiellement se développer en graines mûres*. Le « pollen de qualité » a été défini comme du pollen conspécifique, viable et compatible par Ne'eman *et al.* (2010). Je propose d'ajouter dans cette définition de « pollen de qualité » le fait que ce pollen doit être « de qualité génétique satisfaisante » car, comme l'ont montré Aizen & Harder (2007), chez les Angiospermes totalement ou partiellement auto-compatibles, des embryons – donc des ovules fécondés – peuvent être avortés si les gamètes mâles qui les ont engendré étaient génétiquement trop proches des ovules. Les ovules fécondés doivent pouvoir « potentiellement » se développer en graines mûres car, comme l'ont souligné Harder & Routley (2006) et Harder & Aizen (2010), les ressources maternelles peuvent être limitantes,

empêchant le développement d'ovules fécondés en graines. De plus, comme nous l'avons montré dans le chapitre II, cette *demande* en pollinisation telle que définie varie avec la température, du fait de la variation de la performance du pollen avec la température. Et comme nous l'avons déduit dans le chapitre II, cette *demande* est également supposée varier avec la taille et la fréquence des dépôts de pollen lorsque la pollinisation se réalise en dépôts successifs, pour les espèces pour lesquelles il y a un effet de population du pollen à faible densité (Brink, 1924 ; Brewbaker & Majumder, 1961 ; Brewbaker & Kwack, 1963 ; Cruzan, 1986 ; Niesenbaum, 1999 ; Zhang *et al.*, 2010 ; Harder *et al.*, 2016a,b). De même, pour les espèces pour lesquelles il y a un effet de population du pollen à faible densité et dotées d'un gynécée syncarpe à stigmates libres, cette *demande* est également supposée varier selon le nombre de stigmates pollinisés.

Par analogie, en économie le pollen serait l'équivalent de la *marchandise*, et le nectar ou le pollen délivré par les anthères comme attractifs pour les pollinisateurs, l'équivalent du *prix*. La sécrétion nectarifère et la production de pollen comme attractifs représentent en effet un coût pour la plante, un investissement pour assurer la pollinisation zoophile. Ce concept de *demande* en pollinisation tel que défini est plus précis que le concept de « *pollination requirements* » utilisé jusqu'à présent pour définir les « besoins en pollinisation » d'une culture (McGregor, 1976 ; Free, 1993 ; Delaplane & Mayer, 2000). Le concept de *pollination requirements* pour les cultures est en effet d'ordre essentiellement qualitatif : il définit simplement le mode de reproduction des cultures (auto-fertile, auto-compatible, auto-incompatible), et leur mode de pollinisation (auto-pollinisation, anémophilie, zoophilie, entomophile, etc.). Le concept de *demande* que nous venons de définir est en revanche d'ordre quantitatif.

D'après l'expérimentation menée dans le chapitre II, la quantité minimale de grains de pollen de colza conspécifiques, compatibles, de qualité génétique satisfaisante (le colza est totalement auto-compatible ; Williams, 1978 ; Williams *et al.*, 1986 ; Ouvrard *et al.*, 2017) et a priori viables devant être déposés sur le stigmate pour que la pollinisation ne soit pas un facteur limitant pour la grenaison est estimée à 44 à la température optimale de 22,5°C et dans le cas où le pollen est déposé en une seule fois sur le stigmate. La variété mâle stérile sur laquelle nous avons travaillé est dotée en moyenne de 35,6 ovules, pour une grenaison moyenne estimée complète dans notre expérimentation de 32,5. La *demande* en pollinisation dans ce cas était en moyenne de 44 / 35,6 ≈ 1,24 grains de pollen par ovule. En moyenne, 3 ovules fécondés ont donc avorté par fleur. Ce résultat est difficile à expliquer étant donné que seules certaines fleurs

de cette lignée mâle stérile ont été pollinisées, impliquant que les ressources maternelles disponibles par embryon devaient être très largement en excès.

L'expérimentation menée dans le chapitre I a permis de mesurer la période effective de pollinisation du colza, c'est-à-dire la « fenêtre temporelle » durant laquelle la *demande* en pollinisation définie précédemment doit être satisfaite. Cette expérimentation a également pu montrer que l'attractivité de la fleur, en termes de sécrétion nectarifère, couvre la totalité de cette période.

1.2. L'offre en pollinisation, l'offre en pollinisateurs

Je propose ensuite de définir l'*offre* en pollinisation pour une culture donnée comme : *le nombre de grains de pollen de qualité, c'est-à-dire conspécifiques, viables, compatibles et de qualité génétique satisfaisante, déposés sur le stigmate d'un carpelle* (ou les stigmates dans le cas de gynécées syncarpes à stigmates libres) *durant la période de réceptivité de ce carpelle, divisé par le nombre d'ovules contenus dans l'ovaire*. Cette définition est assez proche de celle du concept d'intensité de la pollinisation, qui est le nombre de grains de pollen conspécifiques viables déposés sur un stigmate réceptif (Snow, 1982 ; Falque *et al.*, 1995), avec l'exception que la charge en pollen déposée sur le stigmate est divisée par le nombre d'ovules disponibles dans l'ovaire dans la définition de *demande*. Le pollen peut être déposé sur le stigmate par contact direct entre anthères et stigmate, par gravité, par le vent ou par des pollinisateurs.

Dans le cas des cultures zoophiles, je propose de définir l'*offre* en pollinisateurs pour une culture donnée comme : *le nombre de pollinisateurs disponibles par fleur, en associant les performances de ces pollinisateurs*. La « performance » d'un pollinisateur a été définie par Ne'eman *et al.* (2010) et comprend son efficacité (respectivement efficience) pollinisatrice individuelle, c'est-à-dire le nombre de grains de pollen (respectivement de qualité) déposés par stigmate par visite de fleur, la quantité de pollen retirée des anthères par visite de fleur, et sa vitesse de butinage. La performance d'un pollinisateur varie à la fois selon les conditions environnementales et selon son propre état. La vitesse de butinage d'un insecte comme organisme ectotherme dépend par exemple en premier lieu, comme corollaire de son état physiologique, de la température, du rayonnement solaire et de la vitesse du vent (Bishop & Armbruster, 1999 ; Colinet *et al.*, 2015 ; Gunderson & Leal, 2016). Sa vitesse devrait dépendre également, d'après le théorème de la valeur marginale de la théorie de l'approvisionnement optimal (Charnov, 1976 ; Calcagno *et al.*, 2014), de la viscosité du nectar disponible dans les fleurs (Heyneman, 1983), qui dépend à la fois de la température ambiante et de sa concentration en sucres totaux dissous (Nicolson & Thornburg, 2007), et de la quantité de nectar disponible,

qui dépend à la fois de la vitesse de la sécrétion nectarifère, dépendant à son tour de la température comme vu dans le chapitre III, et de l'abondance locale d'insectes butinant l'espèce végétale en question. L'efficacité pollinisatrice individuelle, l'efficience pollinisatrice individuelle et la quantité de pollen retirée par visite dépendent quant à elles de nombreuses variables, par exemple pour les productions de semence hybride : le nombre de fleurs mâle stériles visitées depuis la dernière visite d'une fleur mâle fertile (voir Carré *et al.*, 1994 ; Cresswell *et al.*, 1995, 2002 ; Richards *et al.*, 2009), le temps écoulé entre l'anthèse d'une fleur mâle fertile et sa visite par un insecte (ex. Bell & Cresswell, 1998), la diversité et la densité d'insectes sauvages par fleur pour l'efficience pollinisatrice des abeilles mellifères (par synergie entre abeilles mellifères et insectes pollinisateur sauvages pour la pollinisation ; DeGrandi-Hoffman & Watkins, 2000 ; Greenleaf & Kremen, 2006 ; Carvalheiro *et al.*, 2011 ; Brittain *et al.*, 2013b ; Sapir *et al.*, 2017), etc.

Dans l'expérimentation du chapitre IV, nous avons proposé une méthode simple qui permet d'approcher l'*offre* en abeilles mellifères adultes contenues dans un cheptel introduit dans une culture de façon plus précise que le simple nombre de colonies. Mais pour approcher cette *offre* de manière encore plus précise, il faudrait pouvoir quantifier le nombre de butineuses d'abeilles mellifères délivré par colonie. Ce nombre dépend tout d'abord évidemment de la taille de population d'abeilles mellifères adultes contenue dans la colonie, comme évoqué en discussion du chapitre IV, mais il dépend ensuite de plusieurs autres facteurs à la fois environnementaux (climat, ressources florales) et internes à la colonie, comme la présence ou non de cellules vides pour que les butineuses puissent déposer leur butin en pollen ou nectar (Rinderer & Baxter, 1978).

2. L'offre et la demande en pollinisation comme cadre conceptuel quantitatif pour la pollinisation intégrée des cultures

2.1. Lacune dans le concept de pollinisation intégrée des cultures

Le concept de pollinisation intégrée des cultures est d'ordre qualitatif, visant à « combiner espèces pollinisatrices introduites et indigènes en adaptant les pratiques agricoles pour promouvoir les populations indigènes » (Isaacs *et al.*, 2017). Garrat *et al.* (2018b) ont souligné pour leur part que la mise en œuvre d'une « *pollinisation intégrée des cultures efficace requiert une base solide de preuves, incluant une compréhension de la demande en pollinisation entomophile d'une culture, la potentielle contribution de pollinisateurs sauvages et une*

stratégie de gestion optimale pour utiliser des espèces introduites si nécessaire ». Les concepts de *demande* en pollinisation et d'*offre* en pollinisateurs comme formulés plus haut pourraient ainsi fournir un cadre d'étude quantitatif à ce concept.

2.2. Vers un modèle de gestion intégrée de la pollinisation

Deux types d'*offres* en pollinisateurs peuvent être dissociés : une *offre* en pollinisateurs sauvages indigènes, fournissant un service écosystémique de pollinisation, et une *offre* en pollinisateurs introduits, comme pratique culturale ou « intrant agronomique » à part entière (voir Garratt *et al.*, 2018a). Il s'agit alors de (1) définir précisément la *demande* en pollinisation d'une culture cible dans un climat donné comme proposé dans le chapitre II, (2) de quantifier l'*offre* en pollinisation fournie par le service écosystémique local (incluant l'*offre* en auto-pollinisation, et en pollinisations anémophile et zoophile) en échantillonnant les charges en pollen de stigmates exposés en pollinisation libre et les grenaisons associées dans une culture sans introduction de pollinisateurs et en tenant compte du contexte climatique et paysager local, puis (3) estimer le complément nécessaire en pollinisation à fournir pour répondre à la *demande* de la culture, par soustraction des deux valeurs précédentes. (4) La dernière étape consisterait à estimer le nombre de pollinisateurs à introduire par unité de surface de culture, connaissant la performance individuelle moyenne des pollinisateurs introduits, c'est-à-dire leur vitesse moyenne de butinage et leur efficience pollinisatrice individuelle moyenne, leur activité de butinage dans la culture, c'est-à-dire la proportion du *pool* d'insectes introduits mise en activité dans la culture (qui dépend du climat et des ressources offertes par la culture), le nombre de fleurs à polliniser par unité de surface de culture, et la période effective de pollinisation de l'espèce cultivée (voir chapitre I), le tout en tenant compte du contexte cultural et climatique de la culture. La connaissance de l'efficience pollinisatrice moyenne du pollinisateur permettrait d'ajuster à la hausse si nécessaire la *demande* en pollinisation, comme vu dans le chapitre II, si par exemple cette efficience se trouve relativement faible. La performance des pollinisateurs pouvant grandement varier selon le contexte environnemental local, c'est là où un modèle mécaniste de gestion intégrée de la pollinisation pourrait se trouver fort utile, comme outil de gestion intégrée (pour des exemples de modèles mécanistes ayant trait à la pollinisation, voir par exemple : Bajcz *et al.*, 2017 ; Qu & Drummond, 2018 ; Sáez *et al.*, 2018b). Ce modèle pourrait intégrer comme variables d'entrée (ou *inputs*) :

- les caractéristiques intrinsèques de la parcelle et de la variété cultivée : taille de la parcelle, masse florale, production pollinique, vitesse de sécrétion nectarifère,

période effective de pollinisation, période de sécrétion nectarifère, *demande* en pollinisation ;

- les conditions climatiques locales : température, vitesse du vent, rayonnement solaire, humidité relative (qui impacte par exemple la concentration du nectar en sucres totaux dissous et donc sa viscosité et son attractivité) ;
- le contexte paysager de la culture : proportion du paysage entourant la culture dans un certain rayon recouvert d'éléments semi-naturels (praires permanentes, haies, bois, bosquets) et d'autres cultures entomophiles pour quantifier le potentiel local en pollinisateurs indigènes et en ressources alternatives, susceptible de diluer les pollinisateurs introduits (voir Garibaldi *et al.* 2017) ;
- les pratiques agricoles : irrigation (impactant la sécrétion nectarifère, voir chapitre III), protocoles de semis (dans le cas des productions de semences) ;
- les caractéristiques des pollinisateurs introduits : activité de butinage, efficience pollinisatrice individuelle, quantité de pollen retirée par visite, vitesse de butinage.

La variable de sortie (*output*) serait le nombre de pollinisateurs à introduire.

Avec ce modèle, différents scénarii d'introduction de pollinisateurs d'élevage, pouvant comprendre différentes espèces, indigènes ou non (ex : Koh *et al.*, 2018 ; Pitts-Singer *et al.*, 2018), pourraient être simulés pour identifier la stratégie la plus efficace, c'est-à-dire la stratégie qui permettrait d'introduire le moins possible de pollinisateurs dans la culture, comme recommandé par Garibaldi *et al.* (2017).

Un tel modèle permettrait également, en changeant la variable de sortie, d'identifier par exemple un protocole de semis optimal pour les productions de semences hybrides, ou un protocole de plantation optimal pour les vergers (par exemple : Sáez *et al.*, 2018a). Il permettrait encore par exemple de prédire pour un couple de lignées parentales données la réponse en termes de fécondation, pour la lignée mâle stérile en production de semences ou pour la lignée productrice en verger, face par exemple à une élévation de la température ou à une augmentation de la sécrétion nectarifère. Ceci pourrait aider à orienter les recherches en sélection variétale.

2.3. La température, facteur prépondérant

Il apparaît clairement que la température est un facteur prépondérant qui régit à la base un grand nombre de processus biologiques et écologiques, et notamment ceux responsables de la pollinisation. Il est donc très probable que ce facteur apparaisse comme la variable la plus influente à travers une analyse de sensibilité. Ceci rejoint les nombreux travaux qui montrent que la phase de reproduction chez les plantes est la phase la plus sensible à la température (voir

revues dans : Hedhly *et al.*, 2009 ; Thakur *et al.*, 2010 ; Zinn *et al.*, 2010 ; Hedhly, 2011 ; Sage *et al.*, 2015 ; Prasad *et al.*, 2017 ; Rosbackh *et al.*, 2018). Mais si la plupart des processus répondent à la température de façon presque symétrique autour d'une température optimale selon une distribution beta, comme c'est le cas pour la sécrétion nectarifère (voir chapitre III), la performance du pollen (voir chapitre II), l'activité et la vitesse de butinage (Bishop & Armbruster, 1999 ; Colinet *et al.*, 2015 ; Gunderson & Leal, 2016 ; voir aussi les références dans le chapitre IV), le fait que la longévité florale réponde selon une fonction inverse (voir chapitre I) implique que la pollinisation et la fécondation sont plus sensibles aux températures élevées qu'aux températures basses. Il y a comme une sorte de compensation aux températures basses par l'allongement de la longévité florale. Ce type de modèle pourrait donc constituer un outil pour adapter les variétés, les pratiques agricoles et l'offre en pollinisateurs au changement climatique en cours (IPCC, 2013).

2.4. Optimiser l'offre en abeilles mellifères

Une autre façon d'optimiser l'*offre* en pollinisateurs serait, dans le cas de l'introduction d'abeilles mellifères, d'optimiser la gestion du cheptel et la conduite des colonies (McGregor, 1976 ; Free, 1993 ; Delaplane & Mayer, 2000), comme par exemple répartir le cheptel à différents endroits de la parcelle (Cunningham *et al.*, 2016), doter les ruches d'une trappe à pollen pour stimuler la récolte en pollen des colonies (Viana *et al.*, 2014), ou d'un distributeur de pollen pour améliorer l'efficience pollinisatrice individuelle des butineuses.

3. Le nectar, « entremetteur » entre offre et demande en pollinisation

Enfin, la sécrétion nectarifère, jouant le rôle d'attractif pour les insectes, ne correspond ni à l'*offre*, ni à la *demande* en pollinisation. Nous proposions plus haut la sécrétion nectarifère comme l'équivalent du *prix* en économie. C'est donc un intermédiaire, ou « entremetteur », entre demandeurs (les fleurs) et offrants (les pollinisateurs), qui permet de distribuer l'*offre* en pollinisation en fonction de la *demande*. On a effectivement vu dans le chapitre I que le dépôt de pollen sur le stigmate ou le retrait du pollen des anthères entraîne très souvent la sénescence précoce de la fleur et l'arrêt de la sécrétion nectarifère : une fois que la *demande* en pollinisation est satisfaite, la sécrétion s'arrête donc chez un grand nombre d'espèces. Par ailleurs, lorsque

les pollinisateurs sont rares, les pollinisateurs butinent en priorité les fleurs les plus offrantes en nectar, comme prédit par la distribution idéale libre de la théorie de l'approvisionnement optimal (Fretwell & Lucas, 1970 ; Dreisig, 1995). Par analogie avec l'économie, les pollinisateurs sont donc prêts à « vendre » leur service de pollinisation aux fleurs qui proposent le *prix* en nectar le plus élevé pour obtenir ce service. La valeur de la quantité de nectar offerte par fleur est donc une notion relative qui dépend de l'environnement (quantité de nectar offerte par les autres fleurs, nombre de pollinisateurs), exactement comme la variable du *prix*.

3.1. Sécrétion nectarifère et fréquentation en production de semence

L'expérimentation menée dans le chapitre III a permis de mesurer la vitesse de sécrétion nectarifère de deux lignées de colza, une lignée mâle fertile et une lignée male stérile, en masse de sucres totaux dissous. Nous avons mis en évidence que la lignée mâle fertile sécrétait entre 1,6 et 3 fois plus de nectar que la lignée mâle stérile selon la température. Ceci peut avoir des conséquences sur le potentiel taux de visites d'insectes reçu par chacune de ces deux lignées si elles sont semées côte à côte, comme c'est le cas classiquement dans les productions de semence hybride. D'après la distribution idéale libre, on s'attend par exemple à ce que la lignée mâle fertile reçoive de 1,6 à 3 fois plus d'abeilles mellifères butineuses de nectar par fleur que la lignée mâle stérile. Dans un contexte où l'on cherche à polliniser uniquement la lignée mâle stérile, et où la lignée mâle fertile sert simplement à charger les abeilles en pollen, cela peut être perçu comme une dilution à perte de l'*offre* en pollinisateurs dans la lignée mâle fertile. De plus, cette différence de sécrétion entre les deux lignées peut pousser les abeilles à se spécialiser, par apprentissage, sur une lignée, conduisant à une forte limitation des transferts de pollinisateurs entre les deux lignées.

C'est ce qui a en quelque sorte été observé dans les productions de semence de tournesol, mais par rapport au pollen. Il a en effet été montré dans ce type de production qu'il y avait une ségrégation des abeilles mellifères butineuses de nectar et des butineuses de pollen entre les deux types de lignées : les butineuses de pollen se concentrent majoritairement dans les lignées mâles fertiles, tandis que les butineuses de nectar se concentrent majoritairement dans les lignées mâle stériles (Greenleaf & Kremen, 2006). Les abeilles sauvages solitaires (femelles), qui s'approvisionnent toutes individuellement en pollen et nectar, se concentrent quant à elles majoritairement dans les lignées mâle fertiles, comme les abeilles mellifères butineuses de pollen (Parker, 1981 ; Greenleaf & Kremen, 2006). Ce type de distribution limite grandement les échanges de pollinisateurs, et donc de pollen, entre les deux types de lignées. Cette situation s'explique par le fait que les abeilles mellifères butineuses de pollen et les abeilles sauvages

solitaires butinent en priorité les lignées mâle fertiles pour le pollen, mais y butinent aussi le nectar, également disponible dans ces lignées. Cette exploitation de la ressource en nectar des lignées mâles fertiles par les autres abeilles pousse les abeilles mellifères butineuses de nectar, par distribution idéale libre, à exploiter majoritairement les lignées mâle stériles.

3.2. Hypothèse pour augmenter les transferts de pollen en production de semence

Cette situation pourrait être quelque peu modifiée si les lignées mâle stériles sécrétaient plus de nectar que les lignées mâle fertiles. C'est ce qui a pu être observé chez l'abeille solitaire *Osmia lignaria* dans une situation qui mérite d'être comparée à une culture de production de semence hybride (Williams & Tepedino, 2003 ; voir aussi le box 8.2, p. 288 dans Ydenberg, 2007). Les femelles d'*O. lignaria*, comme toutes les femelles d'abeilles solitaires sauvages, récoltent nectar et pollen : le pollen qu'elles ramènent au nid pour leur progéniture, et le nectar qu'elles récoltent pour leur progéniture et pour elles-mêmes. Williams & Tepedino (2003) ont placé des nids d'*O. lignaria* entre deux types de *patches* de fleurs : un *patche* d'*Hydrophyllum capitulum*, pauvre en pollen et riche en nectar, et un *patche* de *Salix spp.*, riche en pollen et pauvre en nectar. Ils se sont aperçus que les femelles d'*O. lignaria* revenaient au nid systématiquement avec les deux types de pollen, indiquant que ces abeilles butinaient systématiquement les deux types de *patches*, même lorsque l'un des deux patches était à grande distance du nid. Williams & Tepedino (2003) ont suggéré qu'il était plus profitable pour les abeilles de butiner sur les deux patches, même à longue distance, car le gain de temps et d'énergie à récolter efficacement du pollen sur *Salix spp.* et du nectar sur *H. capitulum* était supérieur à la perte de temps et d'énergie à réaliser des allers-retours entre les deux patches. Si l'on transpose cette situation aux productions de semence hybride, on pourrait donc s'attendre à ce que les abeilles sauvages solitaires et les abeilles mellifères butineuses de pollen (qui butinent aussi le nectar) visitent plus fréquemment les lignées mâles stériles si ces lignées sécrétaient plus de nectar que les lignées mâle fertiles. Ceci pourrait avoir pour avantage de faire contribuer à la pollinisation des lignées mâle stériles directement les abeilles sauvages, qui sont généralement des pollinisateurs plus efficients que les abeilles mellifères (Garibaldi *et al.*, 2013). Le fait que les abeilles solitaires sauvages soient plus efficientes que les abeilles sociales et en particulier les abeilles mellifères peut s'expliquer par le fait que les abeilles solitaires récoltent et accumulent le pollen dans leurs scopae en gardant ce pollen sec, tandis que les abeilles sociales (abeilles mellifères et bourdons) accumulent le pollen dans leurs corbiculae en l'humectant avec du nectar, ce qui affecte la viabilité du pollen (Parker *et al.*, 2015).

Cette hypothèse montre l'intérêt qu'il y aurait, si cela se confirme possible, de sélectionner des lignées mâles stériles plus productives en nectar que les lignées actuelles (voir discussion dans le chapitre III) pour le producteur de semences oléagineuses en termes de retombées pour le rendement. Cela aurait également un intérêt pour l'apiculteur, qui loue ses colonies d'abeilles mellifères à l'agriculteur pour la pollinisation, en permettant d'augmenter potentiellement ses rendements en miel sur ce type de culture, et ce qui en retour pourrait avoir pour conséquence de faire baisser le prix des colonies pour l'agriculteur…

3.3. Apiculture : ajuster la demande à l'offre en nectar

Côté apiculture enfin, la connaissance de la vitesse de sécrétion nectarifère moyenne par fleur d'une culture donnée en fonction de la température et de l'humidité du sol (chapitre III) permettrait, en multipliant par la masse florale journalière et par la période de sécrétion nectarifère (chapitre I) sur les heures de jour pour les nectaires dépendants de la photosynthèse (chapitre III), d'estimer un potentiel nectarifère, c'est-à-dire la quantité totale de sucres produits par unité de surface de culture par jour, pour un contexte pédoclimatique donné. Cette connaissance pourrait permettre d'optimiser la charge en colonies d'abeilles mellifères par unité de surface de culture, de façon à ce qu'il y ait suffisamment de colonies pour exploiter au mieux le potentiel nectarifère disponible de la culture, et à ce qu'il n'y en ait pas trop pour empêcher les colonies de rentrer en compétition les unes avec les autres. Les apiculteurs pourraient alors répartir leurs cheptels de façon plus rationnelle, de façon à optimiser les miellées en milieu agricole. Dans cette perspective, on pourrait de même introduire les concepts d'*offre* et de *demande*, mais pour l'approvisionnement des colonies. Le nectar ne serait cette fois plus le *prix*, mais la *marchandise*. Le nectar étant librement accessible aux insectes, la *marchandise* serait dans cette situation gratuite. Et il ne faudrait plus ajuster l'*offre* à la *demande* comme pour la pollinisation, mais ajuster cette fois la *demande* en nectar (c'est-à-dire le nombre d'abeilles mellifères) à l'*offre* en nectar.

BIBLIOGRAPHIE

Abele D, Heise K, Pörtner HO, Puntarulo S. 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. *The Journal of Experimental Biology*, 205: 1831-1841.

Abou-Shaara HF, Owayss AA, Ibrahim YY, Basuny NK. 2017. A review of impacts of temperature and relative humidity on various activities of honey bees. *Insectes Sociaux*, 64(4): 455-463.

Acar I, Kakani VG. 2010. The effects of temperature on *in vitro* pollen germination and pollen tube growth of *Pistacia* spp. *Scientia Horticulturae*, 125(4): 569-572.

Adler A. 2017. lamW: Lambert-W Function. R package version 1.3.0. http://CRAN.R-project.org/package=lamW

Aguilar-Alberola JA, Mesquita-Joanes F. 2014. Breaking the temperature-size rule: Thermal effects on growth, development and fecundity of a crustacean from temporary waters. *Journal of Thermal Biology*, 42: 15-24.

Aizen MA, Basilio A. 1998. Sex differential nectar secretion in protandrous *Alstroemeria aurea* (Alstroemeriaceae): is production altered by pollen removal and receipt?. *American Journal of Botany*, 85(2): 245-252.

Aizen MA, Harder LD. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology*, 88: 271-281.

Aizen MA, Morales CL, Vázquez DP, Garibaldi LA, Sáez A, Harder LD. 2014. When mutualism goes bad: density-dependent impacts of introduced bees on plant reproduction. *New Phytologist*, 204(2): 322-328.

Akaike H. 1973. Information theory as an extension of the maximum likelihood principle. In: Petrov BN, Csaki F (eds). *Proceedings Second International Symposium on Information Theory*. Budapest, Hungary: Akademiai Kiado, pp. 267-281.

Akamine EK, Girolami G. 1959. Pollination and fruit set in the yellow passion fruit. *Technical Bulletin of the Hawaii Agricultural Experiment Station, University of Hawaii*, 39.

Allen RG, Balin AK. 2002. Metabolic Rate, Free Radicals and Aging. Volume I. In: Cutler RG, Rodriguez H. (eds). *Critical Reviews of Oxidative Stress and Aging: Advances in Basic Science. Diagnostics and Intervention*. Singapore: World Scientific, pp. 3-23.

Allen AM, Hiscock SJ. 2008. Evolution and phylogeny of self-incompatibility systems in angiosperms. In: Franklin-Tong VE (ed). *Self-Incompatibility in Flowering Plants*. Berlin, Germany: Springer, pp. 73-101.

Alonso C. 2005. Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*. *American Journal of Botany*, 92(8): 1264-1269.

Alonso C, Herrera CM, Ashman TL. 2012. A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. *New Phytologist*, 193(2): 532-542.

Anderson GJ, Bernardello G, Lopez PS, Crawford DJ, Stuessy TF. 2000a. Reproductive biology of *Wahlenbergia* (Campanulaceae) endemic to Robinson Crusoe Island (Chile). *Plant Systematics and Evolution*, 223(1): 109-123.

Anderson GJ, Bernardello G, Lopez P, Stuessy TF, Crawford DJ. 2000b. Dioecy and wind pollination in *Pernettya rigida* (Ericaceae) of the Juan Fernández Islands. *Botanical Journal of the Linnean Society*, 132(2): 121-141.

Anderson GJ, Bernardello G, Stuessy TF, Crawford DJ. 2001. Breeding system and pollination of selected plants endemic to Juan Fernández Islands. *American Journal of Botany*, 88(2): 220-233.

Andreucci MP, Moot DJ, Black AD, Sedcole R. 2016. A comparison of cardinal temperatures estimated by linear and nonlinear models for germination and bulb growth of forage brassicas. *European Journal of Agronomy*, 81: 52-63.

Andrikopoulos CJ, Cane JH. 2018. Two prolonged bee visits suffice to maximize drupelet set for red raspberry. *HortScience*, 53(10): 1404-1406.

Angilletta MJ. 2009. Evolutionary thermal biology. In: *Thermal Adaptation: A Theoretical and Empirical Synthesis*. New York, NY, USA: Oxford University Press, pp. 1-18.

Aras P, de Oliveira D, Savoie L. 1996. Effect of a honey bee (Hymenoptera: Apidae) gradient on the pollination and yield of lowbush blueberry. *Journal of Economic Entomology*, 89(5): 1080-1083.

Armbruster WS. 2012. Evolution and ecological implications of "specialized" pollinator rewards. In: Patiny S (ed). *Evolution of Plant-Pollinator Relationships*. New York, NY, USA: Cambridge University Press, pp. 44-67.

Arroyo MT, Dudley LS, Jespersen G, Pacheco DA, Cavieres LA. 2013. Temperature-driven flower longevity in a high-alpine species of *Oxalis* influences reproductive assurance. *New Phytologist*, 200: 1260-1268.

Ashman TL. 2004. Flower longevity. In: Noodén LD (ed). *Plant Cell Death Processes.* San Diego, CA, USA: Elsevier Academic Press, pp. 349-362.

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, 85: 2408-2421.

Ashman TL, Schoen DJ. 1994. How long should flowers live?. *Nature*, 371: 788-791.

Ashman TL, Schoen DJ. 1996. Floral longevity: fitness consequences and resource costs. In: Lloyd DG, Barrett SCH (eds). *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants*. New York, NY, USA: Chapman & Hall, pp. 112-139.

Bajcz AW, Hiebeler D, Drummond FA. 2017. Grid-Set-Match, an agent-based simulation model, predicts fruit set for the lowbush blueberry (*Vaccinium angustifolium*) agroecosystem. *Ecological Modelling*, 361: 80-94.

Balakhnina TI. 2015. Plant responses to soil flooding. In: Tripathi BN, Müller M (eds). *Stress Responses in Plants: Mechanisms of Toxicity and Tolerance*. Cham, Switzerland: Springer International Publishing, pp. 115-142.

Baskin JM, Baskin CC. 2015. Pollen (microgametophyte) competition: an assessment of its significance in the evolution of flowering plant diversity, with particular reference to seed germination. *Seed Science Research*, 25(1): 1-11.

Bates D, Mächler M, Bolker BM, Walker SC. 2015a. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1): 1-48.

Bates D, Mächler M, Bolker BM, Walker SC. 2015b. lme4: linear mixed-effects models using 'Eigen' and S4. R package version 1.1-14. URL http://cran.r-project.org/web/packages/lme4/index.html

Bates DM, Watts DG. 1988. *Nonlinear Regression Analysis and Its Applications*. New York, NY, USA: John Wiley & Sons.

Batra SWT. 1995. Bees and pollination in our changing environment. *Apidologie*, 26(5): 361-370.

Baty F, Ritz C, Charles S, Brutsche M, Flandrois JP, Delignette-Muller ML. 2015. A toolbox for nonlinear regression in R: the package nlstools. Jounral of Statistical Software, 66: 1-21.

Baude M, Kunin WE, Boatman ND, *et al*. 2016. Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature*, 30(7588):85-88.

Beale KM, Leydon AR, Johnson MA. 2012. Gamete fusion is required to block multiple pollen tubes from entering an *Arabidopsis* ovule. *Current Biology*, 22(12): 1090-1094.

Becher MA, Grimm V, Thorbek P, Horn J, Kennedy PJ, Osborne JL. 2014. BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. *Journal of Applied Ecology*, 51(2): 470-482.

Beckman KB, Ames BN. 1998. The free radical theory of aging matures. *Physiological Reviews*, 78: 547-581.

Bell SA, Cresswell JE. 1998. The phenology of gender in homogamous flowers: temporal change in the residual sex function of flowers of Oil-seed Rape (*Brassica napus*). *Functional Ecology*, 12: 298-306.

Bell CD, Soltis DE, Soltis PS. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany*, 97(8): 1296-1303.

Benedek P, Manninger S, Nagy B. 1972. The number of colonies and the density of honeybees in sunflower fields in relation to the pollination of the crop. *Zeitschrift für Angewandte Entomologie*, 71(1-4): 385-389.

Benjamin FE, Winfree R. 2014. Lack of pollinators limits fruit production in commercial blueberry (*Vaccinium corymbosum*). *Environmental Entomology*, 43(6): 1574-1583.

Bernardello G. 2007. A Systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E (eds). *Nectaries and Nectar*. Dordrecht, Netherlands: Springer, pp. 19-128.

Bernardello G, Anderson GJ, Stuessy TF, Crawford DJ. 2001. A survey of floral traits, breeding systems, floral visitors, and pollination systems of the angiosperms of the Juan Fernández Islands (Chile). *Botanical Review*, 67(3): 255-308.

Bernardello G, Galetto L, Anderson GJ. 2000. Floral nectary structure and nectar chemical composition of some species from Robinson Crusoe Island (Chile). *Canadian Journal of Botany*, 78(7): 862-871.

Berry J, Björkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiol*ogy, 31(1): 491-543.

Bertin RI. 1982. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). *American Journal of Botany*, 69(1): 122-134.

Bertin RI. 1990. Effects of pollination intensity in *Campsis radicans*. *American Journal of Botany*, 77(2): 178-187.

Bishop JA, Armbruster WS. 1999. Thermoregulatory abilities of Alaskan bees: effects of size, phylogeny and ecology. *Functional Ecology*, 13(5): 711-724.

Blitzer EJ, Gibbs J, Park MG, Danforth BN. 2016. Pollination services for apple are dependent on diverse wild bee communities. *Agriculture, Ecosystems & Environment*, 221: 1-7.

Blüthgen N, Klein AM. 2011. Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic and Applied Ecology*, 12(4): 282-291.

Boavida LC, McCormick S. 2007. Temperature as a determinant factor for increased and reproducible in vitro pollen germination in *Arabidopsis thaliana*. *The Plant Journal*, 52(3): 570-582.

Bolker BM. 2008. *Ecological models and data in R*. Princeton, NJ, USA: Princeton University Press.

Bommarco R, Marini L, Vaissière BE. 2012. Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia*, 169(4): 1025-1032.

Bonhomme R. 2000. Bases and limits to using 'degree.day' units. *European Journal of Agronomy*, 13: 1-10.

Bonnet A. 1779. Œuvres d'histoire et de philosophie. i. Traité d'insectologie. Neuchâtel, Switzerland: Samuel Fauche, libraire du roi.

Bonnier G. 1879. Les nectaires : étude critique, anatomique et physiologique. PhD thesis, Faculté des Sciences de Paris, Paris.

Boose DL. 1997. Sources of variation in floral nectar production rate in *Epilobium canum* (Onagraceae): implications for natural selection. *Oecologia*, 110(4): 493-500.

Borghi M, Fernie AR, Schiestl FP, Bouwmeester HJ. 2017. The sexual advantage of looking, smelling, and tasting good: the metabolic network that produces signals for pollinators. *Trends in Plant Science*, 22(4): 338-350.

Bosch M, Waser NM. 2001. Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia*, 126(1): 76-83.

Bosi G. 1973. Méthode rapide pour la détermination par chromatographie en phase gazeuse des glucides du nectar : technique de prélèvement du nectar et de préparation des éthers triméthylsilyles en présence d'eau. *Apidologie*, 4(1): 57-64.

Branstetter MG, Danforth BN, Pitts JP, Faircloth BC, Ward PS, Buffington ML, Gates MW, Kula RR, Brady SG. 2017. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Current Biology*, 27(7): 1019-1025.

Breeze TD, Vaissière BE, Bommarco R, Petanidou T, Seraphides N, Kozák L, Scheper J, Biesmeijer JC, Kleijn D, Gyldenkærne S *et al*. 2014. Agricultural policies exacerbate honeybee pollination service supply-demand mismatches across Europe. *PloS one*, 9(1): e82996.

Brewbaker JL, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany*, 50(9): 859-865.

Brewbaker JL, Majumder SK. 1961. Cultural studies of the pollen population effect and the self-incompatibility inhibition. *American Journal of Botany*, 48: 457-464.

Brink RA. 1924. The physiology of pollen IV. Chemotropism; effects on growth of grouping grains; formation and function of callose plugs; summary and conclusions. *American Journal of Botany*, 11(7): 417-436.

Brittain C, Kremen C, Klein AM. 2013a. Biodiversity buffers pollination from changes in environmental conditions. *Global Change Biology*, 19(2): 540-547.

Brittain C, Williams N, Kremen C, Klein AM. 2013b. Synergistic effects of non-*Apis* bees and honey bees for pollination services. *Proceedings of the Royal Society of London B: Biological Sciences*, 280(1754): 20122767.

Bronstein JL, Alarcón R, Geber M. 2006. The evolution of plant–insect mutualisms. *New Phytologist*, 172(3): 412-428.

Burgett M, Burikam I. 1985. Number of adult honey bees (Hymenoptera: Apidae) occupying a comb: a standard for estimating colony populations. *Journal of Economic Entomology*, 78(5): 1154-1156.

Burgos L, Egea J. 1993. Apricot embryo-sac development in relation to fruit set. *Journal of Horticultural Science*, 68: 203-208.

Burgos L, Egea J, Dicenta F. 1991. Effective pollination period in apricot (*Prunus armeniaca* L.) varieties. *Annals of Applied Biology*, 119: 533-539.

Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: A practical information-theoretic approach*. Berlin, Germany: Springer.

Búrquez A, Corbet SA. 1991. Do flowers reabsorb nectar?. *Functional Ecology*, 5: 369-379.

Button L, Elle E. 2014. Wild bumble bees reduce pollination deficits in a crop mostly visited by managed honey bees. *Agriculture, Ecosystems & Environment*, 197: 255-263.

Calcagno V, Mailleret L, Wajnberg É, Grognard F. 2014. How optimal foragers should respond to habitat changes: a reanalysis of the Marginal Value Theorem. *Journal of Mathematical Biology*, 69(5): 1237-1265.

Campbell DR. 1986. Predicting plant reproductive success from models of competition for pollination. *Oikos*, 47(3): 257-266.

Cane JH, Schiffhauer D. 2003. Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* [Ericaceae]). *American Journal of Botany*, 90(10): 1425-1432.

Cappellari SC, Schaefer H, Davis CC. 2013. Evolution: pollen or pollinators - Which came first?. *Current Biology*, 23(8): R316-R318.

Carbonell-Bejerano P, Urbez C, Granell A, Carbonell J, Perez-Amador MA. 2011. Ethylene is involved in pistil fate by modulating the onset of ovule senescence and the GA-mediated fruit set in *Arabidopsis*. *BMC Plant Biology*, 11: 84.

Cardinal S, Danforth BN. 2013. Bees diversified in the age of eudicots. *Proceedings of the Royal Society of London B: Biological Sciences*, 280(1755): 20122686.

Carré S, Badenhausser I, Taséi JN, Le Guen J, Mesquida J. 1994. Pollen deposition by *Bombus terrestris* L, between male-fertile and male-sterile plants in *Vicia faba* L. *Apidologie*, 25(3): 338-349.

Carroll AB, Pallardy SG, Galen C. 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany*, 88(3): 438-446.

Carvalheiro LG, Veldtman R, Shenkute AG, Tesfay GB, Pirk CWW, Donaldson JS, Nicolson SW. 2011. Natural and within-farmland biodiversity enhances crop productivity. *Ecology Letters*, 14(3): 251-259.

Cerović R, Ružić Đ. 1992. Senescence of ovules at different temperatures and their effect on the behaviour of pollen tubes in sour cherry. *Scientia Horticulturae*, 51: 321-327.

Cerović R, Ružić Đ, Mićić N. 2000. Viability of plum ovules at different temperatures. Annals of Applied Biology, 137: 53-59.

Chabert S, Lemoine T, Cagnato MR, Morison N, Vaissière BE. 2018. Flower age expressed in thermal time: is nectar secretion synchronous with pistil receptivity in oilseed rape (*Brassica napus* L.)?. *Environmental and Experimental Botany*, 155: 628-640.

Chambó ED, de Oliveira NTE, Garcia RC, Ruvolo-Takasusuki MCC, de Toledo VAA. 2017. Statistical modeling of insect behavioral response to changes in weather conditions in *Brassica napus* L. *Arthropod-Plant Interactions*, 11(4): 613-621.

Charnov EL. 1976. Optimal Foraging, the Marginal Value Theorem. *Theoretical Population Biology*, 9(2): 129-136.

Chautá-Mellizo A, Campbell SA, Bonilla MA, Thaler JS, Poveda K. 2012. Effects of natural and artificial pollination on fruit and offspring quality. *Basic and Applied Ecology*, 13(6): 524-532.

Chaves MM, Flexas J, Pinheiro C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, 103(4): 551-560.

Chen YF, Matsubayashi Y, Sakagami Y. 2000. Peptide growth factor phytosulfokine-α contributes to the pollen population effect. *Planta*, 211(5): 752-755.

Chifflet R. 2010. *Faune pollinisatrice, paysage et echelle spatiale des flux de pollen chez Brassica napus L. (Brassicaceae)*. PhD thesis, Université d'Avignon et des Pays de Vaucluse, Avignon, France.

Clarke D, Robert D. 2018. Predictive modelling of honey bee foraging activity using local weather conditions. *Apidologie*, 49: 386-396.

Clarke D, Whitney H, Sutton G, Robert D. 2013. Detection and learning of floral electric fields by bumblebees. *Science*, 340(6128): 66-69.

Colinet H, Sinclair BJ, Vernon P, Renault D. 2015. Insects in fluctuating thermal environments. *Annual Review of Entomology*, 60: 123-140.

Collins TJ. 2007. ImageJ for microscopy. *Biotechniques*, 43(1): 25-30.

Cook AJ. 1897. Value of bee-keeping on the ranch. *American Bee Journal*, 30: 470-471.

Corbet SA. 1991. Applied pollination ecology. *Trends in Ecology & Evolution*, 6(1): 3-4.

Corbet SA. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. *Apidologie*, 34: 1-10.

Corbet SA, Fussell M, Ake R, Fraser A, Gunson C, Savage A, Smith K. 1993. Temperature and the pollinating activity of social bees. *Ecological Entomology*, 18: 17-30.

Corless RM, Gonnet GH, Hare DE, Jeffrey DJ, Knuth DE. 1996. On the Lambert *W* function. *Advances in Computational Mathematics*, 5(1): 329-359.

Costa CM, Yang S. 2009. Counting pollen grains using readily available, free image processing and analysis software. *Annals of Botany*, 104(5): 1005-1010.

Couvillon MJ, Walter CM, Blows EM, Czaczkes TJ, Alton KL, Ratnieks FL. 2015. Busy bees: variation in insect flower-visiting rates across multiple plant species. *Psyche: A Journal of Entomology*, ID134630: 1-7.

Crane E. .1999. History of the use of bees for crop pollination. In: *The World History of Beekeeping and Honey Hunting*. New York, NY, USA: Routledge, pp. 472-482.

Cresswell JE, Bassom AP, Bell SA, Collins SJ, Kelly TB. 1995. Predicted pollen dispersal by honey-bees and three species of bumble-bees foraging on oil-seed rape: a comparison of three models. *Functional Ecology*, 9: 829-841.

Cresswell JE, Osborne JL, Bell SA. 2002. A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. *Oikos*, 98(3): 375-384.

Cruden RW, Hermann SM. 1983. Studying nectar? Some observations on the art. In: Bentley B, Elias T (eds). *The Biology of Nectaries*. New York, NY, USA: Columbia University Press, pp. 223-241.

Cruden RW, Hermann SM, Peterson S. 1983. Patterns of nectar production and plant-pollinator coevolution. In: Bentley B, Elias T (eds). *The Biology of Nectaries*. New York, NY, USA: Columbia University Press, pp. 80-125.

Cruzan MB. 1986. Pollen tube distributions in *Nicotiana glauca*: evidence for density dependent growth. *American Journal of Botany*, 73(6): 902-907.

Cunningham SA, Fournier A, Neave MJ, Le Feuvre D. 2016. Improving spatial arrangement of honeybee colonies to avoid pollination shortfall and depressed fruit set. *Journal of Applied Ecology*, 53(2): 350-359.

Dafni A. 1992. Pollen and stigma biology. In: *Pollination Ecology: A Practical Approach*. New York, NY, USA: Oxford University Press, pp. 59-89.

Dafni A, Firmage D. 2000. Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant Systematics and Evolution*, 222: 113-132.

Dafni H, Lensky Y, Fahn A. 1988. Flower and nectar characteristics of nine species of Labiatae and their influence on honeybee visits. *Journal of Apicultural Research*, 27(2): 103-114.

Dafni A, Pacini E, Nepi M. 2005. Pollen and stigma biology. In: Dafni A, Kevan PG, Husband BC (eds). *Practical Pollination Biology*. Cambridge, Ontario, Canada: Enviroquest, pp. 83-146.

Damos P, Savopoulou-Soultani M. 2012. Temperature-driven models for insect development and vital thermal requirements. *Psyche*, 2012: 123405.

Darwin C. 1859. *On the Origin of Species by Means of Natural Selection*. London, UK: John Murray.

Darwin C. 1876. *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. London, UK: John Murray.

Davis AR. 2001. Searching and breeding for structural features of flowers correlated with high nectar-carbohydrate production. *Acta Horticulturae*, 561: 107–121.

Davis AR, Peterson RL, Shuel RW. 1986. Anatomy and vasculature of the floral nectaries of *Brassica napus* (Brassicaceae). *Canadian Journal of Botany*, 64: 2508-2516.

Davis AR, Pylatuik JD, Paradis JC, Low NH. 1998. Nectar-carbohydrate production and composition vary in relation to nectary anatomy and location within individual flowers of several species of Brassicaceae. *Planta*, 205: 305-318.

DeGrandi-Hoffman G, Hoopingarner R, Pulcer R. 1987. REDAPOL: pollination and fruit-set prediction model for 'Delicious' apples. *Environmental Entomology*, 16(2): 309-318.

DeGrandi-Hoffman G, Watkins JC. 2000. The foraging activity of honey bees *Apis mellifera* and non-*Apis* bees on hybrid sunflowers (*Helianthus annuus*) and its influence on cross-pollination and seed set. *Journal of Apicultural Research*, 39(1-2): 37-45.

Delaplane KS, Mayer DF. 2000. *Crop Pollination by Bees*. Wallingford, UK: CABI Publishing.

Delaplane KS, van der Steen J, Guzman-Novoa E. 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicultural Research*, 52(1): 1-12.

de Pinto MC, Locato V, De Gara L. 2012. Redox regulation in plant programmed cell death. *Plant, Cell & Environment*, 35: 234-244.

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, *et al*. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, 36(1): 27-46.

Doyle JA. 2012. Molecular and fossil evidence on the origin of angiosperms. *Annual Review of Earth and Planetary Sciences*, 40: 301-326.

Dreisig H. 1995. Ideal free distributions of nectar foraging bumblebees. *Oikos*, 72: 161-172.

Dresselhaus T, Franklin-Tong N. 2013. Male–female crosstalk during pollen germination, tube growth and guidance, and double fertilization. *Molecular Plant*, 6(4): 1018-1036.

Dumas C. 1984. De l'ovule à la graine : bases cytologiques et physiologiques de la fécondation. In: Pesson P, Louveaux J (eds). *Pollinisation et Productions Végétales*. Paris, France: INRA, pp. 47-72.

Dumas C, Zandonella P. 1984. Évolution des processus sexués chez les végétaux et notion d'angiospermie. In: Pesson P, Louveaux J (eds). *Pollinisation et Productions Végétales*. Paris, France: INRA, pp. 3-12.

Eaton LJ, Nams VO. 2012. Honey bee stocking numbers and wild blueberry production in Nova Scotia. *Canadian Journal of Plant Science*, 92(7): 1305-1310.

Eisikowitch D. 1981. Some aspects of pollination of oil-seed rape (*Brassica napus* L.). *Journal of Agricultural Science*, 96: 321-326.

Ellis CR, Feltham H, Park K, Hanley N, Goulson D. 2017. Seasonal complementary in pollinators of soft-fruit crops. *Basic and Applied Ecology*, 19: 45-55.

Endress PK. 1982. Syncarpy and alternative modes of escaping disadvantages of apocarpy in primitive angiosperms. *Taxon*, 31: 48-52.

Endress PK. 2001. The flowers in extant basal angiosperms and inferences on ancestral flowers. *International Journal of Plant Sciences*, 162(5): 1111-1140.

Endress PK. 2010. The evolution of floral biology in basal angiosperms. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365(1539): 411-421.

Endress PK, Igersheim A. 2000. Gynoecium structure and evolution in basal angiosperms. *International Journal of Plant Sciences*, 161(S6): S211-S223.

Erbar C. 2003. Pollen tube transmitting tissue: place of competition of male gametophytes. *International Journal of Plant Sciences*, 164(S5): S265-S277.

Faegri K, van der Pijl L. 1979. *The Principles of Pollination Ecology*. Third Revised Edition. Oxford, UK: Pergamon Press.

Falque M, Vincent A, Vaissiere BE, Eskes AB. 1995. Effect of pollination intensity on fruit and seed set in cacao (*Theobroma cacao* L.). *Sexual Plant Reproduction*, 8(6): 354-360.

Farmer KJ, Sohal RS. 1987. Effects of ambient temperature on free radical generation, antioxidant defenses and life span in the adult housefly, *Musca domestica*. *Experimental Gerontology*, 22: 59-65.

Farrar CL. 1931. The evaluation of bees for pollination. *Journal of Economic Entomology*, 24(3): 622-627

Farrar CL. 1937. The influence of colony populations on honey production. *Journal of Agricultural Research*, 54(12): 945-954.

Fernando DD, Lazzaro MD, Owens JN. 2005. Growth and development of conifer pollen tubes. *Sexual Plant Reproduction*, 18(4): 149-162.

Ferrer MM, Good SV. 2012. Self-sterility in flowering plants: preventing self-fertilization increases family diversification rates. *Annals of Botany*, 110(3): 535-553.

Fetscher AE, Kohn JR. 1999. Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). *American Journal of Botany*, 86(8): 1130-1135.

Field DL, Pickup M, Barrett SC. 2012. The influence of pollination intensity on fertilization success, progeny sex ratio, and fitness in a wind-pollinated, dioecious plant. *International Journal of Plant Sciences*, 173(2): 184-191.

Findlay N, Reed ML, Mercer FV. 1971. Nectar production in *Abutilon* III. Sugar secretion. *Austral Journal of Biological Science*, 24(3): 665-675.

Fischer AM. 2007. Nutrient remobilization during leaf senescence. In: Gan S (ed). *Senescence Processes in Plants*. *Annual Plant Reviews*, 26: 87-107.

Foyer CH, Noctor G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment*, 28: 1056-1071.

Franchi GG, Nepi M, Pacini E. 2014. Is flower/corolla closure linked to decrease in viability of desiccation-sensitive pollen? Facts and hypotheses: a review of current literature with the support of some new experimental data. *Plant Systematics and Evolution*, 300: 577-584.

Franklin-Tong VE. 2008. *Self-Incompatibility in Flowering Plants*. Berlin, Germany: Springer.

Free JB. 1993. *Insect Pollination of Crops*. Second Edition. Cambridge, UK: Academic Press.

Friedman WE. 1987. Growth and development of the male gametophyte of *Ginkgo biloba* within the ovule (*in vivo*). *American Journal of Botany*, 74(12): 1797-1815.

Fretwell SD, Lucas HL. 1969. On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica*, 19(1): 16-36.

Friedman WE. 1990. Double fertilization in *Ephedra*, a nonflowering seed plant: its bearing on the origin of angiosperms. *Science*, 247(4945): 951-954.

Friedman WE. 1993. The evolutionary history of the seed plant male gametophyte. *Trends in Ecology & Evolution*, 8(1): 15-21.

Friedman WE, Carmichael JS. 1996. Double fertilization in Gnetales: implications for understanding reproductive diversification among seed plants. *International Journal of Plant Sciences*, 157(S6): S77-S94.

Friis EM, Crane PR, Pedersen KR. 2011a. Introduction to angiosperms. In: *Early Flowers and Angiosperm Evolution*. New York, NY, USA: Cambridge University Press, pp. 1-21.

Friis EM, Crane PR, Pedersen KR. 2011b. Origin and age of angiosperms. In: *Early Flowers and Angiosperm Evolution*. New York, NY, USA: Cambridge University Press, pp. 141-162.

Friis EM, Crane PR, Pedersen KR. 2011c. History and evolution of pollination in angiosperms. In: *Early Flowers and Angiosperm Evolution*. New York, NY, USA: Cambridge University Press, pp. 415-443.

Friis EM, Endress PK. 1990. Origin and evolution of angiosperm flowers. *Advances in Botanical Research*, 17: 99-162.

Friis EM, Pedersen KR, Crane PR. 2006. Cretaceous angiosperm flowers: innovation and evolution in plant reproduction. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 232(2-4): 251-293.

Fründ J, Dormann CF, Holzschuh A, Tscharntke T. 2013. Bee diversity effects on pollination depend on functional complementarity and niche shifts. *Ecology*, 94(9): 2042-2054.

Fründ J, Dormann CF, Tscharntke T. 2011. Linné's floral clock is slow without pollinators–flower closure and plant-pollinator interaction webs. *Ecology Letters*, 14: 896-904.

Fulton M, Jesson LK, Bobiwash K, Schoen DJ. 2015. Mitigation of pollen limitation in the lowbush blueberry agroecosystem: effect of augmenting natural pollinators. *Ecosphere*, 6(11): 1-19.

Gaines-Day HR, Gratton C. 2016. Crop yield is correlated with honey bee hive density but not in high-woodland landscapes. *Agriculture, Ecosystems & Environment*, 218: 53-57.

Galetto L, Bernardello G. 1995. Characteristics of nectar secretion by *Lycium cestroides*, *L. ciliatum* (Solanaceae), and their hybrid. *Plant Species Biology*, 11: 157-163.

Galetto L, Bernardello G. 2004. Floral nectaries, nectar production dynamics and chemical composition in six *Ipomoea* species (Convolvulaceae) in relation to pollinators. *Annals of Botany*, 94(2): 269-280.

Galetto L, Bernardello G, Isele IC, Vesprini J, Speroni G, Berduc A. 2000. Reproductive biology of *Erythrina crista-galli* (Fabaceae). *Annals of the Missouri Botanical Garden*, 87: 127-145.

Gallagher MK, Campbell DR. 2017. Shifts in water availability mediate plant–pollinator interactions. *New Phytol*ogist, 215(2): 792-802.

Gallai N, Salles JM, Settele J, Vaissière BE. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68(3): 810-821.

Gan S, Amasino RM. 1997. Making sense of senescence. Molecular genetic regulation and manipulation of leaf senescence. *Plant Physiology*, 113: 313-319.

Garibaldi LA, Aizen MA, Cunningham S, Klein AM. 2009. Pollinator shortage and global crop yield: looking at the whole spectrum of pollinator dependency. *Communicative & Integrative Biology*, 2(1): 37-39.

Garibaldi LA, Carvalheiro LG, Vaissière BE, Gemmill-Herren B, Hipólito J, Freitas BM, Ngo HT, Azzu N, Sáez A, Åtsröm J *et al*. 2016. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science*, 351(6271): 388-391.

Garibaldi LA, Requier F, Rollin O, Andersson GK. 2017. Towards an integrated species and habitat management of crop pollination. *Current Opinion in Insect Science*, 21: 105-114.

Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O *et al*. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, 339(6127): 1608-1611.

Garratt MP, Bishop J, Degani E, Potts SG, Shaw RF, Shi A, Roy S. 2018a. Insect pollination as an agronomic input: Strategies for oilseed rape production. *Journal of Applied Ecology*, 55(6): 2834-2842.

Garratt MP, Brown R, Hartfield C, Hart A, Potts SG. 2018b. Integrated crop pollination to buffer spatial and temporal variability in pollinator activity. *Basic and Applied Ecology*, 32: 77-85.

Garratt MP, Coston DJ, Truslove CL, Lappage MG, Polce C, Dean R, Biesmeijer JC, Potts SG. 2014. The identity of crop pollinators helps target conservation for improved ecosystem services. *Biological Conservation*, 169: 128-135.

Gelbart G, von Aderkas P. 2002. Ovular secretions as part of pollination mechanisms in conifers. *Annals of Forest Science*, 59(4): 345-357.

Geslin B, Aizen MA, Garcia N, Pereira AJ, Vaissière BE, Garibaldi LA. 2017. The impact of honey bee colony quality on crop yield and farmers' profit in apples and pears. *Agriculture, Ecosystems and Environment*, 248: 153-161.

Gibbs PE. 2014. Late-acting self-incompatibility–the pariah breeding system in flowering plants. *New Phytologist*, 203(3): 717-734.

Gillespie S, Long R, Williams N. 2015. Indirect effects of field management on pollination service and seed set in hybrid onion seed production. *Journal of Economic Entomology*, 108(6): 2511-2517.

Goulson D, Nicholls E, Botías C, Rotheray EL. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347: 1255957.

Grass I, Meyer S, Taylor PJ. Foord SH, Hajek P, Tscharntke T. 2018. Pollination limitation despite managed honeybees in South African macadamia orchards. *Agriculture, Ecosystems & Environment*, 260: 11-18.

Greenleaf SS, Kremen C. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences*, 103(37): 13890-13895.

Greggers U, Koch G, Schmidt V, Dürr A, Floriou-Servou A, Piepenbrock D, Göpfert MC, Menzel R. 2013. Reception and learning of electric fields in bees. *Proceedings of the Royal Society of London B: Biological Sciences*, 280(1759): 20130528.

Grimm SS, Jones JW, Boote KJ, Herzog DC. 1994. Modeling the occurrence of reproductive stages after flowering for four soybean cultivars. *Agronomy Journal*, 86: 31-38.

Gu S. 2016. Growing degree hours - a simple, accurate, and precise protocol to approximate growing heat summation for grapevines. *International Journal of Biometeorology*, 60: 1123-1134.

Guerra ME, Wünsch A, López-Corrales M, Rodrigo J. 2011. Lack of fruit set caused by ovule degeneration in Japanese plum. *Journal of the American Society of Horticultural Science*, 136: 375-381.

Guiboileau A, Sormani R, Meyer C, Masclaux-Daubresse C. 2010. Senescence and death of plant organs: nutrient recycling and developmental regulation. *Comptes Rendus Biologies*, 333: 382-391.

Gunderson AR, Leal M. 2016. A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Ecology Letters*, 19(2): 111-120.

Harder LD, Aizen MA. 2010. Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365(1539): 529-543.

Harder LD, Aizen MA, Richards SA. 2016a. The population ecology of male gametophytes: the link between pollination and seed production. *Ecology Letters*, 19(5): 497-509.

Harder LD, Aizen MA, Richards SA, Joseph MA, Busch JW. 2016b. Diverse ecological relations of male gametophyte populations in stylar environments. *American Journal of Botany*, 103(3): 484-497.

Harder LD, Routley MB. 2006. Pollen and ovule fates and reproductive performance by flowering plants. In: Harder LD, Barrett SCH (eds). *Ecology and Evolution of Flowers.* New York, NY, USA: Oxford University Press, pp. 61-80.

Hargreaves AL, Harder LD, Johnson SD. 2009. Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biological Reviews*, 84(2): 259-276.

Hargreaves AL, Harder LD, Johnson SD. 2010. Native pollen thieves reduce the reproductive success of a hermaphroditic plant, *Aloe maculata*. *Ecology*, 91(6): 1693-1703.

Harman D. 1956. Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology*, 11: 298-300.

Hartley G, Nell TA, Leonard RT, Barrett JE, Boyle TH. 1995. Effect of interior light and temperature on longevity of *Rhipsalidopsis*. *Acta Horticulturae*, 405: 164-169.

Hebbar KB, Rose HM, Nair AR, Kannan S, Niral V, Arivalagan M, Gupta A, Samsudeen K, Chandran KP, Chowdappa P *et al*. 2018. Differences in *in vitro* pollen germination and pollen tube growth of coconut (*Cocos nucifera* L.) cultivars in response to high temperature stress. *Environmental and Experimental Botany*, 153: 35-44.

Hedhly A. 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environmental and Experimental Botany*, 74: 9-16.

Hedhly A, Hormaza JI, Herrero M. 2003. The effect of temperature on stigmatic receptivity in sweet cherry (*Prunus avium* L.). *Plant, Cell & Environment*, 26: 1673-1680.

Hedhly A, Hormaza JI, Herrero M. 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). *American Journal of Botany*, 91(4): 558-564.

Hedhly A, Hormaza JI, Herrero M. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology*, 7: 476-483.

Hedhly A, Hormaza JI, Herrero M. 2009. Global warming and sexual plant reproduction. *Trends in Plant Science*, 14(1): 30-36.

Henselek Y, Eilers EJ, Kremen C, Hendrix SD, Klein AM. 2018. Pollination requirements of almond (*Prunus dulcis*): combining laboratory and field experiments. *Journal of Economic Entomology*, 111(3): 1006-1013.

Herrero M, Hormaza JI. 1996. Pistil strategies controlling pollen tube growth. *Sexual Plant Reproduction*, 9(6): 343-347.

Heslop-Harrison JS. 1992. Pollen capture, adhesion and hydration. In: Cresti M, Tiezzi A (eds). *Sexual Plant Reproduction*. Berlin, Germany: Springer, pp. 81-88.

Heyneman AJ. 1983. Optimal sugar concentrations of floral nectars - dependence on sugar intake efficiency and foraging costs. *Oecologia*, 60(2): 198-213.

Higashiyama T, Takeuchi H. 2015. The mechanism and key molecules involved in pollen tube guidance. *Annual Review of Plant Biology*, 66: 393-413.

Higashiyama T, Yabe S, Sasaki N, Nishimura Y, Miyagishima SY, Kuroiwa H, Kuroiwa T. 2001. Pollen tube attraction by the synergid cell. *Science*, 293(5534): 1480-1483.

Hiroi K, Sone M, Sakazono S, Osaka M, Masuko-Suzuki H, Matsuda T, Suzuki G, Suwabe K, Watanabe M. 2013. Time-lapse imaging of self-and cross-pollinations in *Brassica rapa*. *Annals of Botany*, 112(1): 115-122.

Hoehn P, Tscharntke T, Tylianakis JM, Steffan-Dewenter I. 2008. Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1648): 2283-2291.

Holm SO. 1994. Pollination density-effects on pollen germination and pollen tube growth in *Betula pubescens* Ehrh. in northern Sweden. *New Phytologist*, 126(3): 541-547.

Hormaza JI, Herrero M. 1992. Pollen selection. *Theoretical and Applied Genetics*, 83(6-7): 663-672.

Hu S, Dilcher DL, Taylor DW. 2012. Pollen evidence for the pollination biology of early flowering plants. In: Patiny S (ed). *Evolution of Plant-Pollinator Relationships*. New York, NY, USA: Cambridge University Press, pp. 165-236.

Huber H. 1956. Die Abhängigkeit der Nektarsekretion von Temperatur, Luft- und Bodenfeuchtigkeit. *Planta*, 48(1): 47-98.

Hudewenz A, Pufal G, Bögeholz AL, Klein AM. 2014. Cross-pollination benefits differ among oilseed rape varieties. *The Journal of Agricultural Science*, 152(5): 770-778.

Imdorf A, Buehlmann G, Gerig L, Kilchenmann V, Wille H. 1987. Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. *Apidologie*, 18(2): 137-146.

Imdorf A, Gerig L. 2001. Course in determination of colony strength. *Swiss Federal Dairy Research Institute, Liebefeld*. *Schweiz Bienen-Zeitung*, 106: 199-204.

Inouye DW, Larson BMH, Ssymank A, Kevan PG. 2015. Flies and flowers III: Ecology of foraging and pollination. *Journal of Pollination Ecology*, 16: 115-133.

IPCC. 2013. Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds). *Climate Change 2013: The physical science basis. Contribution of working group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK & New York, NY, USA: Cambridge University Press.

Isaacs R, Kirk AK. 2010. Pollination services provided to small and large highbush blueberry fields by wild and managed bees. *Journal of Applied Ecology*, 47(4): 841-849.

Isaacs R, Williams N, Ellis J, Pitts-Singer TL, Bommarco R, Vaughan M. 2017. Integrated Crop Pollination: Combining strategies to ensure stable and sustainable yields of pollination-dependent crops. *Basic and Applied Ecology*, 22: 44-60.

Iwano M, Takayama S. 2012. Self/non-self discrimination in angiosperm self-incompatibility. *Current Opinion in Plant Biology*, 15(1): 78-83.

Jauker F, Bondarenko B, Becker HC, Steffan-Dewenter I. 2012. Pollination efficiency of wild bees and hoverflies provided to oilseed rape. *Agricultural and Forest Entomology*, 14(1): 81-87.

Johnson SD. 2006. Pollinator-driven speciation in plants. In: Harder LD, Barrett SCH, eds. *Ecology and Evolution of Flowers.* New York, NY, USA: Oxford University Press, 295-310.

Johnson VE. 2013. Revised standards for statistical evidence. *Proceedings of the National Academy of Sciences*, 110(48): 19313-19317.

Kaczorowski RL, Juenger TE, Holtsford TP. 2008. Heritability and correlation structure of nectar and floral morphology traits in *Nicotiana alata*. *Evolution*, 62(7): 1738-1750.

Kakani VG, Prasad PV, Craufurd PQ, Wheeler TR. 2002. Response of in vitro pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. *Plant, Cell & Environment*, 25(12): 1651-1661.

Kakani VG, Reddy KR, Koti S, Wallace TP, Prasad PVV, Reddy VR, Zhao D. 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Annals of Botany*, 96(1): 59-67.

Kamler F. 1984. Selection of lines of winter rape (*Brassica napus* v. *arvensis*) for nectar production. *V^ème^ Symposium International sur la Pollinisation*. 27-30 Septembre 1983, Versailles. *Les Colloques de l'INRA*, 21: 135-139.

Kearns CA, Inouye DW. 1993. *Techniques for Pollination Biologists*. University Press of Colorado.

Keil G, Cummings E, de Magalhaes JP. 2015. Being cool: how body temperature influences ageing and longevity. *Biogerontology*, 16: 383-397.

Kellar BM. 2018. *Honey Bees and Apple Trees: Hood River, Oregon as a Case Study for the Creation of the Honey Bee Pollination Industry*. PhD Thesis, Oregon State University.

Kelly MA, Zieba AP, Buttemer WA, Hulbert AJ. 2013. Effect of temperature on the rate of ageing: an experimental study of the blowfly *Calliphora stygia*. *PloS one*, 8: e73781.

Kerner von Marilaun A. 1896. The natural history of plants: their forms, growth, reproduction and distribution. London, UK: Blackie & Son.

Kessler SA, Grossniklaus U. 2011. She's the boss: signaling in pollen tube reception. *Current Opinion in Plant Biology*, 14(5): 622-627.

Kevan PG, Clark EA, Thomas VG. 1990. Insect pollinators and sustainable agriculture. *American Journal of Alternative Agriculture*, 5(1): 13-22.

King C, Ballantyne G, Willmer PG. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, 4(9): 811-818.

Kirk WD, Ali M, Breadmore KN. 1995. The effects of pollen beetles on the foraging behaviour of honey bees. *Journal of Apicultural Research*, 34(1): 15-22.

Kirkegaard JA, Sprague SJ, Lilley JM, McCormick JI. Virgona JM, Morrison MJ. 2012. Physiological response of spring canola (*Brassica napus*) to defoliation in diverse environments. *Field Crops Research*, 125: 61-68.

Klass MR. 1977. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mechanisms of Ageing and Development*, 6: 413-429.

Klein AM, Boreux V, Fornoff F, Mupepele AC, Pufal G. 2018. Relevance of wild and managed bees for human well-being. *Current Opinion in Insect Science*, 26: 82-88.

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London B: Biological Sciences*, 274(1608): 303-313.

Koh I, Lonsdorf EV, Artz DR, Pitts-Singer TL, Ricketts TH. 2018. Ecology and economics of using native managed bees for almond pollination. *Journal of Economic Entomology*, 111(1): 16-25.

Koh I, Lonsdorf EV, Williams NM, Brittain C, Isaacs R, Gibbs J, Ricketts TH. 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. *Proceedings of the National Academy of Sciences*, 113(1): 140-145.

Knauer AC, Schiestl FP. 2015. Bees use honest floral signals as indicators of reward when visiting flowers. *Ecology Letters*, 18(2): 135-143.

Knight TM, Steets JA, Ashman TL. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany*, 93(2): 271-277.

Koh I, Lonsdorf EV, Artz DR, Pitts-Singer TL, Ricketts TH. 2017. Ecology and economics of using native managed bees for almond pollination. *Journal of Economic Entomology*, 111(1): 16-25.

Kohn JR, Waser NM. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. *American Journal of Botany*, 72(7): 1144-1148.

Kremen C. 2018. The value of pollinator species diversity. *Science*, 359(6377): 741-742.

Kremen C, Williams NM, Thorp RW. 2002. Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences*, 99(26): 16812-16816.

Krupnick GA, Weis AE, Campbell DR. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology*, 80(1): 125-134.

Labandeira CC, Kvaček J, Mostovski MB. 2007. Pollination drops, pollen, and insect pollination of Mesozoic gymnosperms. *Taxon*, 56(3): 663-695.

Labarca C, Loewus F. 1973. The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum*: II. Production and utilization of exudate from stigma and stylar canal. *Plant Physiology*, 52(2): 87-92.

Labouche AM, Richards SA, Pannell JR. 2017. Effects of pollination intensity on offspring number and quality in a wind-pollinated herb. *Journal of Ecology*, 105(1): 197-208.

Langenberger MW, Davis AR. 2002. Temporal changes in floral nectar production, reabsorption, and composition associated with dichogamy in annual caraway (*Carum carvi*; Apiaceae). *American Journal of Botany*, 89(10): 1588-1598.

Leiss KA, Klinkhamer PGL. 2005. Genotype by environment interactions in the nectar production of *Echium vulgare*. *Functional Ecology*, 19(3): 454-459.

Leonard AS, Francis JS. 2017. Plant–animal communication: past, present and future. *Evolutionary Ecology*, 31(2): 143-151.

Leroy B. 2016. Rarity: calculation of rarity indices for species and assemblages of species. R package version 1.3-6. URL http://cran.r-project.org/web/packages/Rarity/index.html

Lindström SA, Herbertsson L, Rundlöf M, Smith HG, Bommarco R. 2016. Large-scale pollination experiment demonstrates the importance of insect pollination in winter oilseed rape. *Oecologia*, 180(3): 759-769.

Lindström SAM, Klatt BK, Smith HG, Bommarco R. 2018. Crop management affects pollinator attractiveness and visitation in oilseed rape. *Basic and Applied Ecol*ogy, 26: 82-88.

Liochev SI. 2013. Reactive oxygen species and the free radical theory of aging. *Free Radical Biology and Medicine*, 60: 1-4.

Little S, Prior N, Pirone C, von Aderkas P. 2014. Pollen-ovule interactions in gymnosperms. In: Ramawat KG, Mérillon JM, Shivanna KR (eds). *Reproductive Biology of Plants*. Boca Raton, FL, USA: CRC Press, pp. 97-117.

Loeb J, Northrop J. 1917. On the influence of food and temperature upon the duration of life. *Journal of Biological Chemistry*, 32: 103-121.

Lopez RG, Runkle ES. 2004. The effect of temperature on leaf and flower development and flower longevity of *Zygopetalum* Redvale 'Fire kiss' orchid. *HortScience*, 39: 1630-1634.

Lora J, Herrero M, Hormaza JI. 2011. Stigmatic receptivity in a dichogamous early-divergent angiosperm species, *Annona cherimola* (Annonaceae): Influence of temperature and humidity. *American Journal of Botany*, 98: 265-274.

Lora J, Hormaza JI, Herrero M. 2016. The diversity of the pollen tube pathway in plants: toward an increasing control by the sporophyte. *Frontiers in Plant Science*, 7(107).

Luo EY, Ogilvie JE, Thomson JD. 2014. Stimulation of flower nectar replenishment by removal: a survey of eleven animal-pollinated plant species. *Journal of Pollination Ecoogyl*, 12(7): 52-62.

Magallón S. 2010. Using fossils to break long branches in molecular dating: a comparison of relaxed clocks applied to the origin of angiosperms. *Systematic Biology*, 59(4): 384-399.

Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL, Hernández-Hernández T. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist*, 207(2): 437-453.

Mallick SA. 2000. Technique for washing nectar from the flowers of Tasmanian leatherwood (*Eucryphia lucida* Eucryphiaceae). *Austral Ecology*, 25(2): 210-212.

Mallinger RE, Gratton C. 2015. Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. *Journal of Applied Ecology*, 52(2): 323-330.

Mallinger RE, Prasifka JR. 2017. Bee visitation rates to cultivated sunflowers increase with the amount and accessibility of nectar sugars. *Journal of Applied Entomology*, 141(7): 561-573.

Marini L, Tamburini G, Petrucco-Toffolo E, Lindström SA, Zanetti F, Mosca G, Bommarco R. 2015. Crop management modifies the benefits of insect pollination in oilseed rape. *Agriculture, Ecosystems & Environment*, 207: 61-66.

Maruyama D, Hamamura Y, Takeuchi H, Susaki D, Nishimaki M, Kurihara D, Kasahara RD, Higashiyama T. 2013. Independent control by each female gamete prevents the attraction of multiple pollen tubes. *Developmental Cell*, 25(3): 317-323.

Mattila HR, Seeley TD. 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science*, 317(5836): 362-364.

McArthur MC, Sohal RS. 1982. Relationship between metabolic rate, aging, lipid peroxidation, and fluorescent age pigment in milkweed bug, *Oncopeltus fasciatus* (Hemiptera). *Journal of Gerontology*, 37: 268-274.

McDade LA, Davidar P. 1984. Determinants of fruit and seed set in *Pavonia dasypetala* (Malvaceae). *Oecologia*, 64(1): 61-67.

McGregor SE. 1976. *Insect Pollination of Cultivated Crop Plants*. Agriculture Handbook No. 496. Washington, DC, USA: USDA.

McKenna MA, Thomson JD. 1988. A technique for sampling and measuring small amounts of floral nectar. *Ecology*, 69(4): 1306-1307.

McShane BB, Gal D, Gelman A, Robert C, Tackett JL. 2017. Abandon statistical significance. arXiv preprint arXiv:1709.07588

Melathopoulos AP, Cutler GC, Tyedmers P. 2015. Where is the value in valuing pollination ecosystem services to agriculture?. *Ecological Economics*, 109: 59-70.

Mesquida J, Marilleau R, Pham-Delègue MH, Renard M. 1988. A study of rapeseed (*Brassica napus* L. var. *oleifera* Metzger) flower nectar secretions. *Apidologie*, 19(3): 307-318.

Mesquida J, Pham-Delègue MH, Marilleau R, Le Métayer M, Renard M. 1991. La sécrétion nectarifère des fleurs de cybrides mâles-stériles de colza d'hiver (*Brassica napus* L). *Agronomie*, 11(3): 217-227.

Mesquida J, Renard M. 1981. Pollinisation du colza d'hiver male-fertile et male-stérile (*Brassica napus* L. var. *oleifera* Metzger) par l'abeille domestique (*Apis m. mellifica* L.). Effets sur la phénologie et le rendement. *Apidologie*, 12(4): 345-362.

Michez D, Vanderplanck M, Engel MS. 2012. Fossil bees and their plant associates. In: Patiny S (ed). *Evolution of Plant-Pollinator Relationships*. New York, NY, USA: Cambridge University Press, pp. 103-164.

Miquel J, Lundgren PR, Bensch KG, Atlan H. 1976. Effects of temperature on the life span, vitality and fine structure of *Drosophila melanogaster*. *Mechanisms of Ageing and Development*, 5: 347-370.

Mitchell RJ. 1997a. Effects of pollination intensity on *Lesquerella fendleri* seed set: variation among plants. *Oecologia*, 109(3): 382-388.

Mitchell RJ. 1997b. Effects of pollen quantity on progeny vigor: evidence from the desert mustard *Lesquerella fendleri*. *Evolution*, 51(5): 1679-1684.

Mitchell RJ. 2004. Heritability of nectar traits: why do we know so little?. *Ecology*, 85(6): 1527-1533.

Morrant DS, Schumann R, Petit S. 2009. Field methods for sampling and storing nectar from flowers with low nectar volumes. *Annals of Botany*, 103(3): 533-542.

Mulcahy DL. 1979. The rise of the angiosperms: a genecological factor. *Science*, 206(4414): 20-23.

Muth F, Papaj DR, Leonard AS. 2015. Colour learning when foraging for nectar and pollen: bees learn two colours at once. *Biology Letters*, 11(9): 20150628.

Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, 82: 591-605.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4: 133-142.

Nasr ME, Thorp RW, Tyler TL, Briggs DL. 1990. Estimating honey bee (Hymenoptera: Apidae) colony strength by a simple method: measuring cluster size. *Journal of Economic Entomology*, 83(3): 748-754.

Ne'eman G, Jürgens A, Newstrom-Lloyd L, Potts SG, Dafni A. 2010. A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews*, 85(3): 435-451.

Nepi M, Guarnieri M, Pacini E. 2001. Nectar secretion, reabsorption, and sugar composition in male and female flowers of *Cucurbita pepo*. *International Journal of Plant Science*, 162: 353-358.

Nepi M, Pacini E. 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Annals of Botany*, 72: 527-536.

Nepi M, Stpiczyńska M. 2008. The complexity of nectar: secretion and resorption dynamically regulate nectar features. *Naturwissenschaften*, 95: 177-184.

Netzer MH, Tittonel E, Merrien A. 1986. Initiation florale du colza d'hiver *Brassica napus* L. cv Jet Neuf en conditions contrôlées. *Informations Techniques du CETIOM*, 96: 3-9.

Nicolson SW. 1995. Direct demonstration of nectar reabsorption in the flowers of *Grevillea robusta* (Proteaceae). *Functional Ecology*, 9(4): 584-588.

Nicolson SW. 2007. Nectar consumers. In: Nicolson SW, Nepi M, Pacini E (eds). *Nectaries and Nectar*. Dordrecht, Netherlands: Springer, pp. 289-342.

Nicolson SW, Thornburg RW. 2007. Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E (eds). *Nectaries and Nectar*. Dordrecht, Netherlands: Springer, pp. 215-264.

Nielsen A, Reitan T, Rinvoll AW, Brysting AK. 2017. Effects of competition and climate on a crop pollinator community. *Agriculture, Ecosystems and Environment*, 246: 253-260.

Niesenbaum RA. 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). *American Journal of Botany*, 86(2): 261-268.

Odoux JF, Aupinel P, Gateff S, Requier F, Henry M, Bretagnolle V. 2014. ECOBEE: a tool for long-term bee colony monitoring at landscape scale in West European intensive agrosystems. *Journal of Apicultural Research*, 53: 57–66.

Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, Kasahara RD, Hamamura Y, Mizukami A, Susaki D *et al*. 2009. Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. *Nature*, 458(7236): 357-361.

Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals?. *Oikos*, 120(3): 321-326.

O'Neill SD. 1997. Pollination regulation of flower development. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48: 547-574.

Ouvrard P, Quinet M, Jacquemart AL. 2017. Breeding system and pollination biology of belgian oilseed rape cultivars (*Brassica napus*). *Crop Science*, 57(3): 1455-1463.

Owens CD. 1971. The thermology of wintering honey bee colonies. *Technical Bulletin, USDA*, 1429: 1-32.

Owens JN, Takaso T, Runions CJ. 1998. Pollination in conifers. *Trends in Plant Science*, 3(12): 479-485.

Pacini E, Nepi M. 2007. Nectar production and presentation. In: Nicolson SW, Nepi M, Pacini E (eds). *Nectaries and Nectar*. Dordrecht, Netherlands: Springer, pp. 167-214.

Pacini E, Nepi M, Vesprini JL. 2003. Nectar biodiversity: a short review. *Plant Systematics and Evolution*, 238: 7-21.

Pakyari H, Fathipour Y, Enkegaard A. 2011. Effect of temperature on life table parameters of predatory thrips *Scolothrips longicornis* (Thysanoptera: Thripidae) fed on two spotted spider mites (Acari: Tetranychidae). *Journal of Economic Entomology*, 104: 799-805.

Pandey AK, Tripathi CPM. 2008. Effect of temperature on the development, fecundity, progeny sex ratio and life-table of *Campoletis chlorideae*, an endolarval parasitoid of the pod borer, *Helicoverpa armigera*. *BioControl*, 53: 461-471.

Park MG, Raguso RA, Losey JE, Danforth BN. 2016. Per-visit pollinator performance and regional importance of wild *Bombus* and *Andrena* (*Melandrena*) compared to the managed honey bee in New York apple orchards. *Apidologie*, 47(2): 145-160.

Parker FD. 1981. Sunflower pollination: abundance, diversity and seasonality of bees and their effect on seed yields. *Journal of Apicultural Research*, 20(1): 49-61.

Parker FD, Batra SWT, Tepedino VJ. 1987. New pollinators for our crops. *Agricultural Zoology Review*, 2: 279-307.

Parker AJ, Tran JL, Ison JL, Bai JDK, Weis AE, Thomson JD. 2015. Pollen packing affects the function of pollen on corbiculate bees but not non-corbiculate bees. *Arthropod-Plant Interactions*, 9(2): 197-203.

Pechan PM. 1988. Ovule fertilization and seed number per pod determination in oil seed rape (*Brassica napus*). *Annals of Botany*, 61: 201-207.

Pelletier G, Budar F. 2015. *Brassica* Ogu-INRA cytoplasmic male sterility: an example of successful plant somatic fusion for hybrid seed production. In: Li X-Q, Donnelly DJ, Jensen TG (eds). *Somatic Genome Manipulation: Advances, Methods and Applications*. New York, NY, USA: Springer, pp. 199-216.

Percival MS. 1961. Types of nectar in angiosperms. *New Phytologist*, 60(3): 235-281.

Peris D, Pérez-de la Fuente R, Peñalver E, Delclòs X, Barrón E, Labandeira CC. 2017. False blister beetles and the expansion of gymnosperm-insect pollination modes before angiosperm dominance. *Current Biology*, 27(6): 897-904.

Perrot T, Gaba S, Roncoroni M, Gautier JL, Bretagnolle V. 2018. Bees increase oilseed rape yield under real field conditions. *Agriculture, Ecosystems & Environment*, 266: 39-48.

Petanidou T. 2007. Ecological and evolutionary aspects of floral nectars in Mediterranean habitats. In: Nicolson SW, Nepi M, Pacini E (eds). *Nectaries and Nectar*. Dordrecht, Netherlands: Springer, pp. 343-375.

Petanidou T, Goethals V, Smets E. 2000. Nectary structure of Labiatae in relation to their nectar secretion and characteristics in a Mediterranean shrub community - does flowering time matter?. *Plant Systematics and Evolution*, 225(1): 103-118.

Petanidou T, Smets E. 1996. Does temperature stress induce nectar secretion in Mediterranean plants?. *New Phytologist*, 133(3): 513-518.

Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, Kozlov A, Podsiadlowski L, Petersen M, Lanfear R, Diez PA, Heraty J, Kjer KM, Klopfstein S, Meier R, Polidori C, Schmitt T, Liu S, Zhou X, Wappler T, Rust J, Misof B, Niehuis O. 2017. Evolutionary history of the Hymenoptera. *Current Biology*, 27(7): 1013-1018.

Petit S, Rubbo N, Schumann R. 2011. Nectar collected with microcapillary tubes is less concentrated than total nectar in flowers with small nectar volumes. *Australian Journal of Botany*, 59(6): 593-599.

Phillips BB, Williams A, Osborne JL, Shaw RF. 2018. Shared traits make flies and bees effective pollinators of oilseed rape (*Brassica napus* L.). *Basic and Applied Ecology*, 32: 66-76.

Pierre J, Mesquida J, Marilleau R, Pham-Delègue MH, Renard M. 1999. Nectar secretion in winter oilseed rape, *Brassica napus* - quantitative and qualitative variability among 71 genotypes. *Plant Breeding*, 118: 471-476.

Pierre J, Renard M. 2010. Bilan de 30 ans de travaux de recherche effectués en France sur la pollinisation du colza. *Oilseeds & fats, Crops and Lipids*, 17(3): 121-126.

Pinzauti M. 1986. The influence of the wind on nectar secretion from the melon and on the flight of bees: the use of an artificial wind-break. *Apidologie*, 17(1): 63-72.

Pisanty G, Afik O, Wajnberg E, Mandelik Y. 2016. Watermelon pollinators exhibit complementarity in both visitation rate and single-visit pollination efficiency. *Journal of Applied Ecology*, 53(2): 360-370.

Pisanty G, Klein AM, Mandelik Y. 2014. Do wild bees complement honeybee pollination of confection sunflowers in Israel?. *Apidologie*, 45(2): 235-247.

Pitts-Singer TL, Artz DR, Peterson SS, Boyle NK, Wardell GI. 2018. Examination of a managed pollinator strategy for almond production using *Apis mellifera* (Hymenoptera: Apidae) and *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology*, 47(2): 364-377.

Pleasants JM. 1983. Nectar production patterns in *Ipomopsis aggregata* (Polemoniaceae). *American Journal of Botany*, 70: 1468-1475.

Poinar GO, Danforth BN. 2006. A fossil bee from Early Cretaceous Burmese amber. *Science*, 314(5799): 614-614.

Postweiler K, Stösser R, Anvari SF. 1985. The effect of different temperatures on the viability of ovules in cherries. *Scientia Horticulturae*, 25: 235-239.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*, 25(6): 345-353.

Prasad PV, Bheemanahalli R, Jagadish SK. 2017. Field crops and the fear of heat stress - opportunities, challenges and future directions. *Field Crops Research*, 200: 114-121.

Primack RB. 1985. Longevity of individual flowers. *Annual Review of Ecology and Systematics*, 16: 15-37.

Proctor M, Yeo P, Lack A. 2012. The study of pollination: a short history. In: *The Natural History of Pollination*. London, UK: HarperCollins.

Prŷs-Jones OE, Corbet SA. 2011. *Bumblebees. Naturalists' Handbooks 6, Ecology and Identification*. Thurd Edition. Pelagic publishing.

Qu H, Drummond F. 2018. Simulation-based modeling of wild blueberry pollination. *Computers and Electronics in Agriculture*, 144: 94-101.

Qu LJ, Li L, Lan Z, Dresselhaus T. 2015. Peptide signalling during the pollen tube journey and double fertilization. *Journal of Experimental Botany*, 66(17): 5139-5150.

Rader R, Edwards W, Westcott DA, Cunningham SA, Howlett BG. 2013. Diurnal effectiveness of pollination by bees and flies in agricultural *Brassica rapa*: implications for ecosystem resilience. *Basic and Applied Ecology*, 14(1): 20-27.

Rader R, Howlett BG, Cunningham SA, Westcott DA, Edwards W. 2012. Spatial and temporal variation in pollinator effectiveness: do unmanaged insects provide consistent pollination services to mass flowering crops?. *Journal of Applied Ecology*, 49(1): 126-134.

Rader R, Howlett BG, Cunningham SA, Westcott DA, Newstrom-Lloyd LE, Walker MK, Teulon DAJ, Edwards W. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology*, 46(5): 1080-1087.

Raghavan V. 2003. Some reflections on double fertilization, from its discovery to the present. *New Phytologist*, 159(3): 565-583.

Raghavan V. 2006. *Double Fertilization: Embryo and Endosperm Development in Flowering Plants*. Berlin, Germany: Springer.

Ramawat KG, Mérillon JM, Shivanna KR. 2014. *Reproductive Biology of Plants*. Boca Raton, FL, USA: CRC Press.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL http://www.R-project.org/.

Réaumur RA. 1735. Observations du thermomètre faites pendant l'année MDCCXXXV comparées à celles qui ont été faites sous la ligne à l'Isle-de-France, à Alger et en quelques-unes de nos Isles de l'Amérique. Mémoires de l'Académie Royale des Sciences, Paris, pp. 545-576.

Reid MS, Chen J-C. 2007. Flower senescence. In: Gan S (ed). *Senescence Processes in Plants*. *Annual Plant Reviews*, 26: 256-277.

Requier F, Odoux JF, Henry M, Bretagnolle V. 2017. The carry-over effects of pollen shortage decrease the survival of honeybee colonies in farmlands. *Journal of Applied Ecology*, 54: 1161-1170.

Richards SA, Williams NM, Harder LD. 2009. Variation in pollination: causes and consequences for plant reproduction. *The American Naturalist*, 174(3): 382-398.

Rinderer TE, Baxter JR. 1978. Effect of empty comb on hoarding behavior and honey production of the honey bee. *Journal of Economic Entomology*, 71(5): 757-759.

Rogers HJ. 2006. Programmed cell death in floral organs: how and why do flowers die?. *Annals of Botany*, 97(3): 309-315.

Rogers HJ. 2012. Is there an important role for reactive oxygen species and redox regulation during floral senescence?. *Plant, Cell & Environment*, 35: 217-233.

Rogers HJ. 2015. Senescence-associated programmed cell death. In: Gunawardena AN, McCabe PF (eds). *Plant Programmed Cell Death*. Cham, Switzerland: Springer International Publishing, pp. 203-233.

Rogers H, Munné-Bosch S. 2016. Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: similar but different. *Plant Physiology*, 171: 1560-1568.

Rollin O, Garibaldi LA. 2018. Impacts of honey bee density on crop yield: a meta-analysis. Eurbee 8, Ghent, Belgium.

Rosbakh S, Pacini E, Nepi M, Poschlod P. 2018. An unexplored side of regeneration niche: seed quantity and quality are determined by the effect of temperature on pollen performance. *Frontiers in Plant Science*, 9: 1036

Rosbakh S, Poschlod P. 2016. Minimal temperature of pollen germination controls species distribution along a temperature gradient. *Annals of Botany*, 117(7): 1111-1120.

Russo L, Park MG, Blitzer EJ, Danforth BN. 2017. Flower handling behavior and abundance determine the relative contribution of pollinators to seed set in apple orchards. *Agriculture, Ecosystems & Environment*, 246: 102-108.

Sabbahi R, de Oliveira D, Marceau J. 2005. Influence of honey bee (Hymenoptera: Apidae) density on the production of canola (Crucifera: Brassicacae). *Journal of Economic Entomology*, 98(2): 367-372.

Sáez A, di Virgilio A, Tiribelli F, Geslin B. 2018a. Simulation models to predict pollination success in apple orchards: a useful tool to test management practices. *Apidologie*, *in press*.

Sáez A, Morales JM, Morales CL, Harder LD, Aizen MA. 2018b. The costs and benefits of pollinator dependence: empirically based simulations predict raspberry fruit quality. *Ecological Applications*, 28(5): 1215-1222.

Sáez A, Morales CL, Ramos LY, Aizen MA. 2014. Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *Journal of Applied Ecology*, 51(6): 1603-1612.

Sage TL, Bagha S, Lundsgaard-Nielsen V, Branch HA, Sultmanis S, Sage RF. 2015. The effect of high temperature stress on male and female reproduction in plants. *Field Crops Research*, 182: 30-42.

Sammataro D, Erickson EH, Garment MB. 1985. Ultrastructure of the sunflower nectary. *Journal of Apicultural Research*, 24(3): 150-160.

Sanzol J, Herrero M. 2001. The "effective pollination period" in fruit trees. *Scientia Horticulturae*, 90: 1-17.

Sapir G, Baras Z, Azmon G, Goldway M, Shafir S, Allouche A, Stern E, Stern RA. 2017. Synergistic effects between bumblebees and honey bees in apple orchards increase cross pollination, seed number and fruit size. *Scientia Horticulturae*, 219: 107-117.

Schemske DW. 1983. Breeding system and habitat effects on fitness components in three neotropical *Costus* (Zingiberaceae). *Evolution*, 37(3): 523-539.

Schielzeth H. 2010. Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, 1(2); 103-113.

Schiestl FP. 2012. Animal pollination and speciation in plants: general mechanisms and examples from the orchids. In: Patiny S (ed). *Evolution of Plant-Pollinator Relationships*. New York, NY, USA: Cambridge University Press, pp. 263-278.

Schmickl T, Crailsheim K. 2007. HoPoMo: A model of honeybee intracolonial population dynamics and resource management. *Ecological Modelling*, 204(1-2): 219-245.

Schmidhuber J, Tubiello FN. 2007. Global food security under climate change. *Proceedings of the National Academy of Science*, 104: 19703-19708.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7): 671-675.

Schoen DJ, Ashman TL. 1995. The evolution of floral longevity: resource allocation to maintenance versus construction of repeated parts in modular organisms. *Evolution*, 49: 131-139.

Schulte PM. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, 218: 1856-1866.

Seeley TD. 1985. Temperature control. In: *Honeybee ecology: a study of adaptation in social life*. Princeton, NJ, USA: Princeton University Press.

Shaw RF, Bercaw BL. 1962. Temperature and life-span in poikilothermous animals. *Nature*, 196: 454-457.

Shi P, Ge F. 2010. A comparison of different thermal performance functions describing temperature-dependent development rates. *Journal of Thermal Biology*, 35: 225-231.

Shibuya K, Ichimura K. 2016. Physiology and molecular biology of flower senescence. In: Pareek S (ed). *Postharvest Ripening Physiology of Crops*. Boca Raton, FL, USA: CRC Press, pp. 109-137.

Shivanna KR. 2014. Biotic pollination: how plants achieve conflicting demands of attraction and restriction of potential pollinators. In: Ramawat KG, Mérillon JM, Shivanna KR (eds). *Reproductive Biology of Plants*. Boca Raton, FL, USA: CRC Press, pp. 218-267.

Shore JS, Barrett SC. 1984. The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). *Canadian Journal of Botany*, 62(6): 1298-1303.

Silander JA, Primack RB. 1978. Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). *The American Midland Naturalist*, 100(1): 213-216.

Singh SK, Kakani VG, Brand D, Baldwin B, Reddy KR. 2008. Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. *Journal of Agronomy and Crop Science*, 194(3): 225-236.

Smith SA, Beaulieu JM, Donoghue MJ. 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences*, 201001225.

Smith MR, Singh GM, Mozaffarian D, Myers SS. 2015. Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. *The Lancet*, 386(10007): 1964-1972.

Smyth DR, Bowman JL, Meyerowitz EM. 1990. Early flower development in *Arabidopsis*. *Plant Cell*, 2: 755-767.

Snow AA. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. *Oecologia* 55(2): 231-237.

Snow AA. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. *American Journal of Botany*, 73(1): 139-151.

Soltani A, Sinclair TR. 2012. Phenology – temperature. In: *Modeling Physiology of Crop Development, Growth and Yield*. Croydon, UK: CABI, pp. 55-72.

Soltis D, Soltis P, Endress P, Chase M, Manchester S, Judd W, Majure L, Mavrodiev E. 2018. Phylogeny of angiosperms: an overview. In: *Phylogeny and Evolution of the Angiosperms. Revised and Updated Edition*. Chicago, IL, USA: The University of Chicago Press, pp. 35-62.

Southwick EE, Heldmaier G. 1987. Temperature control in honey bee colonies. *BioScience*, 37(6): 395-399

Speakman JR, Selman C. 2011. The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *Bioessays*, 33: 255-259.

Spielman M, Scott RJ. 2008. Polyspermy barriers in plants: from preventing to promoting fertilization. *Sexual Plant Reproduction*, 21(1): 53-65.

Stanley DA, Gunning D, Stout JC. 2013. Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland: ecological and economic incentives for pollinator conservation. *Journal of Insect Conservation*, 17(6): 1181-1189.

Stanton-Geddes J, de Freitas CG, de Sales Dambros C. 2014. In defense of P values: comment on the statistical methods actually used by ecologists. *Ecology*, 95(3): 637-642.

Stead AD. 1992. Pollination-induced flower senescence: a review. *Plant Growth Regulation*, 11: 13-20.

Stead AD, van Doorn WG. 1994. Strategies of flower senescence - a review. In: Scott RJ, Stead AD (eds). *Molecular and Cellular Aspects of Plant Reproduction*. Cambridge, UK: Cambridge University Press, pp. 215-238.

Steffan-Dewenter I. 2003. Seed set of male-sterile and male-fertile oilseed rape (*Brassica napus*) in relation to pollinator density. *Apidologie*, 34(3): 227-235.

Steinacher G, Wagner J. 2010. Flower longevity and duration of pistil receptivity in high mountain plants. *Flora*, 205: 376-387.

Stephens PA, Buskirk SW, del Rio CM. 2007. Inference in ecology and evolution. *Trends in Ecology and Evolution*, 22(4): 192-197.

Stephenson AG. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics*, 12: 253-279.

Stösser R, Anvari SF. 1982. On the senescence of ovules in cherries. *Scientia Horticulturae*, 16: 29-38.

Sumpter DJT, Broomhead DS. 2000. Shape and dynamics of thermoregulating honey bee clusters. *Journal of Theoretical Biology*, 204(1): 1-14.

Szabo TI. 1980. Effect of weather factors on honeybee flight activity and colony weight gain. *Journal of Apicultural Research*, 19(3): 164-171.

Takayama S, Isogai A. 2005. Self-incompatibility in plants. *Annual Review of Plant Biology*, 56: 467-489.

Takhtajan AL. 1980. Outline of the classification of flowering plants (Magnoliophyta). *The Botanical Review*, 46(3): 225-359.

Takkis K, Tscheulin T, Tsalkatis P, Petanidou T. 2015. Climate change reduces nectar secretion in two common Mediterranean plants. *AoB Plants*, 7: plv111.

Taylor LP, Hepler PK. 1997. Pollen germination and tube growth. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48: 461-491.

Teixido AL, Valladares F. 2015. Temperature-limited floral longevity in the large-flowered mediterranean shrub *Cistus ladanifer* (Cistaceae). *International Journal of Plant Science*, 176: 131-140.

Teuber LR, Albertsen MC, Barnes DK, Heichel GH. 1980. Structure of floral nectaries of alfalfa (*Medicago sativa* L.) in relation to nectar production. *American Journal of Botany*, 67(4): 433-439.

Thakur P, Kumar S, Malik JA, Berger JD, Nayyar H. 2010. Cold stress effects on reproductive development in grain crops: an overview. *Environmental and Experimental Botany*, 67(3): 429-443.

Thien LB. 1980. Patterns of pollination in the primitive angiosperms. *Biotropica*, 12(1): 1-13.

Thien LB, Azuma H, Kawano S. 2000. New perspectives on the pollination biology of basal angiosperms. *International Journal of Plant Sciences*, 161(S6): S225-S235.

Thien LB, Bernhardt P, Devall MS, Chen ZD, Luo YB, Fan JH, Yuan LC, Williams JH. 2009. Pollination biology of basal angiosperms (ANITA grade). *American Journal of Botany*, 96(1): 166-182.

Thompson MM, Liu LJ. 1973. Temperature, fruit set, and embryo sac development in 'Italian' prune. *Journal of the American Society of Horticultural Science*, 98: 193-197.

Thorp RW. 2000. The collection of pollen by bees. *Plant Systematics and Evolution*, 222: 211-223.

Tromp J, Borsboom O. 1994. The effect of autumn and spring temperature on fruit set and on the effective pollination period in apple and pear. *Scientia Horticulturae*, 60: 23-30.

Trudgill DL, Honek A, Li D, van Straalen NV. 2005. Thermal time – concepts and utility. *Annals of Applied Biology*, 146: 1-14.

Vaissière BE. 1991. Measure of pollination intensity and associated parameters. In: *Honey bees, Apis mellifera L. (Hymenoptera: Apidae), as pollinators of upland cotton, Gossypium hirsutum L. (Malvaceae), for hybrid seed production*. PhD thesis, Texas A&M University, College Station, Texas, USA, 23-111.

Vaissière BE, Froissart R. 1996. Pollination of cantaloupes under spun bonded row cover by honey bees (Hymenoptera: Apidae) in West Africa. *Journal of Economic Entomology*, 89(5): 1213-1222.

Vaissiere BE, Malaboeuf F, Rodet G. 1996. Viability of cantaloupe pollen carried by honeybees *Apis mellifera* varies with foraging behavior. *Naturwissenschaften*, 83(2): 84-86.

Valenta K, Nevo O, Martel C, Chapman CA. 2017. Plant attractants: integrating insights from pollination and seed dispersal ecology. *Evolutionary Ecology*, 31(2): 249-267.

Van Breusegem F, Dat JF. 2006. Reactive oxygen species in plant cell death. *Plant Physiology*, 141: 384-390.

van Dooremalen C, Cornelissen B, Poleij-Hok-Ahin C, Blacquière T. 2018. Single and interactive effects of *Varroa destructor*, *Nosema* spp., and imidacloprid on honey bee colonies (*Apis mellifera*). *Ecosphere*, 9(8): e02378.

van Doorn WG. 1997. Effects of pollination on floral attraction and longevity. *Journal of Experimental Botany*, 48: 1615-1622.

Van Voorhies WA, Ward S. 1999. Genetic and environmental conditions that increase longevity in *Caenorhabditis elegans* decrease metabolic rate. *Proceedings of the National Academy of Science*, 96: 11399-11403.

Varga S, Nuortila C, Kytöviita M-M. 2013. Nectar sugar production across floral phases in the gynodioecious protandrous plant *Geranium sylvaticum*. PLoS ONE, 8(4): e62575.

Vasilakakis MD, Porlingis IC. 1985. Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. *HortScience*, 20: 733-735.

Vesprini JL, Pacini E. 2005. Temperature-dependent floral longevity in two *Helleborus* species. *Plant Systematics and Evolution*, 252: 63-70.

Viana BF, da Encarnação Coutinho JG, Garibaldi LA, Gastagnino B, Laercio G, Peres Gramacho K, Oliveira da Silva F. 2014. Stingless bees further improve apple pollination and production. *Journal of Pollination Ecology*, 14(25): 261-269.

Vicens N, Bosch J. 2000. Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). *Environmental Entomology*, 29(3): 413-420.

Villarreal AG, Freeman CE. 1990. Effects of temperature and water stress on some floral nectar characteristics in *Ipomopsis longiflora* (Polemoniaceae) under controlled conditions. *Botanical Gazette*, 151(1): 5-9.

Vuletin Selak G, Cuevas J, Goreta Ban S, Pinillos V, Dumicic G, Perica S. 2014. The effect of temperature on the duration of the effective pollination period in 'Oblica' olive (*Olea europaea*) cultivar. *Annals of Applied Biology*, 164: 85-94.

Waite MB. 1898. *Pollination of Pomaceous Fruits*. Washington, DC, USA: USDA.

Wang JJ, Tsai JH. 2001. Development, survival and reproduction of black citrus aphid, *Toxoptera aurantii* (Hemiptera: Aphididae), as a function of temperature. *Bulletin of Entomological Research*, 91: 477-487.

Waser NM. 1986. Flower constancy: definition, cause, and measurement. *The American Naturalist*, 127(5): 593-603.

Waser NM, Price MV. 1991. Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set. *Ecology*, 72(1): 171-179.

Waser NM, Price MV. 2016. Drought, pollen and nectar availability, and pollination success. *Ecology*, 97(6): 1400-1409.

Wheeler T, von Braun J. 2013. Climate change impacts on global food security. *Science*, 341: 508-513.

Wilcock C, Neiland R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science*, 7: 270-277.

Williams RR. 1966. Pollination studies in fruit trees. II. The effective pollination period for some apple and pear varieties. Reports of the Long Ashton Research Station for 1965, pp. 136-138.

Williams IH. 1978. The pollination requirements of swede rape (*Brassica napus* L.) and of turnip rape (*Brassica campestris* L.). *The Journal of Agricultural Science*, 91(2): 343-348.

Williams IH, Martin AP, White RP. 1986. The pollination requirements of oil-seed rape (*Brassica napus* L.). *The Journal of Agricultural Science*, 106(1): 27-30.

Williams JH, Mazer SJ. 2016. Pollen—Tiny and ephemeral but not forgotten: New ideas on their ecology and evolution. *American Journal of Botany*, 103(3): 365-374.

Williams NM, Tepedino VJ. 2003. Consistent mixing of near and distant resources in foraging bouts by the solitary mason bee *Osmia lignaria*. *Behavioral Ecology*, 14(1): 141-149.

Willmer PG, Cunnold H, Ballantyne G. 2017. Insights from measuring pollen deposition: quantifying the pre-eminence of bees as flower visitors and effective pollinators. *Arthropod-Plant Interactions*, 11(3): 411-425.

Willmer PG, Stone GN. 2004. Behavioral, ecological, and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior*, 34: 347-466.

Winfree R, Reilly JR, Bartomeus I, Cariveau DP, Williams NM, Gibbs J. 2018. Species turnover promotes the importance of bee diversity for crop pollination at regional scales. *Science*, 359(6377): 791-793.

Wist TJ, Davis AR. 2006. Floral nectar production and nectary anatomy and ultrastructure of *Echinacea purpurea* (Asteraceae). *Annals of Botany*, 97(2): 177-193.

Wist TJ, Davis AR. 2008. Floral structure and dynamics of nectar production in *Echinacea pallida* var. *angustifolia* (Asteraceae). *International Journal of Plant Science*, 169(6): 708-722.

Woodcock BA, Edwards M, Redhead J, Meek WR, Nuttall P, Falk S, Nowakowski M, Pywell RF. 2013. Crop flower visitation by honeybees, bumblebees and solitary bees: behavioural differences and diversity responses to landscape. *Agriculture, Ecosystems & Environment*, 171: 1-8.

Wyatt R, Broyles SB, Derda GS. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *American Journal of Botany*, 79(6): 636-642.

Yamori W, Hikosaka K, Way DA. 2014. Temperature response of photosynthesis in C_3, C_4, and CAM plants: temperature acclimation and temperature adaptation. *Photosynthesis Res*earch, 119(1-2): 101-117.

Yan W, Hunt LA. 1999. An equation for modelling the temperature response of plants using only the cardinal temperatures. *Annals of Botany*, 84: 607-614.

Yasaka M, Nishiwaki Y, Konno Y. 1998. Plasticity of flower longevity in *Corydalis ambigua*. *Ecological Research*, 13: 211-216.

Ydenberg RC. 2007. Provisioning. In: Stephens DW, Brown JS, Ydenberg RC (eds). *Foraging Behavior and Ecology*. Chicago, IL, USA: The University of Chicago Press, pp. 273-303.

Yin X, Kropff MJ, McLaren G, Visperas RM. 1995. A nonlinear model for crop development as a function of temperature. *Agricultural and Forest Meteorology*, 77: 1-16.

Zentgraf U. 2007. Oxidative stress and leaf senescence. In: Gan S (ed). *Senescence Processes in Plants*. *Annual Plant Reviews*, 26, 69-86.

Zentgraf U, Hemleben V. 2008. Molecular cell biology: are reactive oxygen species regulators of leaf senescence?. In: Lüttge U, Beyschlag W, Murata J (eds). *Progress in Botany*, 69: 117-138.

Zhang C, Tateishi N, Tanabe K. 2010. Pollen density on the stigma affects endogenous gibberellin metabolism, seed and fruit set, and fruit quality in *Pyrus pyrifolia*. *Journal of Experimental Botany*, 61(15): 4291-4302.

Zinn KE, Tunc-Ozdemir M, Harper JF. 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany*, 61(7): 1959-1968.

Zuberi MI, Dickinson HG. 1985. Pollen-stigma interaction in *Brassica*. III. Hydration of the pollen grains. *Journal of Cell Science*, 76: 321-336.