Finding new targets to screen oilseed rape (Brassica napus) resistance to pollen beetle (Brassicogethe aeneus) : from metabolomics to the field

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Recherche de nouveaux leviers pour cribler la résistance du colza (*Brassica napus*) au méligèthe (*Brassicogethes aeneus*) : de la métabolomique au champ

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Table of contents

Introduction

General introduction

1. General context and main objectives of the thesis

Arthropods (mainly insects and mites) are estimated to destroy 13-16% of annual crop production worldwide (Oerke, 2006; Culliney, 2014). Insects damage crops in a variety of ways: they attack growing plants and reduce their photosynthetic activity, disturb root systems and destroy harvested parts such as fruits and grains. Insects are also vectors of pathogens and viruses that can infect plants and reduce yield and marketability (Culliney, 2014).

Modern agriculture relies primarily on insecticides to control insect pests but over-utilisation of these compounds led to environmental issues such as impact on human health and biodiversity (Devine and Furlong 2007). Agronomical efficiency of these compounds is also decreasing as numerous pest species are becoming resistant to insecticides (Sparks, 2013). Alternatives to pesticides need to be found. Based on the ecology of insects several approaches have been developed such as behavioural perturbation using pheromones, biological control or trap cropping. These strategies are mainly implement in small surfaces like vegetable fields or orchards but are more complicated and costly to deploy in large fields (Shelton and Badenes-Perez, 2006).

Another strategy could be to use plants with enhanced resistances against insects. Such strategy was efficient in controlling some insects in field crops such as wheat, rice or sorghum for example (Wilde, 2002). Interest about this strategy is increasing and may benefit from basic researches on insect-plant interactions. However, breeding plants for their resistances to insects remains methodologically complex. One of the major issue concerns the phenotyping of plant resistances. Technical development, especially using chemical analysis could help to circumvent these problems by identifying biomarkers of resistances. Knowledge on insect ecology remains essential to identify target life history traits that could efficiently reduce insect damage. Insect feeding behaviour is of special interest as it causes damage to the plant and it is also strongly related to insect fitness.

In the present work, we targeted such questions by working on the pollen beetle (*Brassicogethe aeneus*), an important pest of oilseed rape (*Brassica napus*) for which new control methods are urgently needed. We focussed on two main objectives: 1/ Develop methods enabling to screen resistance against the pollen beetle in the field and identify biochemical biomarkers of this resistance. 2/ Better understand key steps in the interaction between the pest and its host plant to identify potential new target traits for resistance.

2. Insect pest management, from ecology to new management practices

2.1. Environmental and agronomic issues related to insecticides

To control insect pests, modern agriculture relies primarily on insecticides. Large adoption of this management practice can be explained by its ease of use, its low price and its rapid curative effect on insects (Teetes, 1985). Even if insecticides have many benefits for farmers, their social, environmental and agronomic consequences may be costly. Negative effects of insecticides for human health and environment are increasingly documented (Devine and Furlong 2007). Costs associated with agricultural water pollution

in the UK are estimated to be 1-2 % of total agricultural value (Pretty et al., 2000). Insecticides also have effects on non-target species and strongly affect biodiversity. Water pollution related to pesticides reduces by 30 % family richness of aquatic macroinvertebrate even at accepted regulatory threshold levels and thus strongly impacts biodiversity (Stehle and Schulz, 2015). Other organisms such as birds, amphibians and arthropods are also severely affected by pesticide applications (Devine and Furlong 2007; Hallmann et al., 2014). Insecticides also have negative agronomical effects by decreasing natural enemies' populations. They reduce biological control potential in agrosystems leading to increased complexity for integrated pest management implementation (Geiger et al., 2009). By its pressure exerted on pest populations, systematic application of insecticides has led to the development of resistances to many active compounds. Since the 50's, insect pest populations resistant to insecticides are increasing and today, more than 550 Arthropod species are known to be resistant to insecticides (Fig.1a; Sparks, 2013). From an economical point of view, profitability of insecticides for companies is decreasing as the number of compounds tested during insecticide development and costs attributed to the development of these compounds is increasing over time (Fig. 1b, 1c; Sparks, 2013). This results in a reduction of efficiency of insecticides and a deficit of newly commercialized compounds.

Limitations of insecticides are increasingly recognized at the regulatory level. Recently, legislation especially in the European Union has shrunk pesticide use by banning utilization of some harmful compounds (Masip et al., 2013). Alternatives to pesticides need to be found for many crops. These alternatives should meet farmers and social needs by being easy to implement, cheap and effective but also by allowing production of healthy products with low impact on the environment and biodiversity. Knowledge on insect-plant interactions and especially on plant defences has been set aside by management strategies primarily based on pesticides but it could help developing new strategies meeting these demands.

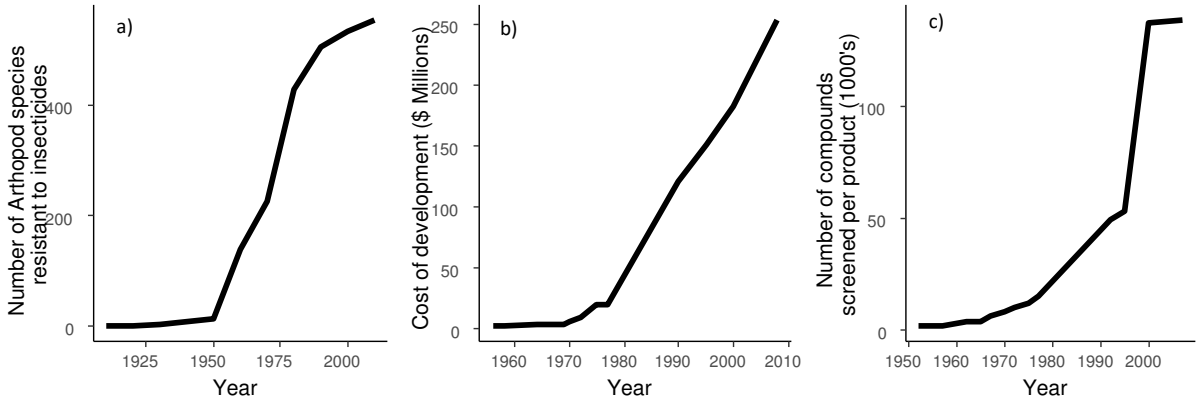

Figure 1: a) Number of referenced Arthropods species resistant to insecticides. b) Cost of development of insecticides in million dollars. c) Number of compounds screened to develop insecticides in thousands (data adapted from Spark, 2013).

2.2. From insect-plant interaction research to new management practices

Plants and herbivorous insects are among the most diverse taxa with an estimate of 1 million herbivore species and 300 000 vascular plant species (Futuyma and Agrawal, 2009). This diversity is the product of at least 400 Ma of evolutionary history (Labanderira, 2007). It resulted in highly diverse utilisation of plants by insects and complex set of defence mechanisms in plants. To efficiently use these mechanisms in an

applied context, it is important to understand the physiological needs of insects and their utilisation of plant tissues but also how plants defend themselves against insects.

2.2.1. Insect utilisation of plants

Insects feed on plants to fulfil their nutritional needs. Amino acids, carbohydrates, sterols, lipids, fatty acids, vitamins and trace elements are essential to their development and survival (Behmer, 2009). However, plant tissues contain low levels of these nutrients. Nitrogen for example, is a major limiting element for insects. It accounts for about 8-14 % of insect mass whereas plants contain only 2-4 % of nitrogen (Mattson, 1980). Furthermore, nutrients are diluted in a matrix of unpalatable cellulose, lignin or secondary metabolites making them difficult to metabolize thus forcing insects to eat large quantities of plant tissues (Schoonhoven et al., 2005). Herbivory is estimated to cause a loss of approximately 20 % of terrestrial plant biomass and thereby strongly affect plant fitness (Agrawal, 2011).

Plants can adapt to defend themselves against insects. Ehrlich and Raven (1964) hypothesized that a "coevolutionary arm race" is ongoing between plants and herbivorous insects. Their hypothesis assumes that in response to herbivory, plants evolve new defence mechanisms, especially by the synthesis of new compounds that are toxic or anti-nutritious for insects. These defences enable plants to escape from herbivory and allow them to radiate in diverse species sharing common defence mechanisms. On the other hand, some insect species colonize these plants and adapt to their defence allowing them to radiate too. Consequently, plant defences may become a double message for insects depending on their specialization: information about toxicity of plants for insects that are not adapted to them and a host plant signal for adapted species.

Insects evolved diverse mechanisms to locate and efficiently use their host plants (i.e. choose the ones with adequate nutrient content and minimal defences). Plant odours and visual cues play an important role in host plant location and quality assessment at long distance (Bruce et al., 2005). After plant contact, insects often probe and taste plants to confirm their choice and feed or lay their eggs on these plants (Schoonhoven et al., 2005). Several strategies allow insects to deal with plant defence mechanisms and better benefit from plant organs. These can be behavioural, such as avoidance (e.g. feeding on toxin-free refuges in the plant) or manipulation (i.e. cutting trenches across leaves to depressurize canals containing toxic compounds and stopping their exudation) (Després et al., 2007). They can also be physiological sequestration or detoxification of toxic compounds (Després et al., 2007). All these adaptations allowed some insects to become phytophagous and use plants for feeding. Nevertheless, plants are still a suboptimal diet. Experiments comparing insects reared on artificial diets to insects fed with plants have shown that even susceptible plants are well defended and their nutritional quality limits insect development (Schoonhoven et al., 2005).

2.2.2. Plant defences against insects

To resist to insect damage, plants developed a vast array of complex and interconnected defence mechanisms. Establishment of a conceptual framework allowing categorizing these defences has been challenging to researchers but is essential to their use in an applied context.

Painter (1951) proposed one of the first conceptual framework used to categorize plant defences. This framework differentiated three major "mechanisms": 1) *Antibiosis*, which is defined as "adverse effects on the insect life history which result when the insect uses a resistant host-plant variety. The effects on the

insect take form of reduced fecundity, decrease size, abnormal length of life, and increase mortality." 2) *Nonpreference* or *antixenosis* (Kogan and Ortman, 1978) which includes "plant characters and insect responses that lead to or away from the use of particular plant or variety, for oviposition, for food, or for shelter, or for combinations of the three". 3) *Tolerance* which refers to the "ability of a plants to grow and reproduce itself or to repair injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host".

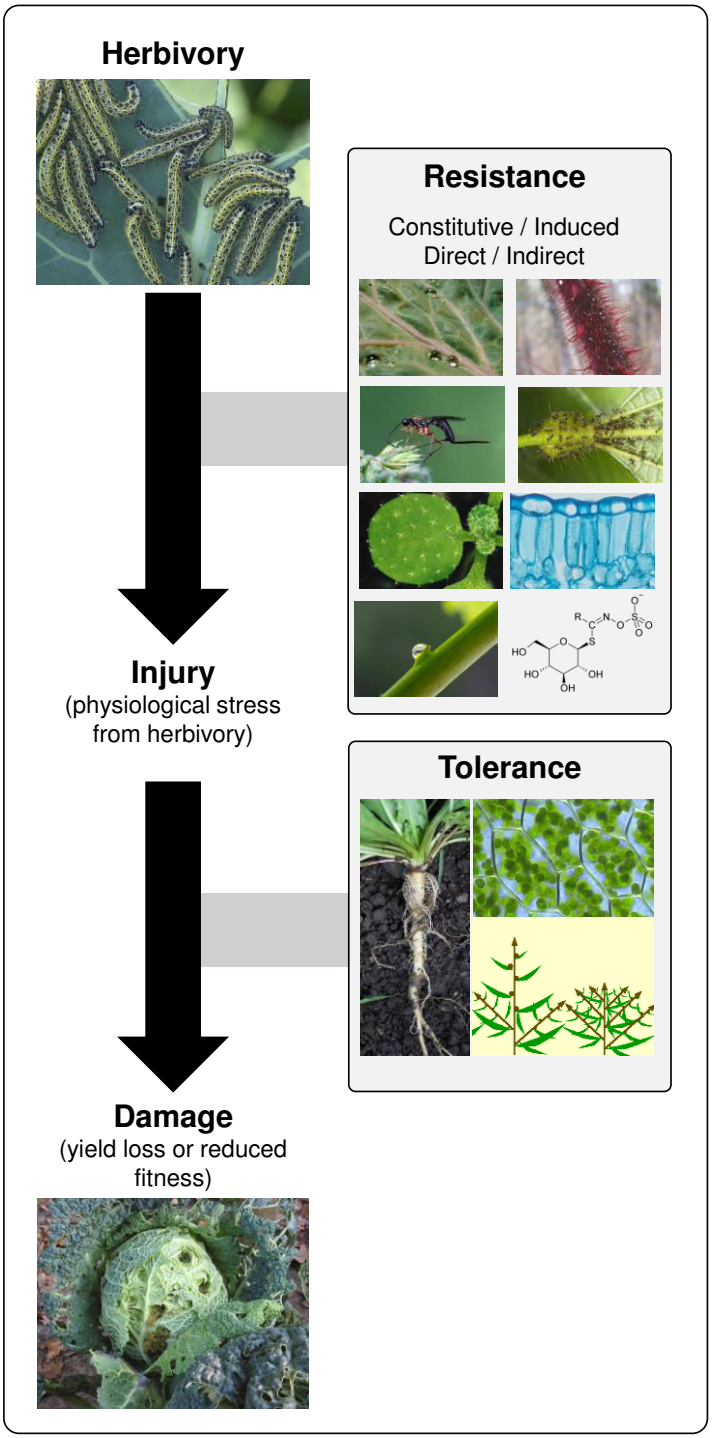

Figure 2: Conceptual framework of plant defence to insects (adapted from Stout, 2013)

This framework is quite simple and has been highly influential for applied entomologists but studies made since the 50's showed its limitations (Stout, 2013). Distinction between antibiosis and antixenosis for example is especially difficult as compounds having a repellent effect on insects can also have a toxic effect on them (Mithöfer and Boland, 2012; Stout, 2013). Moreover, new mechanisms of defence have been identified since that time that cannot be included in this framework: indirect defences, which are the recruitment, feeding or sheltering of natural enemies of herbivorous insects leading to an increased predation or parasitism (Heil, 2008). Stout (2013) proposed a different conceptual framework taking into account actual knowledge of plant defence mechanisms. He established a dichotomy between *resistance* defined as "plant traits that reduce the extent of injury done to plant by an herbivore" and *tolerance* referring to "plant traits or physiological processes that lessen the amount of damage resulting per unit injury" (Fig. 2). Resistance can rely on *constitutive* defences (i.e. produced even in the absence of insect attack) or induced defences (i.e. produced or enhanced only after insect attack). Both resistances can also be *direct* and have an unmediated effect on herbivores or *indirect* and be dependent of the action of natural enemies such as parasitoids or predators (Fig. 2).

These two frameworks show the multiplicity of defence strategies developed by plants to

cope with insect attacks. However, plant defences act together in response to a complex environment where plants are submitted to biotic (e.g. insects, bacteria, virus and nematodes) and abiotic stresses (e.g. nutrient deficiencies, drought, extreme temperatures and salinity). These stresses interact with insect resistance and can involve similar signalling pathways that allow plants to modulate their response to a specific environment (Thoen et al., 2017).

2.2.3. Manipulating plant-insect interactions in agrosystems

This basic knowledge of plant-herbivorous insect interactions indicates that plant defence mechanisms could be used to control insect pests in agrosystems. Management strategies involving manipulation of insect behaviour or plant defences have already been developed. Trap cropping for example, is an interesting strategy which involves plant stands deployed to attract, divert, intercept, and/or retain targeted insects in order to reduce damage to the main crop (Shelton and Badenes-Perez, 2006). This strategy has been implement in cotton fields in California since the 60's where alfalfa, an attractive plant, is used to retain lygus bugs out of cotton crops (Godfrey and Leigh, 1994). Strategies that are more complex include "push-pull" strategies where attractive and repellent plants are used together. Repellent plants are placed in the field to push pests away from the crop of interest and attractive plants are deployed at the border of the field to pull them (Khan et al., 2016). VOCs emitted by plants can also be used in this kind of system to attract insects out of the field and catch them in traps (Gregg et al., 2010; Szendrei and Rodriguez-Soana, 2010) or to repel them and protect crops (Lamy et al., 2017).

All these strategies involve complex set ups with specific cropping arrangements and the use of specific equipment such as traps or volatile dispensers. They can be implemented in small surfaces like vegetable fields or orchards but are more complicated and costly to deploy in large fields (Shelton and Badenes-Perez, 2006). Modifications of insect infestations through plant mediated mechanisms could also be directly used in agrosystems and even enhanced through plant breeding. This strategy does not require specific arrangement or equipment and could be used at large scale.

3. Breeding plants for resistance to insects

3.1. Impact of domestication on insect-plant interactions

Plants have developed different defence mechanisms to resist and tolerate insect attacks. During plant domestication and breeding by farmers, plants have been primarily selected for traits related to quality and quantities of harvested parts and not for resistance to biotic stresses (especially since the wide implementation of pesticides). Plant defences could have been substantially affected during this process. To breed plant resistant to insects, it is important to know how domestication and breeding affected plant defences.

Crop domestication modified plants in a variety of ways (Whitehead et al., 2016). Changes occurred in plant genome through hybridizations that changed their level of polyploidy for example (Olsen and Wendel, 2013). Plant genetic diversity was also affected since domestication was accompanied by the use of a limited number of individuals as progenitors (Doebley et al., 2006). This process considerably reduced plant genetic diversity (Doebley et al., 2006) and could impact defence variability. Farmers have directly altered plant defence by counter selecting resistance traits detrimental to human use such as hardness of seed coats or toxic compounds present in harvested organs (Meyer et al., 2012). Counter selection of undesirable traits which genes are located near genes coding for resistance has resulted in changes in plant defences. This has

for example been observed in the sunflower (*Helianthus annuus*) where genes coding for seed coat are located on the same region as apical branching (Tang et al., 2006). Plant breeding for reduction of branching has decreased seed coat thickness and therefore plant physical defence (Chen et al., 2015). Farmers have also indirectly changed these resistances by increasing the size and nutritional quality of harvested organs for their consumption, resulting in plants that are more nutritious to insects thereby improving their development (Delgado-Baquerizo et al., 2016). Indirect counter selection can also be observed if a trade-off between allocation to defences and nutriment provision to harvested organs exists (Rosenthal and Dirzo, 1997). Consequently, increase in harvested organ size and nutritional quality often results in reduction of defences. Finally, in agrosystems plants evolved in conditions where insects were partly managed by farmers. Their evolution with reduced constraints on defences may have reduced their ability to defend themselves (Milla et al., 2015).

The reduction of plant defences due to domestication has often been advocated (Whitehead et al., 2016) but such conclusion was based on a small number of domestication events and this may not be true for all crops. A recent meta-analysis made by Whitehead et al. (2016) investigated this question by comparing 632 domesticated plants and their wild relatives. They found negative effect of domestication on plant defences in 38 % of these studies, positive and no effect on respectively 26 and 27 % of the comparisons. Overall, this meta-analysis found negative effects of domestication on plant defence against insects but this relationship appears much more complex than previously thought. There doesn't seem to be a general effect of domestication on defence traits such as secondary metabolites or physical barriers, but this effect could be present when considering harvested organs suggesting that for example, reduced defences could be found in fruits or seeds while high levels of defence could still be present in leaves.

Even if domestication and breeding has led to an overall reduction in plant defence and genetic variability, defence mechanisms and variability still exist. In maize for example, over 400 hybrid genotypes commercialized in the United States, 90 % have resistance to the European corn borer (*Ostrinia nubilalis*) (Barry and Darrah, 1991). Also in this species, differences in egg induced VOCs attracting egg parasitoids have been shown in over 77 maize genotypes comprising landrace, hybrids and inbred lines (Tamiru et al., 2015). This variability in defence mechanisms offers opportunities to use and select plants more resistant to insects and thereby less dependent on pesticides.

3.2. Past and current utilisation of crop resistances to insects

Breeding crops for resistance to insects has a long history. Earliest documented observations of differences in susceptibility of a crop to insects were made by Haven in 1792 who identified the wheat cultivar "Underhill" as resistant to the Hessian fly (*Mayetiola destructor*) (Haven, 1792). In 1941, Snelling (1941) already identified 254 publications dealing with breeding for resistance to insects and almost one hundred plant species where differences in resistance to insects have been studied. One of the first insect resistant species widely used was the American grape (*Vitis labrusca*) resistant to the grape phylloxera (*Daktulosphaira vitifoliae*). This insect was introduced in France in about 1860 and caused heavy damage to French vineyards (Granett et al., 2001). The use of *V. labrusca* as rootstock of *V. vinifera* allowed producers to recover from this crisis (Granett et al., 2001). The first resistant genotype bred to express resistances was a wheat cultivar commercialized in 1944 and resistant to the Hessian fly (Painter, 1958). Since 1941 scientific interest on plants resistant to insects increased and large screenings of germplasms were made in different countries (Reed et al., 1980; Agrawal and Abraham, 1984; Heinrichs, 1986). These screenings allowed the development of resistant cultivars such as the rice genotype IR36 produced by the

International Rice Research Institute which was resistant to four species of leafhoppers and a stem borer (*Nilaparvata lugens*, *Nephotettix nigropictis*, *N. malayanus*, *N. virescens* and *Scirpophaga incertulas*) (Brar et al., 2009). This genotype was widely used during the "Green Revolution" in South-East Asia and was estimated to pay an additional $1 billion each year to farmers (Heinrichs, 1986). Between 1975 and 1996, more than 500 resistant genotypes were commercialized in the United States (Smith, 1989; Stoner, 1996). Today, resistant genotypes are known in almost all economically important crops (Wilde, 2002). Use of these resistances still goes on as most rice genotypes cultivated in Asia display resistance to one or more insects and hybrid sorghum resistant to greenbug (*Schizaphis graminum*) represents 80 % of sorghum surface in the United States (Wilde, 2002). However, it is difficult to have a comprehensive view of this strategy. Most recent reviews were made on data collected during the 90's and originating mainly from the United States (Wilde, 2002; Smith, 2005). Moreover, numerous studies identified resistant plants but only a minority of these resistances were used in breeding programs and commercialized (Teetes, 1993).

Interest about conventional breeding for resistance to insects decreased in the 90's with the development of GM crops resistant to insects (Stout, 2014). The main GM technology used to manage insects is the addition of genes encoding the entomocidal toxin Cry from *Bacillus thuringiensis* (Bt) (Gatehouse et al., 2011). Surfaces planted with Bt crops worldwide increased from 1.1 M ha in 1996 to 98.5 M ha in 2016 (Tabashnik and Carrière, 2017). However, restrictions in the use of GMO in some countries (for example in the European Union), high cost of GM crop development, emergence of insects resistant to Bt crops and necessity to develop alternatives to pesticides revive the interest on conventional breeding of resistant plants. Several reviews and perspective papers have been written on this subject recently (Broekgaarden et al., 2011; VanDoom and de Vos, 2013; Stenberg et al., 2015; Tamiru et al., 2015; Fatouros et al., 2016; Mitchell et al., 2016) showing a growing interest in the scientific community.

3.3. Overview of methods used to breed plants for resistance to insects

Even if interest and economic implications are high, technical problems often limit the development of this strategy. Major limitations arise during plant phenotyping, as it is difficult to estimate plant resistances to insects. Below is a brief overview of the current technics and constraints related to identification and development of resistance and how new technics could overcome these issues.

3.3.1. Searching for sources of resistance

Most technical limitations in breeding for resistance to insects arise from the fact that resistance traits are rare. Hundreds or even thousands of genotypes need to be screened to identify good candidates in germplasms and screenings need to be repeated during the breeding process. Smith (2005) reviewed screening programs on different crops and insects. He found between 1 % and 10 % of resistance in most of the germplasms studied but a large part of these studies focused on high levels of resistance. This kind of resistance often relies on a small number of genes and is governed by gene-for-gene interactions (Yencho et al., 2000). In this kind of resistances, a specific gene in the plant recognizes products of the avirulence gene in the insect that triggers a specific response from the plant (Stuart, 2015). This phenomenon is similar to the one found in plant-pathogen interactions but it is less frequently observed for insects than for pathogens (Stout and Davis, 2009). Most of the times, it is found for insects having intimate relationships with the plant such as piercing-sucking or galling insects (Yencho et al., 2000; Stout and Davis, 2009; VanDoom and de Vos, 2013). High to low levels of resistance can also be found in polygenic resistances (Smith and Clement, 2012). Indeed, basic researches have shown that most defence mechanisms are

quantitatively controlled by regions of the genome and not by a small number of specific genes (Kliebenstein et al., 2009; Meihls et al., 2013). Quantitative Trait Loci (QTL) for plant resistance are frequently found but breeding for these resistances traits is difficult (Smith and Clement, 2012). Difficulties arise because each QTL represents a small proportion of resistance and a robust and stable resistance can be achieve only by adding several of these QTL. Such pyramiding of small resistances is difficult to reach by random crossing. However, recent development in genetics allowed utilization of DNA based Marker-Assisted Selection (MAS). This method consists in breeding plants based on the allelic content at a given locus as described by a molecular marker. This can either be a substitute for, or assist the phenotypic selection (Collar et al., 2005). These markers are DNA sequences tagging genes or QTL linked to the expression of resistance. MAS thus allows easier breeding for quantitative resistance as the crossings are not done randomly anymore and the progeny can be screened very early in plant development. However, even with large screenings, it is sometimes not possible to find interesting levels of resistances in germplasms. Resistances against the rice water weevil (*Lissorhoptrus oryzophilus*) have been sought for over 40 years within 8000 rice lines and high levels of resistance has not been found (Stout, 2014).

Resistance can also be found in other related species (wild or already cultivated) and introgressed into crops. As explained above (see 3.1), high levels of resistance exist in non-domesticated relatives of most crops (Mirnezhad et al., 2009; Maharijaya and Vosman, 2015). Such plants have been extensively used to incorporate desirable traits into crops and especially resistance traits to biotic stresses (Dempewolf et al., 2017). Most of these resistances concern pathogens such as *Phytophthora infestans* (an oomycete causing the late blight) for which the wild potato *Solanum demissum* harboured a resistance (Hajjar and Hodgkin, 2007). While less frequent, successful introgressions of resistance to insects in elite material has also been made. It has for example been made by hybridization between oilseed rape (*Brassica napus*) and white mustard (*Sinapis alba*) which was resistant to the cabbage seed weevil (*Ceutorhynchus assimilis*) (Dosdall and Kott, 2006). Introgression of resistance traits from a wild relative remains challenging. Improvement of in-vitro tissue culture such as embryo rescue has allowed performing distant hybridizations that produce non-germinating seeds (Sharma et al., 1996). However, hybridization between two related species is not always feasible and can lead to retention of undesirable agronomic traits. Backcrossing process is thus necessary but it is time consuming and sometimes even not practicable (Hajjar and Hodgkin, 2007). For example, lines of hops (*Humulus lupulus*) expressing resistance to spider mites conferred by a wild relative have exhibited extreme hairiness and could not be commercialized (Hajjar and Hodgkin, 2007).

3.3.2. Measuring plant resistance to insects

Once genotypes to test have been chosen, their resistances need to be measured. Different experimental procedures and measurement methods have been developed to screen genotypes. Here is an overview of technics usually employed for that purpose.

Experimental conditions

Screening large collections of plants for resistance to insects is more challenging than screening for other traits such as defence to pathogens. One of the main issues arise from the fact that insect behaviour can strongly bias comparisons. Insects are generally more mobile than pathogens and contrary to them, they can choose between plants. Specific experimental designs are thus necessary to control these behavioural biases and compare genotypes for their susceptibility to insect pests not for their differential attractiveness.

Experimental designs vary according to the objectives of the study and possible constraints concerning the species studied (crop and insect). Plant resistance can be studied in field trials under uncontrolled conditions where different genotypes are sown in a field and exposed to natural infestation. The greatest benefit of this method is that resistances are observed in realistic agricultural conditions. Moreover, this design also allows to test environmental variability of defence expression. Plant phenotypes and defences are polygenic traits (Smith and Clement, 2012). They can be highly dependent on environmental factors such as temperature, drought, nutrients availability in the soil and even soil microbiota (Prudic et al., 2005; Grinnan et al., 2013; Tao et al., 2016). To consider these effects, field trials can be performed at different locations with contrasted climates. This allows testing the effect of an interaction between environments and genotypes on resistance expression. Field trials allow testing a large number of genotypes and several successful screenings of germplasms have been done this way (Sharma et al., 1992). Major drawbacks of this method are related to the variability of insect infestations. Insect infestations can differ between localities, years and even inside the trial. Before conducting such experiments, it is important to find hotspots of insect infestation where pest pressure is high every year. Finally, even if conditions in field trials are close to those in commercial fields, an important difference is that these trials offer a choice among varieties to insects which they do not have in commercial fields usually consisting in monocultures of a single genotype. Among the varieties tested, presence of highly attractive ones can lead to biased estimation of resistance. Moreover, plant susceptibility period of different genotypes tested needs to fit insect infestation to allow comparison of meaningful traits (i.e. not plant phenology). These constraints can be managed by specific field designs like those proposed by Chesnokov (1953) where experimental plots are surrounded by different strips of susceptible plants used to attract and homogenise insects on the genotypes tested. These designs remain complex to implement and they are not always effective.

Infestations of insect in the field can also be partly controlled. This can be done by enhancing insect populations by attracting them with baits. This method has been used in India for screening resistance to the Sorghum Shoot Fly (*Atherigona soccata*) in sorghum fields where fishmeal was spread to attract this insect (Taneja and Leuschner, 1985). Reared insects or plant parts already infested can also be spread to homogenise insect infestations. Such an approach was used for screening resistance to lepidopteran larvae in maize. Manual dispersers of larvae distributing precise numbers of insects on large numbers of plants were developed (Wiseman et al., 1980). In another example, Chinese cabbage plants pre-infested with aphids (*Myzus persicae*) were disseminated in a field to enhance aphid density in potato trials (Tingey and van de Klashorst, 1976). Insect populations can also be more directly controlled by caging plots or plant parts and adding a controlled number of insects in the cage (Sharma and Lopez, 1992). This kind of design may be informative as insect infestation is more controlled and interactions between the environment and the genotype can be tested. Specific equipment is often needed to develop these experiments and they often need insect mass rearing. This is problematic as a many pest species cannot be reared (Stout and Davis, 2009).

Experiments can also be carried out in complete controlled conditions in the laboratory or in greenhouses by confronting plants or detached plant parts to insects. This design allows controlling insect infestation and environmental conditions. It can be useful to study more specific traits and mechanisms of resistance. Experiments under controlled conditions can allow screening large numbers of genotypes, as for example in the case of rice and the brown planthopper (*Nilaparvata lugens*) where more than 68 000 accessions were tested (Brar et al., 2009). Such experiments require mass rearing of insects to screen a large numbers of genotypes. Resistance observed in laboratory conditions may not be expressed in the field. As explained

above, most resistance traits to insects are quantitative and are thus partly dependant on environmental conditions. Growth conditions can influence insects by modifying plant architecture, chemistry or physiology and change interactions with other biotic stresses. Resistances identified using this method need to be confirmed under realistic cropping conditions.

Measuring resistance

Once the experimental condition chosen, specific methods need to be created to phenotype for plant resistance. This is especially complex as insects attack different organs (leaves, stems, roots and meristems) in a variety of ways (chewing, piercing and digging). Consequently, methods developed to measure damage caused by one insect species may not be usable for others. Measurement methods can be dissociated between those that measure insect damage to plants and those that measure insect populations development or behaviour.

Phenotyping for plant resistance can be based on plant yield but it is a complex trait influenced by many environmental factors such as climatic conditions, fertilization, biotic and abiotic stresses (Shi et al., 2009). Studies focussing on this measurement often involve the addition of control plots sprayed with insecticide to estimate the difference between yields with and without insects. Due to these difficulties, direct measurement of insect attacks is often preferred over quantification of yield losses. Insects causing visible damage such as leaf chewing, destruction of meristem or stem mining leading to stem break can be directly quantified. Leaf area chewed by insects, count of damaged meristems or length of larval tunnels is easily quantifiable but time consuming. Standardised damage rating scales can also be used to quickly quantify damage but these methods are not accurate and lack statistical power. These scales can be useful at the beginning of a breeding program to screen large numbers of plants and remove the most susceptible genotypes.

Measurements can also be done on insects. This is of special interest for insects that do not lead to direct, visible damage. Aphids for example cause hardly quantifiable damage. In such a case, counting the number of individuals or the number of colonies on a plant is much more effective in quantifying plant resistance (Singh et al., 2014). Insect behaviour can also be monitored to quantify plant resistance. Electrical Penetration Graph (EPG) allows monitoring the feeding activity of sap-sucking insects by placing electrodes on the insect and on the plant. This method has been widely used to screen resistance to sap-sucking insects and allows accurate quantification of behaviours (Caillaud et al., 1995; Klinger et al., 1998; Greenslade et al., 2016).

Even if specific methods have been developed to quantify damage caused by a variety of insects, plant phenotyping remains a time consuming and labour intensive process. Phenotyping is increasingly recognized as one of the main bottleneck in plant breeding programs (Furbank and Tester, 2011; Fiorani and Schurr, 2013) and this is particularly true concerning resistance to insects (Goggin et al., 2015). However, recent technical developments could help designing new methodologies that are more rapid and efficient than the ones presented above.

3.4. How to relieve the phenotyping bottleneck?

Technical developments over the last decades dramatically increased phenotyping opportunities. Costs of sensors and chemical analyses have substantially decreased and led to an increased affordability of these methods (Fernie and Schauer, 2009; Tsaftaris and Noutsos, 2009). Three classes of sensors are extensively

used for plant phenotyping: RGB (Red, Green and Blue) sensors which detect visual wavelengths, fluorescence sensors that are used to measure chlorophyll and polyphenol content and spectral measurement such as near-infrared (NIR) that quantify water content of plant tissues, temperature and stomatal conductance (Fiorani and Schurr, 2013). Plant chemistry on the other hand is mainly analysed using mass spectrometry (MS) coupled with gas or liquid chromatography or nuclear magnetic resonance (NMR) (Hall, 2006). These technics could help breeders and entomologists to quantify damages caused by insects or morphological and chemical factors related to plant defences.

3.4.1. Quantifying damage caused by insects

If insects cause visible damage on plants, they can be monitored directly. In such a case, analysis of RGB image is better adapted to quantify leaf surface defoliated, losses of pigments related to cell feeding, leaf mining and stem boring. Digital images have been used to quantify plant damage or identifying plants attacked by insects for damage caused by *Spodoptera frugiperda* on maize or *Cameraria ohridella* on horse chestnut (Gilbert and Grégoire, 2003, Sena et al., 2003; Johnson et al., 2016).

As explained in the previous section, some insects do not cause easily detectable damage. Insect counts can be an alternative way to estimate plant resistance. Digital images have for example been used to count whiteflies (*Trialeurodes vaporariorum*) (Boissard et al., 2008) and video tracking can efficiently monitor aphid feeding behaviour and discriminate resistant plants (Kloth et al., 2015). Herbivory can generate local drought stress on leaves and impact plant photosynthesis (Tang et al., 2006). This can be measured using fluorescence and even NIR sensors to detect subtle changes in temperature (Tang et al., 2006; Nabity et al., 2009). Herbivory has also more systemic consequences on plants such as deformation of plant organs or specific pigmentation that can be monitored using RGB images (Goggin et al., 2015).

Measuring plant damage is still complex as most of the methods are adapted for measurements on flat surfaces. Utilisation of these methods has been limited to laboratory and greenhouse experiments until now. Another limitation of these technics is that identification of resistant plants by measuring damage caused by insects is dependent on the latter. This limits screening possibilities especially for insects that cannot be reared.

3.4.2. Measuring plant defences

Another approach to screen for plant resistance is to measure morphological or chemical factors correlated to resistance or directly involved in plant defence or susceptibility mechanisms. Once these factors have been identified, measurement of plant traits or concentration of chemical compounds can be used as biomarkers to rapidly find potential resistant plants (Smith, 2005). This approach is less dependent of insect populations than quantification of damage as measurement of biomarkers can be done without confronting plants and insects.

Using biomarkers that are not directly related to defence mechanisms is also possible. Shaw et al. (2009) for example, used this method to identify a flavonoid correlated with resistance against the cabbage seedpod weevil (*Ceutorhynchus obstrictus*) without demonstrating a direct effect of this molecule on the insect. Such a method needs to be carefully conducted as biomarkers can be identified in specific experimental conditions and not be confirmed in other environments.

Biomarkers directly involved in defences may be preferred over markers simply correlated with them. They give a more reliable estimation of plant resistance. Identification of these makers can be done by

measurement of morphological factors. Trichome density, leaf toughness or stem size are linked to plant resistance or tolerance to insects and have been used to screen genotypes (Kitch et al., 1985; Berguinson et al., 1995; Ekuere et al., 2005). Utilisation of chemical analysis is also a powerful tool to discriminate resistant and susceptible plants and predict plant susceptibility (Malchev et al., 2010; Wang et al., 2017). Once compounds related to resistance have been identified, breeding for enhanced defence is possible. Such an approach has been implemented in maize where maysin concentration (a defence compound acting on *Helicoverpa zea*) was enhanced though recurrent breeding (Widstrom and Snook, 2001). Most of the time, quantification of biochemical biomarkers is performed using MS but NMR or NIR also have been used (Rutherford, 1998; Leiss et al., 2011). Handheld fluorescence sensors can be an interesting alternative to detect polyphenols (Muñoz-Huerta et al., 2013). These compounds are known to be an important part of defence against biotic agents such as insects and have been identified as markers of resistance (Wang et al., 1999; Leiss et al., 2009; Anyanga et al., 2017).

Chemical analyses remain more expensive and variable according to experimental conditions than genotyping (Guo et al., 2016). A second step in developing breeding programs using biomarkers is to identify QTL related to expression of these chemical markers (Lee et al., 2014). Once QTL of biomarkers have been found, screening of plants for resistance can be performed through genotyping. This method increases breeding programs speed as genotypes can be sampled even at an early development stage.

3.4.3. Understanding mechanisms behind insect-plant interactions

As explained in the previous section (see 3.4.2), measuring mechanisms of defence could help breeders and entomologists developing resistant plants. Historically, applied researches on plant resistance have focused on the development, characterisation and utilisation of resistant varieties (Stout, 2013). Little attention has been paid to the understanding of the mechanistic basis of these resistances (Stout, 2013). In its influential work, Painter (1951) considered that such understanding was complex and could be of little use. Maybe because of this, breeding for insect resistances has long been performed without trying to understand the mechanisms involved. However, Painter's view must be regarded as a reflection of the knowledge and means of the 50's which limited the elucidation of mechanisms. Today, theoretical knowledge, equipment and technics such as chemical analysis, genetics and GMOs could allow to better understand the resistance mechanisms. Development of "-omics" approaches give valuable information on these resistances and is increasingly used for breeding (Fernandez et al., 2016). Integrative approaches combining genomic, transcriptomic, proteomic and metabolomics could facilitate identification of genetic traits linked to plant phenotype and predict complex traits (Barah and Bones, 2015).

Benefits brought by the elucidation of mechanisms of resistance are multiple. First, they could help breeders and entomologists during their work. Understanding mechanisms involved allows to develop adapted and specific screening methods. For example, if the resistances identified are based on induced or indirect mechanisms, induction of defence or presence of natural enemies are necessary to the screening. Second, they allow building resistances that are effective in diverse environments and durable. Plants that produce induced defences may be more useful against insects having relatively long interactions with a specific plant than insects highly mobile moving rapidly from plant to plant. Similarly, plants expressing higher indirect defences may be more likely to be effective in conditions where natural enemies are abundant. Finally, all plant defences do not affect insect populations in the same way. Plants that have toxic effects on insects may exert more pressure on pest populations than repellent plants and thus may be more rapidly by-passed by insects.

4. Conclusion

In his book, Smith (2005) concluded his chapter dedicated to phenotyping by: "New techniques to accurately identify plant resistance to arthropods must be a dominant element of future crop cultivar improvement research efforts." This problem remains central to research in this area today. Even if drastic technical improvements have been made on image, chemical and genetic analyses, applications of these techniques to develop cops resistant to insects remain rare. Place importance on understanding life history traits of target insects and developing biomarkers may be part of the solution to breed for complex resistances.

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Study system

1. Oilseed rape

Oilseed rape (OSR, *Brassica napus* L.) seems to originate from a spontaneous cross between a turnip rape (*B. rapa*) and a cabbage (*B. oleracea*) which occurred during the Middle Ages (Iñiguez Luy and Federico, 2011). This relatively recent cross has led to a reduced genetic diversity and modern breeding of OSR for low erucic acid and low glucosinolates content again has reduced this diversity (Becker et al., 1995; Bus et al., 2011). Development of OSR cultivation is relatively recent and has been possible thanks to the creation of cultivars containing low erucic acid and glucosinolates in seeds. These two events have increased its commercialisation as oil for human consumption and "oil cake" for animal feed (Hebinger, 2013). Moreover, incentive policies on biodiesel have increased market needs of oil and supported increase in acreage (Hebinger, 2013). OSR is the world's second oilseed crop (Carré and Pouzet, 2014) and the most cultivated oilseed crop in Europe with 6.46 M ha grown in 2015 (Eurostat, 2015). OSR is mainly grown in the European Union (EU), Canada, China and India (Carré and Pouzet, 2014). In the EU, France and Germany are the main producers with 1.5 M ha and 1.28 M ha in France and Germany respectively (Eurostat, 2015).

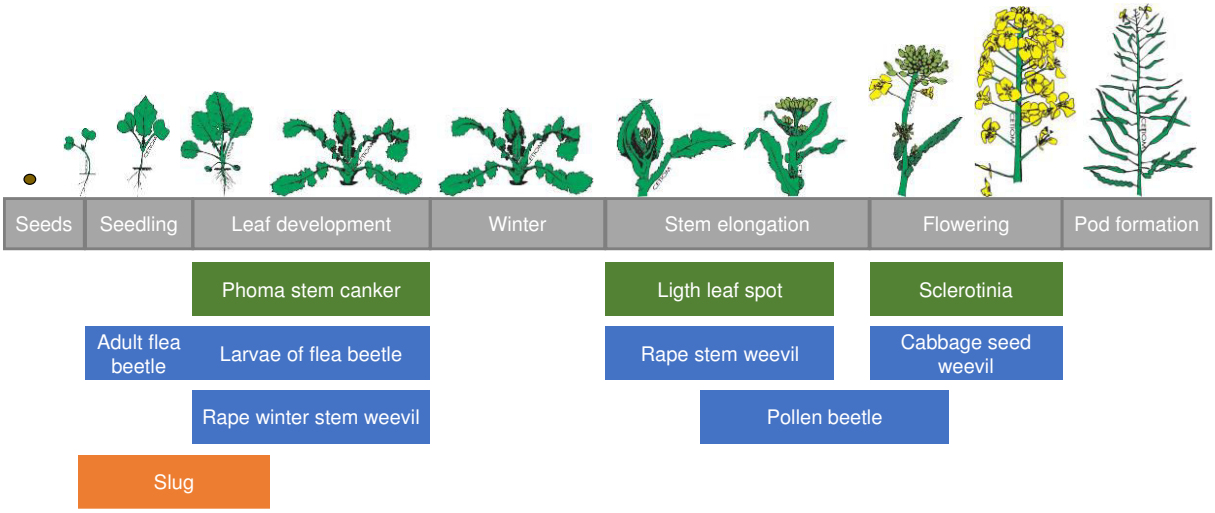

Figure 1: Phenology of OSR during the season. Coloured boxes illustrate periods of occurrence of main pests of this crop: plant diseases in blue, insects in green and gastropods in orange (adapted from Terres Inovia).

Three major types of OSR are cultivated. Winter OSR, which is the most grown in the EU, is sown in August-September and harvested in June-August and necessitates a vernalisation period in order to initiate flowering. Semi-winter OSR is sown in September-October and harvested in April-May and is the most cultivated in China. Finally, spring OSR is grown in North America, Australia and Northern Europe. It is sown in March-April and harvested in August-September in the northern hemisphere. Cultivation of winter OSR is long (between 270 and 300 days). During this period plants are submitted to multiple biotic stresses such as pathogens, slugs and insects (Fig. 1). Autumn and spring are especially suitable for insect attacks. During the autumn, plants can be attacked by the cabbage-stem flea beetle (*Psylliodes chrysocephala*) and the rape winter stem weevil (*Ceutorhynchus picitarsis*) that destroy vegetative plant parts. In the spring, the rape stem weevil (*C. napi*), the pollen beetle (*Brassicogethe aeneus*), the cabbage seed weevil (*C. assimilis*

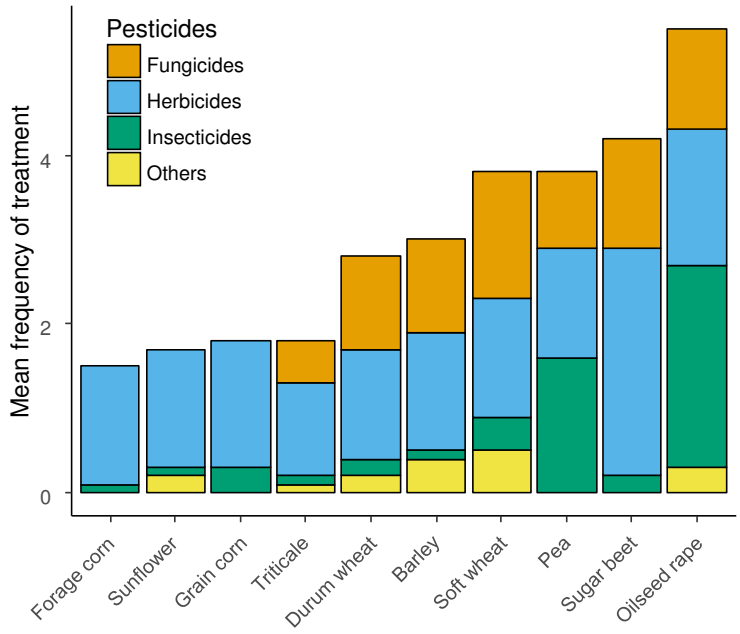

Figure 2: Frequency of pesticide treatments of different crops in France during the year 2011 (AGRESTE, 2013).

syn. *C. obstrictus*) and the brassica pod midge (*Dasineura brassicae*) are the main insects damaging OSR leading to lodging or reduction in pod production. Numerous pesticide sprays are needed to protect this crop against insects and the treatment frequency of insecticides in OSR is the highest of field crop plants in France (Fig. 2).

As explained in part *General Introduction*, pesticides are problematic for human health and the environment. The use of insecticides on OSR is also a major concern due to its impact on bee populations. OSR is a mass flowering crop, intensively used by bees (honey bees and wild bees) for foraging (Westphal et al., 2003; Hayter and Cresswell, 2006). Utilisation of insecticides in OSR crops has been advocated to be an important factor affecting reduction of bee populations (Cresswell, 2010; Rundlöf et al., 2015). Moreover, efficiency of insecticides is diminishing as populations of insect pests resistant to them are increasingly observed. Populations of pollen beetles resistant to pyrethroids have been first observed in 1999 in France (Zimmer and Nauen 2011) and are now found all over Europe (Slater et al., 2011). Resistances to pyrethroids have also been reported in populations of cabbage flea beetles and cabbage seed weevil (Heimbach and Müller, 2012; Zimmer et al., 2014; Højland et al., 2015). Consequently, new management strategies, less dependent of pesticides need to be developed to reduce the environmental impact of OSR crops.

2. Pollen beetle

2.1. Pollen beetle ecology

Brassicogethes aeneus (formerly *Meligethes aeneus*) Fabricius (Coleoptera: Nitidulidae) is the dominant species of pollen beetle found in OSR crops in Europe (Metspalu et al., 2011; Tóth et al., 2013; Ouvrard et al., 2016). Other species such as *B. viridescens* can be observed but they constitute a small minority (Ouvrard et al., 2016). Adult pollen beetles emerge from the soil and litter of forest in early spring when mean temperatures rise above 10 °C (Fig. 3; Nilsson, 1994). Once temperatures reach 12 °C, insects fly and disperse to find flowering plants. Adult pollen beetles are pollinivorous and begin feeding on diverse spring flowers after emergence before moving to OSR fields (Free and Williams, 1978). Insects colonize OSR fields to feed and oviposit (Fig. 3). Pollen beetle arrival takes place between the end of February and early March in France (source: *www.terresinovia.fr*). Pollen beetles arrive on OSR when plants are at the green bud stage or even before bud apparition and destroy floral buds to reach the pollen inside (Veromann et al., 2012). This insect is specialized on Brassicaceae for oviposition and oviposits on mid-sized floral buds (Nilsson, 1988). Between two and three eggs (occasionally up to ten) are deposited in each bud and one female can produce up to 250 eggs during its life. Complete development of the insect takes about one

month. Two larval instars have been identified (Fig. 3; Osborne, 1965). The first instar develops in floral buds eating pollen while the second develops in open flowers, occasionally moving from flower to flower (Free and Williams, 1978). Once mature, larvae drop from the plant canopy, bury themselves in the first centimetres of soil and pupate (Williams, 2010). A new generation of adult pollen beetles emerge from the soil during the following summer (Fig. 3). These insects need to overwinter to be fertile and thus do not reproduce. They disperse and feed on available flowers before moving to forests where they start overwintering at the beginning of the autumn (Fig. 3).

2.2. Damage caused by the pollen beetle

Most of the damage caused by the pollen beetle is made by adult feeding during the green bud stage and lasts about 1-3 weeks. Adults usually destroy bud perianths (petal and sepal) and carpels to reach the pollen leading to bud abortion and thus affecting grain yield. Insects feeding on open flowers do not cause much damage. Oviposition can also cause abortion of floral buds if the carpel is damaged while larvae can destroy flowers if they are too numerous. However, William and Free (1978) did not find an impact of larval

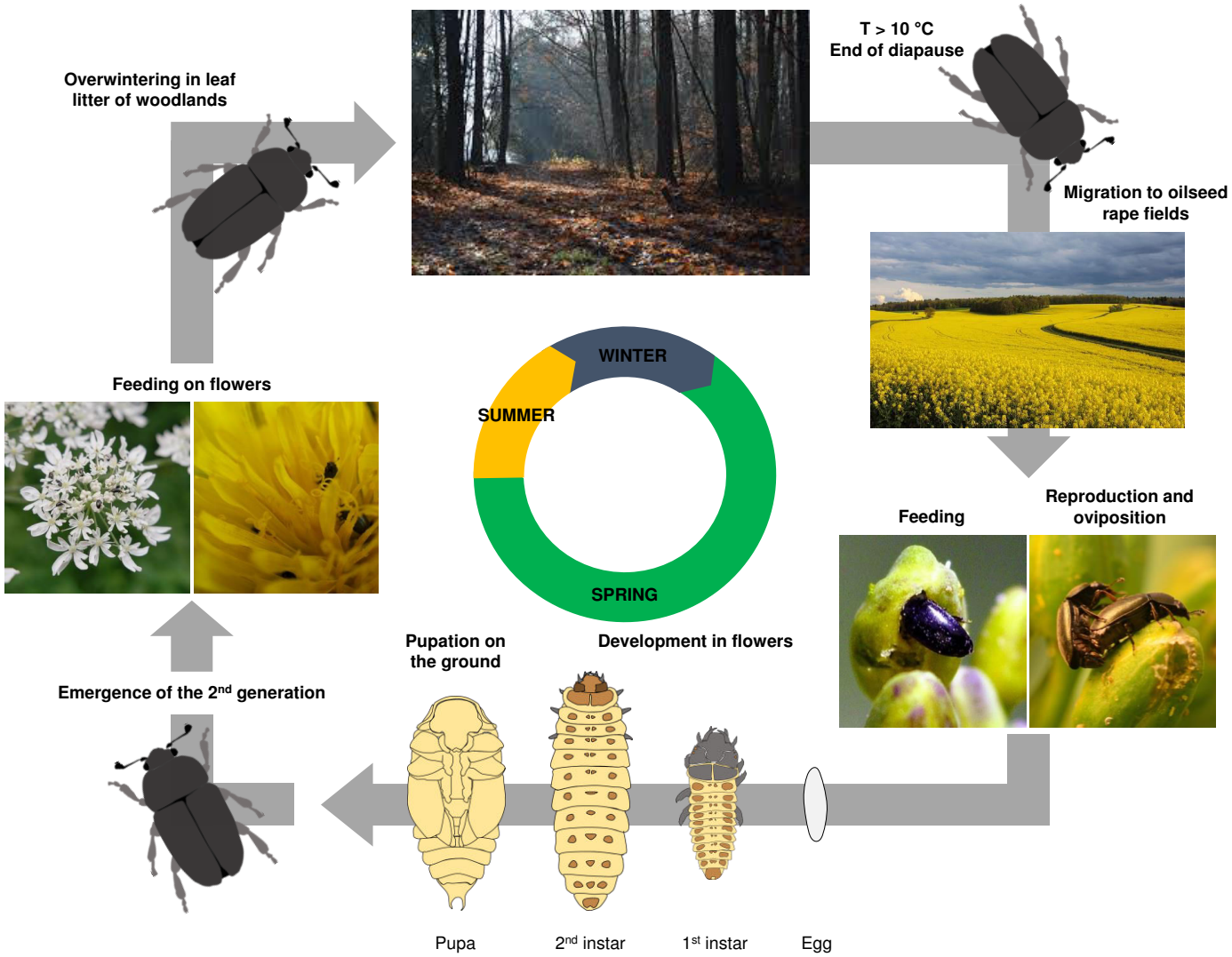

Figure 3: Life cycle of the pollen beetle (*Brassicogethes aeneus*).

infestation on pod sets. Pollen beetle damage can lead to more than 80 % yield reduction if fields are not sprayed with insecticides and insect pressure is high (Nilsson, 1994; Hansen, 2004). Recent observations made in Sweden classed the pollen beetle as the main insect pest damaging OSR (Gagic et al., 2016). However, no such data are available in major producing countries such as France and Germany. Even if pollen beetle can be highly damaging, OSR has an important tolerance (Pinet et al., 2015). Damages can be compensated by the development of supplementary pods. However, plants facing other stresses (attacks from other insects, cold or drought episodes) may not recover from pollen beetle attacks.

Treatment thresholds recommended in several European countries have been reviewed by Richardson (2008) and Williams (2010). These thresholds are highly variable depending on countries (1-15 insects per plant) and have been established between the 1960's and early 2000's. Thresholds established in different countries are not consistent with each other and are not based on the same traits. The most recent control threshold was established in the United Kingdom using experiments in controlled conditions and field observations (Ellis and Berry, 2012). Based on this work, these authors proposed a threshold of 20 insects per plant on winter OSR and 18 insects per plant for spring OSR which is higher than current control thresholds in Europe.

2.3. Pollen beetle management

Insecticides are widely used to control the pollen beetle. More than 90 % of OSR cropping areas are treated for pollen beetles in the EU and 1-4 treatments per year are necessary to control this pest (Richardson, 2008). Insecticides, especially pyrethroids have been used prophylactically during more than 25 years. This over-utilisation has led to the development of resistance to pyrethroids and reduced their efficiency. Other compounds such as neonicotinoids are increasingly used to control this pest (Zimmer and Nauen, 2011) but they are progressively recognized as harmful for pollinators such as bees (Cresswell, 2010; Whitehorn et al., 2012; Rundlöf et al., 2015; Woodcock et al., 2017). Environmental impact of neonicotinoids progressively leads to restrictions and banning of their use in the EU and leaves famers without efficient control methods.

Biological control of pollen beetle populations by natural enemies has received special interest as important levels of parasitism have sometimes been observed in the field (Williams, 2010). The pollen beetle is parasitized by nine hymenopteran endoparasitoid species (Ulber et al., 2010) that are all egg or larval parasitoids. The main species of parasitoids observed on winter OSR are *Tersilochus heterocerus*, *Phradis interstitialis* and *Phradis morionellus* while *Diospilus capito* is abundant on spring OSR. Levels of parasitism are very variable and can by locally high for *Tersilochus heterocerus* that can parasitize up to 97 % of the larvae (Ulber et al., 2010). Ichneumonid parasitoids (*T. heterocerus*, *P. interstitialis* and *P. morionellus*) are univoltine and diapause as adults in the soil of the field where they developed. These species are very sensitive to tillage, particularly ploughing (Nilsson, 2010). Even if high levels of parasitism are reached, efficiency of a strategy relying on these natural enemies is questioned as studies seeking to disentangle the effect of several factors on pollen beetle mortality showed that parasitism explains only a small percentage of this mortality. Exclusion experiments showed that mortality due to parasitoids accounts for only 1-2 % of pollen beetle mortality whereas predators cause 16-27 % mortality (Büchi, 2002). These results are in line with a more recent study that could not identify a relationship between parasitism levels and mortality of pollen beetles but found a good correlation between abundance of ground-dwelling predators and decreased emergence (Riggi et al., 2017). Predators such as carabids and spiders may be of higher interest to limit pollen beetle populations (Haschek et al., 2011; Öberg et al., 2011).

Pollen beetle populations are impacted by the landscape. Landscape complexity and proportion of semi-natural habitats increase pollen beetle abundance in OSR field (Zaller et al., 2008; Rusch, 2010). These observations can be explained by the higher proportion of wintering sites in more complex habitats (Rusch et al., 2011). Parasitoids such as *T. heterocerus* are also more abundant in landscapes with high proportions of semi-natural habitat (Thies and Tscharntke, 1999; Büchi, 2002) where they find increased availability of food sources such as nectar from flowers (Rusch et al., 2013). This may lead to an ambivalent impact of landscape on pollen beetle populations and limits the development of management strategies based on landscape structure.

At the field scale, control using trap-cropping based on pollen beetle behaviour was also proposed. Pollen beetles are known to be more attracted to flowering than non-flowering plants (Cook et al., 2007). They can be diverted from OSR crops by early flowering plants. In France, *Terres Inovia* prescribes to mix OSR with a tall and early flowering OSR genotype in small proportion to divert pollen beetle from target OSR (Fig. 4a, Richardson, 2008). This technic seems to be effective at low to medium levels of infestation. Using turnip rape (*B. rapa*) or other early flowering species such as trap-crops has also been investigated (Cook et al., 2006; Veroman et al., 2012). Implantation of these plants at the border of the field can decrease pollen beetle abundance on OSR (Fig. 4b). However, field experiments showed that growth stage differences between OSR and trap-crops are very variable according to field location and year (Cook et al., 2013). Trap-crop strategy is only occasionally effective in reducing pollen beetle infestations (Cook et al., 2013).

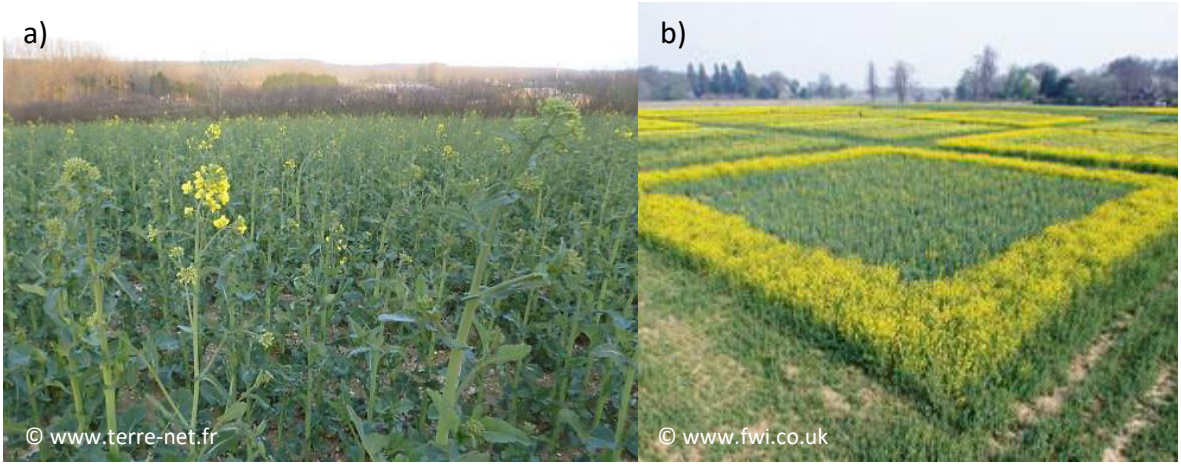

Figure 4: a) Early flowering OSR (ES-Alicia) sown in OSR field. b) Trap-cropping using a border of early flowering turnip rape.

Other strategies such as the use of entomopathogenic fungi (Butt et al., 1998) and nematodes (Nielsen and Philipsen, 2005), transgenic plants (Lehrman et al., 2008) and repellent volatiles (Mauchline et al., 2017) have also been tested but yield mitigated results. An efficient and sustainable strategy is still sought to control pollen beetle populations.

2.4. Breeding OSR for enhancing resistance to pollen beetle

Thus, so far, it seems that no single management strategy can be completely efficient against the pollen beetle. Breeding OSR resistant to this insect could be an important complementary approach. This strategy has been attempted or is ongoing for several OSR pests including pollen beetle (reviewed by Hervé, 2018). To date, no OSR variety with a resistance trait against insects has been commercialized. Several life history

traits could be targeted to reduce pollen beetle incidence on the plant (reviewed by Hervé and Cortesero, 2016):

- *Plant attractiveness*. Differences in OSR attractiveness were observed in laboratory (Cook et al., 2006; Hervé et al., 2017) and field experiments (Giamoustaris and Mithen, 1996; Tölle, 2011). However, the least preferred plants identified in these experiments were still attractive to pollen beetles and no repellent effects have been observed. If such plants are cultivated in monocropping, they will not prevent insect colonisation and will probably be ineffective to reduce damage.

- *Adult survival*. Differences in survival have been observed among genotypes in laboratory tests (Hervé et al., 2016). However, it seems unlikely that variability in such trait could help manage pollen beetle. Damage on OSR are caused at the beginning of insect field colonisation and occur on a relatively short period. No toxic effect that could rapidly kill adults has been observed and survival variability was observed only after several days of starvation which would limit the efficiency of such strategy.

- *Oviposition*. Differences in oviposition levels have been observed among OSR genotypes but they seem to be related to differences in feeding and not directly to genotypes (Hervé et al., 2014a). If it was found, a trait reducing oviposition will not have a direct effect on damage but could reduce infestation for the following year.

- *Adult feeding*. Important variability in adult feeding has been observed among OSR genotypes and this trait could be used for breeding (Hervé et al., 2014b). Reduced adult feeding could be of special interest as most of damage are caused by adults when feeding.

- *Larval development*. Intraspecific variability of these trait is known in OSR (Tölle, 2011; Hervé et al., 2016). It will not directly impact damage on plants but it could reduce pollen beetle populations and decrease infestations the following year.

Adult feeding and to a lesser extent larval development seem to be the most interesting targets to reduce pollen beetle damage.

To identify resistant genotypes, large numbers of genotypes need to be screened. This step of the breeding process is challenging, especially to study resistance to adult feeding. Firstly, because pollen beetle cannot be mass reared (Broman, 1988). This limits screening possibilities as insects can be collected in the field during only 4-5 months (March-July). Beside temporal limitations, insects collected in the field at different times may have different physiological needs or originate from different generations and bias results obtained. Secondly, because experiments on detached buds are not always representative of experiments made on complete plants. As shown by Hervé et al. (2017), susceptibility of plants estimated using detached buds and laboratory experiments conducted on complete plants showed different results (Fig. 5a). Thirdly, when screening is made in the field, plant phenology can introduce an important bias in resistance estimations. Pollen beetles are more attracted to flowering plants than non-flowering ones (Fig. 5b; Cook et al., 2007; Hervé et al., 2017) and plant growth stage also influences pollen beetle infestation as pollen beetles prefer the most advanced plants (Ferguson et al., 2003; Frearson et al., 2005). Moreover, plants whose susceptible growth stage does not occur at the same time are not submitted to equivalent pressures. Pollen beetle activity is highly dependent on weather conditions (Ferguson et al., 2015). If mean temperatures are different at the two periods of susceptibility, insects infesting these plants could have different activities

resulting in different levels of damage (Fig. 5c). Consequently, screening needs to be performed during a short time period, using field populations of insects, testing insects on entire plants and, if conducted in the field, the experimental design needs to prevent phenological biases.

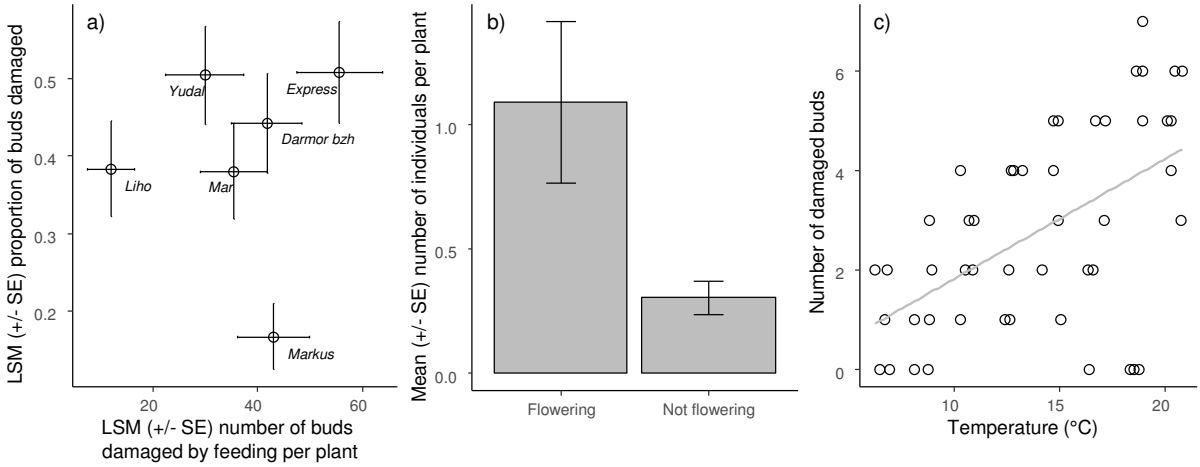

Figure 5: a) Correlation between feeding intensity estimated in complete plants and plant-parts experiments (adapted from Hervé et al., 2017). b) Effect of flowering on pollen beetle attraction (adapted from Hervé et al., 2017). c) Relationship between temperature and pollen beetle bud damage on oilseed rape racemes (adapted from Ferguson et al., 2015).

To effectively screen a large number of genotypes, an alternative approach is to understand the mechanisms at the basis of resistances (Hervé and Cortesero, 2016). Once identified, traits related to resistance could be used as biomarkers for breeding and large collections of genotypes could be screened without constraints related to the insect. To implement such approach for resistance to feeding, a first step was made by Hervé et al. (2014b). This study analysed pollen and perianth chemistry of buds from six OSR genotypes. Quantifications of free amino acids, carbohydrates, polyols, organic acids, glucosinolates and flavonols were performed on these plants. Concentrations of five compounds in the perianth seem to be related to pollen beetle feeding and could be used as potential biomarkers. Three of them were positively related to damages (i.e. sucrose, proline and serine) and two flavonols were negatively linked to feeding (i.e. quercetin-3-O-sophoroside and kaempferol-3-O-sophoroside). This variability in feeding was observed in laboratory conditions on a limited group of genotypes and need to be confirmed in field experiments on larger genotype set.

In addition to issues related to screening possibilities, information about larval development, insect feeding behaviour and their relationships with pollen beetle fitness and damage on the plant are rare. These information remain essential to understand resistance mechanisms and identify new targets for breeding.

As explained in previous sections (see 2.1), pollen beetle larvae feed in flowers on pollen and frequently move from flower to flower (Free and Williams, 1978). Larvae are suspected to eat nectar in flowers as flowers where they are feeding contain less nectar (Kirk et al., 1995). At the end of the flowering period, when all flowers are faded, they can also eat the inflorescence stem (Winfield, 1961). Pollen has a positive effect on insect development (i.e. reduction of developmental time and mortality and increase of larval weight) but larvae can develop without it (Cook et al., 2004). Consequently, they seem to be adapted to deal with different food sources present in flowers. This is in line with transcriptomic analyses that revealed that

larvae overexpress genes related to catabolism compared to adults (Vogel et al., 2014). However, how larvae develop without pollen and if they use other resources present in flowers such as nectar to complete their nutrient needs remain to be studied.

Similar gaps exist for adult pollen beetle feeding. As explained above, adults clearly prefer to feed on flowering plants. Perianth seems to have important effect on its feeding behaviour maybe because it constrains their access to the pollen. Moreover, chemical biomarkers identified by Hervé et al (2014b) show that perianth composition greatly affects pollen beetle behaviour. Insect feeding is also constrained by oviposition. OSR inflorescences are used both for feeding and oviposition but insects avoid buds containing eggs for feeding. Oviposition occurs mainly on medium sized buds (2-5 mm) deflecting feeding on young small buds (1-2 mm) and older large buds (5-7 mm) (Nilsson, 1988). However, it seems that most of feeding attacks occur on small buds (Ferguson et al., 2015) which is surprising as they contain small quantities of pollen. How bud perianth impact adult feeding on OSR and feeding strategies within one inflorescence is not understood.

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Objectives and organisation of the manuscript

To identify biochemical biomarkers which would allow large scale screening of oilseed rape for resistance against the pollen beetle, suitable screening methods need to be developed in the field. As we have seen, most agronomical problems caused by the pollen beetle to oilseed rape crops come from its feeding stages. Feeding is therefore a particularly interesting trait to target for breeding varieties resistant to this pest. While quite an important number of studies have been conducted on this insect, its feeding behaviour is still not fully understood.

Both aspects constitute the main objectives of the present work and they will be presented in 2 chapters where the most important results obtained during this PhD appear as scientific papers (submitted or in preparation).

***Chapter 1*: Screening oilseed rape for pollen beetle resistance**

Paper 1: "Field identification of biochemical biomarkers for screening plant resistance to insects: an example from the pollen beetle – oilseed rape interaction", in prep. for Pest Management Science.

Over two years and locations, oilseed rape genotypes were screened for their resistances to pollen beetles in the field. These experiments aimed at investigating if compounds previously identified as potential biomarkers are good predictors of resistance and identifying potential new biomarkers. The environmental variability of plant chemistry was also assessed.

Short note 1: "Exploring relationship between perianth and floral bud chemistry of oilseed rape to facilitate plant material sampling".

Perianth sampling for chemical analyses is a labour intensive and time consuming process. It greatly limits the number of sampled genotypes. This experiment investigated if chemistry of whole buds was a good proxy of perianth composition.

Short note 2: "Effect of host plant genotype on pollen beetle larval development in field conditions".

A field trial was conducted to develop a method allowing to assess pollen beetle larval infestation and adult emergence in different oilseed rape genotypes.

***Chapter 2*: Feeding ecology of the pollen beetle**

Paper 2: "Impact of flower rewards on phytophagous insects: importance of pollen and nectar for the development of the pollen beetle (*Brassicogethes aeneus*)", submitted to Arthropod-Plant Interactions.

Pollen beetle larvae can develop without pollen but what they eat during their development beside pollen is not known. Laboratory experiments were conducted to identify whether or not nectar could be an important resource for larvae during their development.

Short note 3: "Effect of nectar on preference and feeding behaviour of the pollen beetle (*Brassicogethes aeneus*)".

Although obviously pollinivorous, adult pollen beetles are suspected to feed on flower nectar of oilseed rape. Laboratory experiments were conducted to assess if pollen beetles feed on nectar and if nectar presence impacts their feeding preferences.

Paper 3: "Effect of availability, quality and accessibility of resources on diet selection by herbivorous insects within a host plant", in prep. for Oecologia.

Previous studies pointed out that pollen beetles preferentially feed on small buds of oilseed rape inflorescences but their precise feeding behaviour on inflorescences and the factors governing their choices are still not well understood. We investigated these 2 questions in laboratory experiments.

The manuscript ends with a *General discussion* where the most significant results from both chapters and their implications for pollen beetle management are discussed.

Chapter 1

Screening oilseed rape for pollen beetle resistance

Chapter 1: Screening oilseed rape for pollen beetle resistance

Context

Screening resistance to pollen beetle in oilseed rape is challenging because this insect cannot be reared, laboratory experiments on detached buds are not reliable to estimate genotype resistance and field experiments are greatly influenced by the genotype flowering period. Identifying biochemical biomarkers related to resistance could be a solution for screening a large number of plants. Laboratory experiments already identified some compounds present in the perianth of oilseed rape buds that are related to plant resistances or susceptibility. Whether these compounds can be used as biomarkers need to be confirmed in field experiments. Furthermore if such biomarkers are confirmed their use for resistance screening will require sampling of high numbers of perianths. However, today technics used to collect these samples are time consuming and labour intensive and need to be improved. Even if reduction of feeding damage remains a major target to enhance oilseed rape resistance to insects, diminishing the number of pollen beetles emerging from oilseed rape field and their fitness is an interesting complementary target. Methods allowing to accurately measure larval infestation and numbers of pollen beetles emerging from different oilseed rape genotypes in the field are currently not available.

Approach

Field trials were conducted during two years in different locations to screen resistances to pollen beetle feeding. To reduce bias related to plant phenology, genotypes screened were chosen according to their flowering period. Feeding damage of these plants were estimated over 19 genotypes by counting damaged buds on plants at the green bud stage. Perianths were also collected in different field trials on these genotypes to analyse their chemistry (i.e. free amino acids, carbohydrates, polyols, organic acids, glucosinolates and flavonols). Relationship between genotype damage levels and perianth chemistry was then assessed.

To facilitate perianths sampling, non-dissected complete buds were also collected in the field. Their chemical composition was analysed and compared to perianth chemistry to know if it could be used as a proxy of perianth composition.

A field trial dedicated to monitor pollen beetle larval development over different genotypes was also conducted. We monitored larval infestation and adult pollen beetle emergence using pitfall traps and photoeclectors.

Results

Strong differences in the level of feeding damage were found between oilseed rape genotypes. These differences were consistent between locations demonstrating that they were reliable. Chemical analyses found differences of perianth composition between genotypes but they failed to confirm the relationships between previously identified putative biochemical biomarkers and damage levels. However, some primary metabolites had interesting relationship with genotype resistance and could be interesting new biomarkers.

Good correlations were found between concentrations of metabolites in the perianths and in complete buds giving hope to develop a faster sampling method. Sampling green buds directly without dissecting them could allow screening a large number of genotypes for chemical analyses.

Pollen beetle development could be assessed on three genotypes only and no differences in larval infestation, number of new generation beetles emerging from plots and adult body weight were found. However, a good correlation between the number of larvae collected and the number of new generation pollen beetles emerging was found, indicating that the sampling method used was reliable. The method developed could allow screening larger genotype sets and identifying genotypes that can impact pollen beetle development.

Limits

Even if 19 genotypes were screened for resistance against pollen beetle feeding, plant phenology always limit the number of genotypes that can be screened. Other methods such as caged field plots could help to partly resolve this bias. Moreover, even if some compounds were correlated to damage levels, these relationships were not strong and the biomarkers identified did not seem to be directly related to plant defences. Further testing is necessary to confirm their importance in bud attack by pollen beetles. Field screening conducted in different locations allowed to estimate the environmental variability of perianth chemistry. The environment and its interaction with the genotype explained most of the variation of these compounds and the genotype explained only a relatively limited proportion of this variation. These variations could constraint the value of plant chemistry as a biomarker of resistance.

Field trials conducted to estimate resistance to larval development show that it is possible to screen genotypes for this trait. However, more information about pollen beetle ecology are needs to efficiently use such resistance. How plant traits during larval development affect adult fitness, and especially their ability to survive to overwintering has received little attention but it could help to assess the effect of these resistances on pollen beetle at the population level.

Paper 1

In preparation for Pest Management Science

Field identification of biochemical biomarkers for screening plant resistance to insects: an example from the pollen beetle – oilseed rape interaction

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Abstract: Pollen beetle (*Brassicogethes aeneus*) is one of the main insect pests affecting oilseed rape crops. Efficiency of insecticides used to control this pest is decreasing due to the development of resistance to compounds such as pyrethroids in many populations. Breeding oilseed rape for resistance to pollen beetle attacks could be an interesting strategy to find alternative control methods. However, screening plants for insect resistance remains complicated as it often involves field tests on large genotype collections which are complicated to carry out without biases. Current knowledge on the chemical ecology of interactions between oilseed rape and pollen beetles could help finding biochemical markers of this resistance and bypass this problematic field screening phase thus allowing an indirect breeding approach. Previous laboratory tests have shown that variations in attack levels among a small set of oilseed genotypes could be explained by the biochemistry of bud tissues. The present study aimed at validating this link under field conditions. For that purpose, we conducted a multi-site experiment in France with 19 genotypes exposed to pollen beetle attacks. We phenotyped pollen beetle damage and sampled buds in the field to assess their chemical composition. Large variability in pollen beetle attacks was observed over the genotypes. These attack levels were consistent between locations. Bud chemistry was highly variable but most compounds were well correlated between locations. Potential biomarkers previously identified in laboratory experiments were not confirmed but new compounds which may be considered interesting markers for resistance screening against the pollen beetle emerged.

Key words: Phenotyping, Oilseed rape, Metabolite, Environmental variability, Pollen beetle

INTRODUCTION

Insect pests are one of the main causes of agricultural yield losses, destroying 13-16 % of annual crop production worldwide (Cullinay, 2014). These pests are mainly managed through the use of synthetic insecticides which are increasingly recognized as harmful for human health and environment (Devine and Furlong, 2007). This management strategy needs to be replaced by more sustainable alternatives, less detrimental to health and environment.

Breeding plants for insect resistance could be an interesting approach to reduce agriculture dependency on pesticides (Wiseman, 1994; Singh and Schwartz, 2011). This strategy has already been implemented in the past and has shown its efficiency in controlling some insect populations (Smith, 2005). Interest about this management strategy is growing as plant resistances to insects are easy to use and mostly compatible with other management practices such as biocontrol (Broekgaarden et al., 2011; VanDoom and de Vos, 2013; Stenberg et al., 2015; Tamiru et al., 2015; Mitchell et al., 2016). Recent developments in genetics through genome sequencing and marker assisted breeding have considerably facilitated plant breeding, allowing more rapid and easier achievement of breeders' goals (Smith and Clement, 2012). However, breeding plants for insect resistance remains challenging. Plant phenotyping is becoming the rate limiting step of breeding programs, and this is especially true for developing plants resistant to insects (Barah and Bones, 2015; Goggin et al., 2015; Mitchell et al., 2016). Most of the time, to identify resistant genotypes, phenotyping is addressed through direct confrontation of insects and plants (Tingey, 1986) but this approach is time consuming and can be highly complex for insect species that cannot be reared (Stout and Davis, 2009). Technical advances in phenotyping such as image processing and metabolomics bring new opportunities to develop more efficient screening methods circumventing the phenotyping bottleneck (Furbank and Tester, 2011, Goggin et al., 2015).

Basic researches demonstrated the importance of plant chemistry as a mediator of plant-insect interactions (Berenbaum and Zangerl, 2008). Secondary metabolites such as alkaloids and glucosinolates are used by plants to protect themselves against insects (Bennett and Wallsgrove, 1994). Other compounds such as primary metabolites are major components of insect choice and use of plants (Berenbaum, 1995; Awmack and Leather, 2002). While the former deter, intoxicate or limit the feeding ability of insects, the latter have a nutritional value and are essential to their survival, growth and development. Therefore, plant metabolites could be used as markers of plant-insect relationships. Identification of compounds related to plant susceptibility or resistance to insects could allow large screenings of genotypes based on plant biochemistry rather than on direct confrontation of plants and insects (Smith, 2005). With the reduction of costs and increase accuracy of chemical analyses, it is becoming easier to analyse large numbers of samples and target such approach (Fernie and Schauer, 2009).

Oilseed rape (OSR, *Brassica napus*) is the most cultivated oilseed crop in Europe with 6.46 M ha grown in 2015 (Eurostat, 2015). Winter OSR is attacked by a vast array of insects (Williams, 2010) and thus, it is one of the most insecticide demanding field crop in Europe (AGRESTE, 2013). Pollen beetle (*Brassicogethes aeneus*, formerly *Meligethes aeneus*) is a major pest of OSR in Europe (Gagic et al., 2016). This insect overwinters in the soil and leaf litter of forests and emerges when mean temperatures rise above 10-12°C (Ferguson et al., 2015). It migrates to OSR fields when plants are at the green bud stage and feeds on pollen by destroying flower buds, leading to decreased yields when populations are high (Williams and Free, 1978). Intraspecific variability in OSR susceptibility to pollen beetle feeding has been found in experiments conducted under controlled conditions (Hervé et al., 2014). Field screening of pollen beetle resistance is

challenging as insect infestation is strongly driven by plant phenology through the flowering period (Frearson et al., 2005; Cook et al., 2007) and early flowering plants can escape attacks. Testing a large number of genotypes with diverse flowering periods for resistance to pollen beetle in the field could lead to biased estimation of resistance (Hervé et al., 2017). Estimating resistance to pollen beetle is not trivial and previous studies have quantified infestation using two methods. The first one is based on counting adult pollen beetles present on the plant (Rusch et al., 2013; Kassik et al., 2014). This measure of abundance is easy to do but is not adapted to screen for resistance as pollen beetles are highly mobile and their abundance depending on the weather, can strongly vary between sampling days or even hours. Abundance and damage of pollen beetle have been shown to be weakly correlated (Hansen, 2003; Rusch et al., 2013; Gagic et al., 2016) which confirms that this metric is not the best estimator of plant resistance. A second method to estimate damage has been proposed by counting podless stalks (Free and Williams, 1978; Gagic et al., 2016). This method has been criticised as podless stalks related to pollen beetle can be confounded with abortion due to physiological factors or damage caused by the brassica pod midge (*Dasineura brassicae*) (Free and Williams, 1978; Tatchell, 1983). New methods need to be developed before susceptibility of different genotypes can be assessed in the field. Chemical analyses made on OSR buds identified five potential biochemical biomarkers present in the perianth and correlated to the pattern of resistance to pollen beetle (Hervé et al., 2014). These compounds are both primary metabolites (i.e. serine, proline and sucrose) and secondary metabolites (i.e. flavonols such as quercetin-3-O-sophoroside and kaempferol-3-O-sophoroside). Primary metabolites of the perianth increased bud attacks while secondary metabolites decreased these attacks. Surprisingly, glucosinolates seemed not to be involved. While these results are encouraging, they need to be confirmed under field conditions. The aims of the present study were: i) to develop a screening method allowing to compare resistance of different OSR genotypes to pollen beetle damage in the field; ii) to validate the biomarkers identified by Hervé et al. (2014) on a new set of genotypes and under field conditions; iii) to identify new potential biomarkers and iv) to set the grounds for a future biomarker assisted screening method for pollen beetle resistance in OSR.

MATERIALS AND METHODS

Plant materials

The set of genotypes used by Hervé et al. (2014) contained genotypes with contrasted flowering periods, mixing spring and winter types, and could not be tested in field screening (Hervé et al., 2017). The genotypes used in our experiments were selected based on their phenology as observed in previous years in the field (Appendix 1) and on the genetic diversity observed after genotyping with the illumina 60k infinium Brassica chip (Clarke et al., 2016).

In 2015-16, twenty genotypes of oilseed rape comprising 10 early flowering genotypes and 10 late flowering genotypes were selected for the trials. This was done to maximize the chance of presence of material at the susceptible stage when the pollen beetle flight occurred. In 2016-17, another set of 20 genotypes was selected, this time spanning only the late flowering window, but maximizing the genetic diversity, with six accessions in common with the 2015-16 trials (Appendix 1).

Field trials

Field trials were conducted in two locations on two consecutive years. They were set up in Cornebarrieu (Occitanie, France) and Liverdy en Brie (Ile de France, France) in 2015-2016 and in Mondonville (Occitanie, France) and Liverdy en Brie in 2016-2017. Field trials were designed in a randomized-block

design with 3 blocks. Each genotype was sown in plots of 6 rows of 4 m length, spaced by 50 cm. To reduce border effects, 6 rows of a late-flowering genotype (Licorne in 2015 and Ariana in 2016) were sown around the experimental area.

Pollen beetle and plant growth monitoring

Pollen beetle arrival on the field was monitored using four yellow water traps filled with water and detergent and placed at each corner of the fields. In the spring 2016, pollen beetles arrived at Cornebarrieu on February 25th and on March 23rd at Liverdy en Brie. In 2017, insects arrived at Mondonville on February 16th and in Liverdy en Brie on March 14th. Oilseed rape plants were checked once a week to monitor BBCH growth stage (Lancashire et al., 1991) of each plot to ensure that damage sampling occurred at BBCH growth stage 51-60 when plants are most susceptible to pollen beetle attacks. Damage sampling started once pollen beetles colonized experimental plots and most genotypes were at BBCH growth stage 55-57.

Pollen beetle damage estimation

The number of attacked buds was counted on the first raceme. All buds with one or more holes through the perianth caused by chewing, fading buds with oviposition hole as well as abscised buds were considered as pollen beetle damage. Pollen beetle damage was estimated by randomly sampling 15 plants at BBCH growth stage 51-60 in each plot (45 plants / genotype). Plant size and growth stage (BBCH 51-53, 55-57, 59, 60-61) were recorded for each plant.

In 2016, plant sampling was carried out on April 6th at Cornebarrieu and on April 18th at Liverdy en Brie. In 2017, it was done on March 28th at Mondonville and between April 10th and 11st at Liverdy en Brie.

Plant sampling for chemical analysis

Plants were sampled in 2017 to analyse perianth chemistry. All plants were collected at the same growth stage (BBCH 59) to avoid potential variations in chemistry caused by differences in growth stage. Sampling occurred between March 22nd and April 6th at Mondonville and between April 10th and April 13rd at Liverdy en Brie. Two samples were prepared in each plot to collect a total of six samples for each genotype and location. To complete one sample, 4 plants were harvested in one plot and 20 buds (length > 3 mm) on the first raceme of each plant were removed. These buds were then dissected to collect perianths and immediately frozen in dry ice. Perianths were stored at -20 °C before being freeze-dried and ground to powder before analysis.

Metabolic profiling

Free amino acids (AA), non-structural carbohydrates, polyols and organic acids (CPOA), glucosinolates (GSL) and flavonols (FO) were analysed on sampled perianths.

Quantification of AA and CPOA was based on Gravot et al. (2010). Extraction of these compounds from freeze-dried perianth powder was made on 10 mg of powder, with a methanol-chloroform-water-based extraction. Plant powder was suspended in 500 µl of methanol containing two internal standards: 200µM of 3-aminobutyric acid (BABA) (for quantification of AA) and 400 µM of adonitol (for quantification of CPOA). This suspension was agitated during 15 min at room temperature and 250 µl of chloroform were added followed by 10 min agitation. Five hundred microliters of water were added and samples were vortexed and centrifuged at 12 000 g for 5 min to induce phase separation. The upper phase was transferred to a clean microtube and used for subsequent analysis.

CPOA were analysed by GC-FID according to Adams et al. (1999) and Lugan et al. (2009). The online derivatization was realized with a Trace 1300 GC-FID (Thermo Scientific) equipped with a Tri Plus RSH (Thermo Scientific). Fifty microliters of the extract were dried under vacuum. The residual was redissolved in 50 µl of pyridine containing 20 $mg.ml^{-1}$ methoxyaminehydrochloride, under orbital shaking at 40 °C for 90 min. Fifty microliters of MSTFA (N-methyl-trimethylsilyltrifluoroacetamide) were added before incubation at 40 °C for 30 min. One microliter of the mixture was injected into the GC-FID with a split/splitless injector (split mode set to 1 : 20) at 260 °C, on a TG-5MS column (30 m × 0.32 mm × 0.25 mm, Thermo Scientific) connected to a flame ionization detector at 300 °C. The temperature gradient of the GC oven was set as follows: 4 min at 100 °C followed by an increase of 10 $°C.min^{-1}$ up to 198 °C and maintained at this temperature for 2 min; an increase of 1 $°C.min^{-1}$ up to 202 °C; then an increase of 15 $°C.min^{-1}$ ramp up to 268 °C and held for 3 min followed by an increase of 1 $°C.min^{-1}$ up to 272 °C and raised 210 °C at 10 $°C.min^{-1}$ maintained for 7 min. Adonitol was used as internal standard.

For AA profiling, 50 µl of methanol-water extract were dried under vacuum. The dry residue was suspended in 50 µl of ultrapure water and 5 µl of this suspension were used for-AccQ-Tag Ultra derivatization (Waters). Derivatizated amino acids were analysed using an Acquity UPLC-DAD system (Waters) according to Jubault et al. (2008) except that the column used for analyses was heated at 53 °C and that amino acids were detected at 265 nm using a photodiode array detector. BABA was used as internal standard.

Extraction and analyse of GSL and FO were based on Hervé et al. (2014). Ten milligrams of freeze-dried powder of perianths were suspended in 1 ml of methanol-formic acid (99 : 1) and agitated using a vortex for 30 s at room temperature. The tubes were placed in an ultra-sonicated bath for 5 min and centrifuged for sedimentation. Six hundreds microliters of the liquid phase were then transferred to a clean microtube and directly used for analyse. An Acquity-TQD UPLC-PDA-MS (Waters) with electrospray ionization in a negative mode was used to analyse GSL and FO. Chromatographic conditions were as follow: column Water Acquity C18 (150 mm × 2.1 mm × 1.7 µm), flow rate 0.4 $ml.min^{-1}$, column oven temperature of 25 °C, injection volume of 2 µl. The A-eluent was water-formic acid solution (99.9 : 0.01) and the B-eluent was acetonitrile-formic acid solution (99.9 : 0.01). The applied gradient was: 0 to 0.2 min 2% B, 0.2 to 3 min 62 % B, 3 to 8 min 90 % B, 8 to 9 min 90 % B, then return to initial conditions 2 % B in 1 min and re-equilibration for 1 min.

Mass spectrometry was used to identify GSL based on the m/z response in negative electrospray mode and their retention time. For quantifications, calibration curves were made from a stock solution at 250 $µmol.l^{-1}$ of 3 commercially available standards (glucoerucin, gluconasturtiin and glucobrassicin) with four different dilutions (3, 5, 10 and 50-fold). Glucoerucin, gluconasturtiin and glucobrassicin calibration curves were respectively used to quantify aliphatic, aromatic and indolyl GSL.

FO were analysed using a photodiode array detector at 350 nm. Identification was made based on UV spectra and m/z response (Velasco et al., 2011). Quantification of FO was made with UV calibration curves from a stock solution at 200 $µmol.l^{-1}$ of 3 commercially available compounds (isorhamnetin-3-O-glycoside, quercetin-3-O-glycoside and kaempferol-3-O-glycoside) at 3 different dilutions (10, 20 and 50-fold). These curves were used to quantify compounds structurally related to isorhamnetin, quercetin and kaempferol.

Data analysis

All statistical analyses were performed using R software (R Core Team, 2016).

Pollen beetle infestation of OSR on different sampling occasions

An ANOVA was performed to compare the damage level of OSR at each sampling occasion. The number of damaged buds of the six genotypes sampled in every location and year (i.e. Bolko, G28, Grizzly, Sarepta, Lembke, Lira) were accounted to allow comparison of pollen beetle infestation. Pairwise comparisons of least squares means (LSM) were performed on this ANOVA (package "lsmeans"; Lenth, 2016) with false discovery rate correction for P-values (Benjamini and Hochberg, 1995).

Genotype resistance to pollen beetle

To test differences between genotypes for resistance to pollen beetle different models were built for each location and year. Models were made as follows:

$$Y_{ijk} = \mu + B_j + Sa_{ijk} + D_{ijk} + G_i + \underline{P_{ij}} + \underline{E_{ijk}} \text{ (model 1)}$$

With random and fixed terms symbolized by underlined and non-underlined letters, respectively; Y_{ijl} is the number of damaged buds of the plant k, in the plot of the genotype i in the block j ; μ is the overall mean; B_j is the block effect; S_{ijk} is the size of the plant considered and the regression coefficient of the plant size effect; D_{ijk} is the effect of growth stage of the plant; G_i is the genotypic effect; $\underline{P_{ij}}$ is the plot effect; and E_{ijk} is the residual error following a normal distribution $N(0, \sigma^2_e)$. Wald χ^2 tests were performed on these models to test significant effect of each fixed terms. Pairwise comparisons of least squares means (LSM) were performed as described in the previous section. Pearson's correlation tests were then performed to obtain correlations between LSM of each genotype from different locations and years.

Differences in perianth chemistry

To investigate differences in perianth chemistry between genotypes and locations for 2017 data, the following model was used for each compound:

$$Y_{ijl} = \mu + L_l + B_{jl} + G_i + GL_{il} + E_{ijl} \text{ (model 2)}$$

Where Y_{ijl} is the mean concentration of samples in the block j, the genotype i and the location l; μ is the overall mean; L_l is the effect of the location; B_{jl} is the effect of the block nested in the location; G_i is the effect of the genotype i; GL_{il} is the effect of the interaction between the genotype and the location; and E_{ijl} is the residual error following a normal distribution $N(0, \sigma^2_e)$. F-tests were performed on these models to test significant effect of each variable.

A model similar to model 2 was built with $L_l, B_{jl}, G_i, GL_{il}$ considered as random effects to obtain the percentage of variance of the concentration of each compound explained by the effects of the genotype, the location, the effect of the block nested in the location, the interaction between location and genotype and the residual variance. Variance components of each factor were extracted using "VarCorr" function (package "lme4"; Bates, 2016).

Relation between perianth chemistry and pollen beetle resistance

To establish a relation between genotype chemistry and resistance, LSM of the concentration of each compound and genotype were extracted from model 2. To compare these LSM to similar values of pollen

beetle susceptibility, a model based on average plot values and accounting for the two locations at the same time was needed. To do so, a first model was built to obtain damage level for each plot:

$Y_k = \mu + D_k + Sa_k + E_k$ (model 3)

Where Y_k is the number of damaged buds on the plant *k*; μ is the overall mean; D_k is the effect of growth stage; S_k is the size of the plant considered and a the regression coefficient of the plant size effect E_k is the residual error following a normal distribution $N(0, \sigma^2_e)$.

A second model was made to compute LSM of the number of damaged buds per genotype over all locations:

$Y'_{ijl} = \mu + L_l + B_{jl} + G_i + GL_{il} + E_{ijl}$ (model 4)

Where Y'_{ijl} is the mean value of residuals from model 3 in a plot *j* of the block *j* plus the mean number of damaged buds over all genotypes in the location *l*; μ is the overall mean; L_l is the effect of the location; B_{jl} is the effect of the block nested in the location; G_i is the effect of the genotype; GL_{il} is the effect of the interaction between the location and the genotype; and E_{ijk} is the residual error following a normal distribution $N(0, \sigma^2_e)$. LSM were then computed based on model 4 as explained above.

To investigate isolate relationship between compounds and resistance, Pearson' correlations between LSM of the number of damaged buds for each genotype (model 4) and LSM of concentration of each compound for each genotype (model 2) were computed. A sparse Partial Least Square (sPLS) regression was also done to identify more complex associations between plant resistance and chemistry. This analysis allows finding variables that contribute the most to the association between the number of damaged buds per genotype and the chemistry (Lê Cao et al., 2008). Selection of variables with the sPLS was limited to 5 compounds using 3 components.

RESULTS

Pollen beetle infestation of OSR on different sampling occasions

The number of damaged buds due to pollen beetles varied between sampling sessions (Fig. 1, $F_{3, 1321} = 562.99$, $P < 0.001$). The greatest damage level was observed during the two years of sampling in Occitanie (Cornebarrieu 2016 and Mondonville 2017). The damage level in 2017 was more than that of 2016 at this location (Fig. 1). No differences were observed between years in Liverdy en Brie (Fig. 1).

Genotype resistance to pollen beetle

In 2016, pollen beetles colonized the field trials late in the season. Early flowering genotypes passed the susceptible bud stage before the main migration of beetles and thus were not sampled (Appendix 1). Due to time constraints, only six genotypes could be sampled for damage in 2016 at the two locations (Appendix 1). In 2017, twenty genotypes were sampled at each location (Appendix 1). However, Goeland had a particular morphology with its primary racemes aborting during plant growth. Thus this genotype was not included into the analyses.

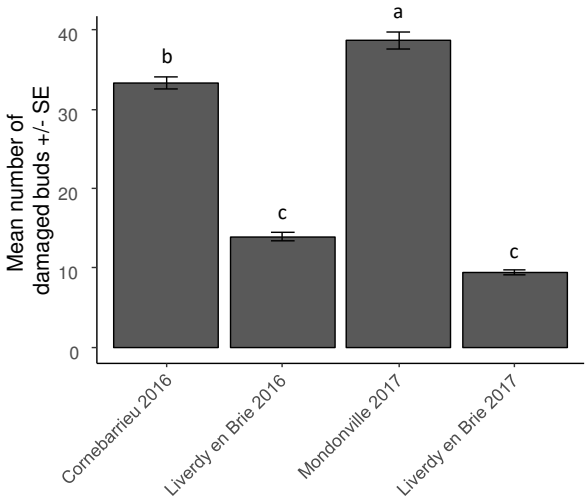

Figure 1: Mean (± SE) number of damaged buds caused by the pollen beetle on in all sites and years sampled. Means are based on data from the six genotypes in common each year and at each location. Different letters indicate significant differences.

Plant size had a significant positive effect on the damage level caused by pollen beetles at every sampling session (Table 1). Plant growth stage had a significant effect on the number of damaged buds for each sampling except at Liverdy en Brie in 2017 (Table 1). The number of damaged buds seemed to increase as plants developed. Significant differences were also observed between blocks in Cornebarrieu 2016 and Mondonville 2017 (Table 1). Genotype had a significant effect on insect attack during each year and at each location except at Liverdy en Brie during the spring 2016 (Table 1, Table 2). Strong correlations were found between average level of damage of different years and locations (Table 3).

Table 1: Values and significance of Type II Wald Chi square tests conducted on fixed effects (block, plant size, growth stage and genotype) in models computed for each sampling session. Significant effect are presented in bold.

Years	Locations	Variables	χ^2	*df*	*P*
2016	Liverdy en Brie	Block	5.51	2	0.064
		Plant size	30.55	1	**< 0.001**
		Growth stage	0.21	1	0.647
		Genotype	7.63	5	0.178
	Cornebarrieu	Block	8.02	2	**0.018**
		Plant size	45.13	1	**< 0.001**
		Growth stage	11.88	2	**0.003**
		Genotype	21.91	5	**< 0.001**
2017	Liverdy en Brie	Block	1.76	2	0.416
		Plant size	67.38	1	**< 0.001**
		Growth stage	6.26	1	**0.012**
		Genotype	85.11	18	**< 0.001**
	Mondonville	Block	11.16	2	**0.004**
		Plant size	214.41	1	**< 0.001**
		Growth stage	169.52	3	**< 0.001**
		Genotype	71.06	18	**< 0.001**

Differences in perianth chemistry

Chemical analysis led to quantification of 38 compounds: 18 AA, 7 CPOA, 7 FO and 6 GSL (Table 4, Appendix 2). To ensure quality of analyses, correlations of LSM values of each genotype between two blocks in each site and year were checked. For most of the compounds, correlations were high (Appendix 3). The mean correlation was $r = 0.68$ (SE ± 0.03) for Mondonville and $r = 0.54$ (SE ± 0.03) for Liverdy en Brie. Mean concentrations by genotype were stable between locations as average correlation coefficient was high ($r = 0.46$, SE ± 0.05) but correlations were variable depending on compounds (Appendix 4).

Significant differences between genotypes were found for most of the compounds (Table 4). Only neoglucobrassicin, methionine, isoleucine and phenylalanine did not vary according to genotype (Table 4). Twenty eight out of the 38 compounds varied according to location and 19 varied according to interactions between genotypes and locations (Table 4). The average variance explained was highest for the location effect which accounted a mean of 44 % of variation in concentration (Table 4). Genotype explained an average of 19 % of the variation but variability was high (min = 0 %, max = 64 %). The interaction between genotypes and locations explained an average of 15 % and variability was also high (min = 0 %, max = 54 %). The interaction between location and block was marginal and explained only 1 % of this variability.

Table 2: Least Square Means (± SE) of the number of damage caused by pollen beetle for each genotype sampled at the two locations in 2016 and 2017. Different letters indicate significant differences in the number of damaged buds between genotypes computed independently according to years and locations.

	Liverdy en Brie 2016		Cornebarrieu 2016		Liverdy en Brie 2017		Mondonville 2017	
Genotypes	**LSM**	**groups**	**LSM**	**groups**	**LSM**	**groups**	**LSM**	**groups**
Akamar					12.21 (1.15)	cde	41.49 (3.71)	bcd
Bolko	12.60 (2.31)	a	27.33 (2.97)	ab	8.70 (1.21)	abcd	33.82 (3.69)	abcd
G5					5.35 (1.21)	ab	18.01 (3.76)	a
Cresus					11.21 (1.14)	cde	40.16 (3.74)	bcd
Debruder Dippes					6.91 (1.14)	abc	40.60 (3.8)	bcd
G10					5.26 (1.17)	a	34.87 (3.88)	abcd
Grizzly	17.14 (1.81)	a	36.41 (2.69)	b	14.56 (1.15)	e	47.73 (3.74)	d
Kombi					11.05 (1.19)	bcde	43.35 (3.74)	bcd
G14					8.12 (1.15)	abcd	34.04 (3.74)	abcd
Lembke	11.03 (2.23)	a	28.07 (2.77)	ab	8.53 (1.17)	abcd	32.74 (3.77)	abcd
Lira	15.96 (1.88)	a	32.12 (2.76)	ab	11.26 (1.14)	cde	37.35 (3.73)	bcd
G18					8.30 (1.14)	abcd	25.55 (3.76)	ab
G19					10.41 (1.15)	abcde	39.53 (3.75)	bcd
Pollux					10.29 (1.17)	abcde	46.09 (3.74)	cd
Quedlinburger Platzester					8.00 (1.14)	abcd	37.58 (3.76)	bcd
G28	9.96 (2.26)	a	22.63 (2.75)	a	8.69 (1.16)	abcd	27.97 (3.83)	abc
Rasant					7.93 (1.13)	abcd	34.59 (3.77)	abcd
Sarepta	15.16 (1.83)	a	37.57 (2.78)	b	7.04 (1.17)	abc	34.93 (3.76)	abcd
Tor					12.93 (1.14)	de	44.86 (3.76)	cd

Relation between perianth chemistry and pollen beetle resistance

To link compounds to plant resistance, correlations between LSM of damage level and LSM of concentrations were computed (Appendix 5). Compounds identified by Hervé et al. (2014) as potential biomarkers of resistance to pollen beetle had low levels of correlations: quercetin-3-O-sophoroside = 0.03, kaempferol-3-O-sophoroside = 0.11, proline = 0.10, serine = 0.30, sucrose = 0.03. Only two compounds had significant correlations (Fig. 2): quinic acid (r = -0.51) and arginine (r = 0.50) (Fig. 3).

The sPLS analysis performed on our dataset did not find an overall relationship between plant chemistry and the number of damaged buds as none of the PLS components had Q2 values higher than 0.0975 (Q2 component 1 = -0.108, Q2 component 2 = -0.295, Q2 component 3 = -0.624).

Table 3: Pearson's correlations coefficients comparing LSM of the number of damage between various locations and years.

		2016		2017	
		Cornebarrieu	Liverdy en Brie	Mondonville	Liverdy en Brie
2016	Cornebarrieu	-	0.89	0.75	0.34
	Liverdy en Brie	-	-	0.87	0.63
2017	Mondonville	-	-	-	0.71
	Liverdy en Brie	-	-	-	-

DISCUSSION

Previous studies showed that substantial differences in resistance to pollen beetle feeding are present in OSR plants when tested in controlled conditions (Hervé et al., 2014). The present experiments revealed that this intraspecific variability also exists among OSR genotypes grown and exposed to pollen beetles in the field. Differences between genotypes in such conditions were also substantial as the most susceptible genotype received more than twice as much attack as the most resistant one. These differences were verified two consecutive years and in different locations, indicating that our observations are reliable and that the gradient of resistance observed is stable in different environments and growing conditions. When screening for resistance to pollen beetle, plant earliness is a major issue as insects are more attracted to the most developed plants (Hervé et al., 2017). Choosing OSR genotypes with simultaneous susceptibility periods in the present study allowed to reduce this bias. Even if we used genotypes with a limited flowering period in our experiments, variations in phenology still existed among genotypes. These variations were integrated in the statistical analyses by taking plant growth stage and size into account in the tests. Screening larger collections of genotypes for pollen beetle resistance seems to be feasible as long as plants with simultaneous susceptible periods are used. Several differences among genotypes could explain the damage gradient observed in our experiments. Genotypes could differ in attractiveness or appetability to pollen beetles. Previous laboratory experiments did not find large variability in OSR attraction to pollen beetle but demonstrated a variability in feeding intensity that could be related to perianth composition (Hervé et al., 2017). Therefore, differences between genotypes observed in our field experiments could be more related to feeding stimulation than to attraction. Semi-field experiments where insects would be introduced into OSR caged plots could help confirming which of these two mechanisms is involved in the gradient we observed in our field trials (Smith, 2005).

Oilseed rape perianths were collected in 2017 at two locations and their composition in different classes of compounds was analysed. High correlations were found between blocks on a single location for all compounds. This indicates that quantification of metabolites was consistent within a location. Differences in perianth chemistry between genotypes were observed for almost every compounds (34 out of 38).

Table 5: *P*-values of the effect of genotype, location, the interaction between genotype and location and the block effect nested in the location on perianth chemistry and variance explained by these variables. Classes: free amino acids (AA), carbohydrates, polyols, organic acids (CPOA), flavonols (FO), glucosinolates (GSL). Unk.FO1 and Unk.FO2 are two unidentified flavonols.

		P				**Variance explained**				
Compounds	**Classes**	**Genotype**	**Location**	**Genotype X Location**	**Block**	**Genotype**	**Location**	**Genotype X Location**	**Block**	**Residuals**
S-methylcysteine sulfoxide (SMCSO)	AA	0	0.001	0.449	0	64%	4%	7%	8%	18%
γ-Aminobutyric Acid (GABA)	AA	0	0	0.066	1	3%	68%	11%	0%	18%
Arginine	AA	0	0	0	1	5%	51%	25%	1%	18%
Asparagine	AA	0	0	0	1	4%	66%	21%	0%	9%
Aspartic Acid	AA	0	0.001	0	1	0%	24%	52%	0%	24%
Glutamic Acid	AA	0	0.903	0	1	4%	9%	54%	0%	32%
Glutamine	AA	0	0	0	1	11%	46%	29%	0%	14%
Histidine	AA	0.005	0	0.004	0.11	0%	61%	16%	4%	20%
Isoleucine	AA	0.81	0	1	1	1%	79%	3%	0%	17%
Methionine	AA	1	0	1	1	1%	83%	1%	0%	16%
Phenylalanine	AA	0.079	0	0.335	1	1%	80%	6%	0%	13%
Proline	AA	0	0.001	0.171	1	45%	20%	12%	0%	24%
Serine	AA	0	0.154	1	1	22%	33%	4%	0%	41%
Threonine	AA	0	0	0.25	1	9%	66%	8%	0%	17%
Tryptophan	AA	0.021	0	0.005	1	0%	77%	10%	0%	13%
Valine	AA	0	0	0.002	1	2%	79%	9%	0%	10%
α-Alanine	AA	0	1	1	1	21%	12%	15%	0%	51%
β-Alanine	AA	0	0	0	0.083	10%	71%	10%	1%	8%
Fructose	CPOA	0	0	0.001	1	5%	88%	4%	0%	4%
Glucose	CPOA	0	0	0.074	1	11%	81%	3%	0%	5%
Glyceric Acid	CPOA	0	0	0.002	1	46%	35%	9%	0%	10%
Malic Acid	CPOA	0	0	0	1	11%	55%	17%	1%	17%
Myo-Inositol	CPOA	0	1	0.001	1	48%	0%	26%	0%	26%
Quinic Acid	CPOA	0	0.033	0.39	1	45%	13%	13%	0%	30%
Sucrose	CPOA	0	0	0	1	11%	54%	23%	0%	13%

Table 5 (continued)

		P				Variance explained				
Compounds	**Classes**	**Genotype**	**Location**	**Genotype X Location**	**Block**	**Genotype**	**Location**	**Genotype X Location**	**Block**	**Residuals**
Unk.FO1	FO	0.001	1	0.052	1	12%	0%	32%	0%	56%
Unk.FO2	FO	0	1	1	1	61%	2%	7%	0%	31%
Isorhamnetin-Di-Glucoside	FO	0	0	1	1	13%	78%	0%	0%	9%
Kaempferol-3-O-Sophoroside	FO	0	0.001	1	0.12	26%	49%	4%	3%	18%
Quercetin-3-O-Caffeoyl-Sophoroside-7-O-Glucoside	FO	0	0	1	1	27%	55%	1%	1%	16%
Quercetin-3-O-Sophoroside	FO	0	0	0	0.025	13%	62%	11%	2%	11%
Quercetin-3-O-Sophoroside-7-O-Glucoside	FO	0	0.008	1	1	30%	23%	11%	0%	37%
Epiprogoitrin	GSL	0	0	0	1	19%	50%	16%	0%	16%
Glucobrassicin	GSL	0	0.364	0	0.01	7%	25%	33%	7%	27%
Gluconapin	GSL	0	0	0	1	18%	51%	24%	0%	8%
Gluconasturtiin	GSL	0	0.938	0.022	1	47%	4%	20%	0%	29%
Neoglucobrassicin	GSL	0.075	1	1	1	16%	10%	5%	1%	68%
Progoitrin	GSL	0	0.726	0.497	1	46%	12%	12%	0%	30%

This result shows that chemical composition of perianths are different among the genotypes tested here. Two compounds were shown to be related to the number of damaged bud caused by pollen beetle (i.e. quinic acid and arginine). The strongest relation was found with quinic acid but this association is mainly driven by genotype G5. High concentrations of quinic acid in perianths of this genotype has been observed in both locations sampled. However, other genotypes expressing high concentration of quinic acid in their perianths need to be identified to confirm the role of this compound. The two compounds identified are not known to be directly related to plant defence (Winter et al., 2015). However, they could be involved in physiological processes correlated to the expression of resistance. Whether such compounds can be used as biomarkers of resistance against the pollen beetle remains to be studied. Concentrations of previously identified biomarkers (i.e. serine, proline, sucrose, quercetin-3-O-sophoroside and kaempferol-3-O-sophoroside) were not correlated to pollen beetle damage in our experiments. This lack of relation does not imply an absence of effect of these molecules but their effect may be minimized in field conditions. Furthermore, differences in biomarkers identified could arise from differences in genotypes tested as none of the genotypes used by Hervé et al. (2014) could be screened in the present study.

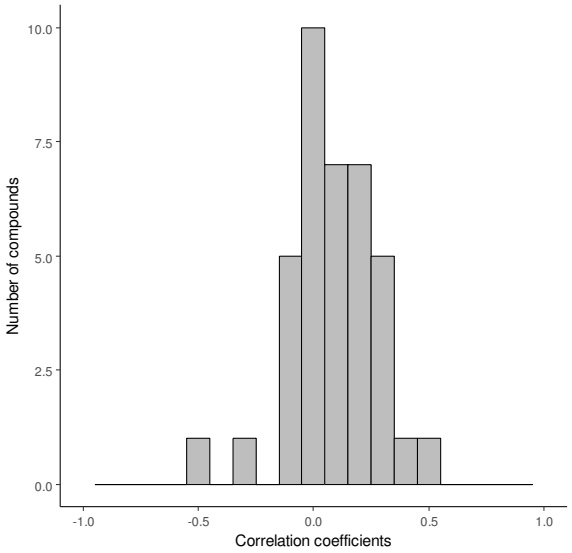

Figure 2: Histogram of coefficients of correlation between the mean concentrations of different compounds per genotype and the mean numbers of damaged buds per genotype.

To our knowledge, most attempts to identify biochemical biomarkers of plant resistance to insects have been conducted in laboratory or greenhouse bioassays (Wang et al., 2005; Omoloye et al., 2007; Leiss et al., 2009; Elek et al., 2013; Wang et al., 2017). Such bioassays allow for experimental conditions to be standardised and they allowed identification of some biochemical biomarkers that were used for screening plant resistance to insects (Shaw et al., 2009). However, whether such biomarkers can also influence levels of attack in the field remains mostly undocumented. Indeed, experimental and environmental conditions can strongly affect other plant traits such as leafs morphology or pigmentation (Mishra et al., 2012) that may influence damage levels by herbivorous insects. Also, light intensity, temperature, and soil conditions can affect plant tissue composition (Jänkänpää et al., 2012; Sun et al., 2016; Sun et al., 2017). Furthermore, most plant resistances to insects have a polygenic basis and are prone to environmental variations (Smith and Clement, 2012). Experiments conducted in laboratory and field conditions may thus lead to different results (Alexandersson et al., 2014). Cystein proteinase inhibitor for example, was shown to be involved in induced defences of soybean against the Mexican bean beetle in greenhouse bioassays but was not related to insect performance in the field (Underwood et al., 2002). Conducting experiment in realistic cropping conditions consequently seems therefore essential to identify robust biomarkers of insect resistance.

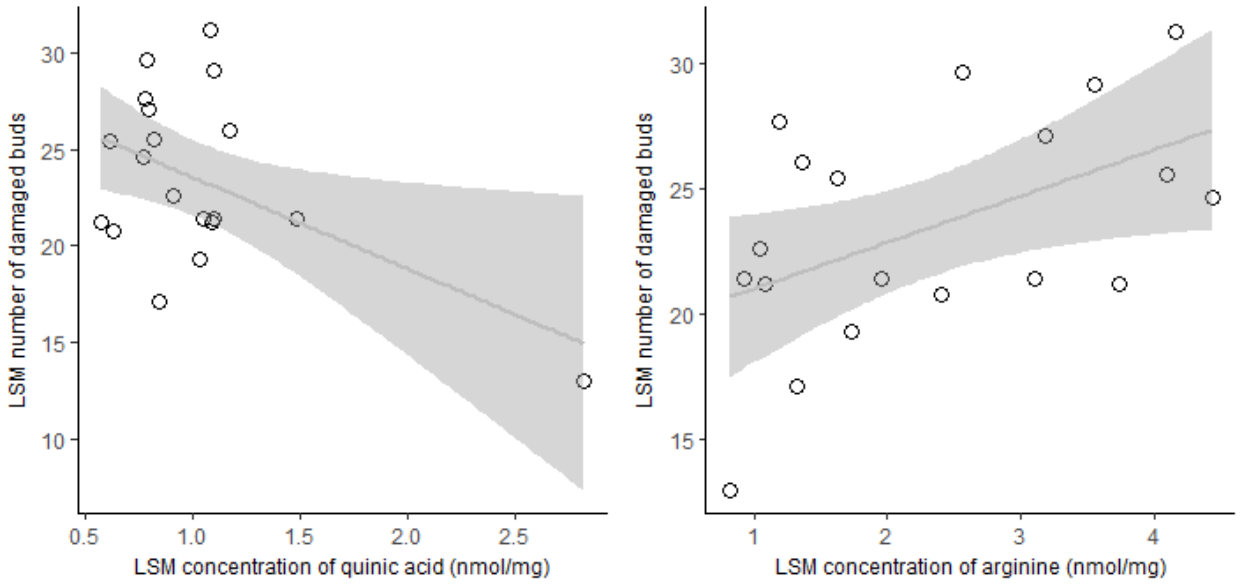

Figure 3: Relation between LSM of the number of damaged buds and concentrations of compounds having significant correlations. Lines and shaded areas show Linear Models predictions ± SE.

Even if reliable quantifications of chemical compounds were performed in our experiments, perianth chemistry was greatly affected by the environment. Correlations of mean concentration per genotype between locations strongly differed according to compounds considered. Some compounds showed very stable gradient of concentration while others did not correlate between locations. Even if significant differences between genotypes were observed for most compounds, a large part of the variability is explained by the environment and its interaction with the genotype. These observations are consistent with data from other studies. Several field experiments showed that the environment and interactions between the environment and the genotype explained most of chemical variability whereas the genotype only accounted for a small proportion of it (Lee et al., 2003; Davies et al., 2010; Chen et al., 2016; Matros et al., 2017). In a study on durum wheat grains for example, most of the variability in chemical composition is explained by the interaction between the genotype and the environment (54 %), followed by the environment (42 %) and finally, the genotype accounts for only 4 % of this variability (Beleggia et al., 2013). These observations highlight that experiments seeking biochemical biomarkers of resistance need to be replicated in different environments and locations to reflect this variability. To date, experiments meeting these criteria are rare (e.g. Abdel Aal et al., 2001; Anyanga et al., 2017) and most field screenings are performed one year at one location (Malchev et al., 2010; Schaefer-Koesterke et al., 2017; Schaefer et al., 2017).

As we have seen, pollen beetle and oilseed rape is a particularly difficult biological model when it comes to screening for resistance because insect and plant phenologies can strongly impact the outcome of field conducted experiments. Potential biases originating from the plant phenology were successfully managed but pollen beetle phenology can also influence this outcome. Screening early flowering plants for example is still risky as showed by our 2015-2016 experiments where early flowering plants escaped from pollen beetle attacks. Our experimental design cannot be used to screen large collections of genotypes but coupled with a biomarker approach, it could be used in future breeding programs. Screening approaches based on biochemical biomarkers have several advantage (reviewed by Fernandez et al., 2016). From a practical perspective, they are less dependent of insect infestations and allow faster identification of resistant

genotypes. In the case of the pollen beetle, this is interesting since controlling insect phenology and its level of infestation is a limiting step for screening. These biomarkers also provide a condensed information of complex traits which is highly useful when studying polygenetic traits such as plant defences. Carbohydrates of miscanthus tissues have for example been used to accurately predict yield of plants harvested six months later (Maddison et al., 2017). Chemical biomarkers also have the advantage to open doors to mechanistic insight (Fernandez et al., 2016). They will allow to dissociate resistance and susceptibility mechanisms observed in gradients of damage and to understand if resistance interferes with other physiological processes in the plant. For now, only moderate levels of correlation were found between metabolites and pollen beetle feeding but analyses of other chemical components such as surface compounds could yield interesting results.

Our study indicates that variability of plant chemistry according to the environment can be a major limitation of biochemical biomarkers utilisation. Therefore, to account for this variability screenings need to be performed as much as possible in the field and in different environmental conditions. A better understanding of the effect of the environment on plant chemistry and especially how experimental conditions affect metabolites could be particularly useful. Approaches based on biochemical biomarkers have been mainly implemented to study plant performances and resistances to pathogens (Fernandez et al., 2016) but they could be highly valuable for developing news control methods of pest insects.

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Appendix 1: Information known about the genotypes used during field trials 2015-2016 and 2016-2017. 0/0: low concentration of erucic acid and glucosinolates in the seeds, 0/+ low concentration of erucic acid and high concentration of glucosinolates in the seeds, +/+ high concentration of both erucic acid and glucosinolates in the seeds. IOC code of the country of origin.

Genotypes	Flowering period	Erucic acid / Glucosinolates	Origin	Field 2015-2016	Field 2016-2017	Sampled in 2016	Sampled in 2017
Akamar	late flowering	NA	FRA		x		x
Aviso	early flowering	0/0	FRA	x			
Bolko	late flowering	0/0	POL	x	x	x	x
Bristol	early flowering	0/0	FRA	x			
G5	late flowering	0/0	FRA		x		x
Cresus	late flowering	+/+	FRA		x		x
Darmor	late flowering	0/0	FRA	x			
Debruder Dippes	late flowering	+/+	NA		x		x
Express	early flowering	0/0	DEU	x			
G10	late flowering	+/+	SWE		x		x
Goeland	late flowering	0/0	FRA		x		
Grizzly	late flowering	0/0	FRA	x	x	x	x
Kombi	late flowering	+/+	UKR		x		x
G14	late flowering	+/+	NA		x		x
Kromerska	early flowering	+/+	NA	x			
Lembke	late flowering	+/+	DEU	x	x	x	x
Lira	late flowering	0/+	DEU/RUS	x	x	x	x
G18	late flowering	0/0	DEU		x		x
G19	late flowering	0/0	DEU		x		x
Montego	early flowering	0/0	GBR	x			
Oleski	early flowering	+/+	POL	x			
G22	late flowering	0/0	DEU	x			
Pollus	late flowering	+/+	SWE		x		x
Prerovska	early flowering	+/+	CSK	x			
Primor	early flowering	0/+	FRA	x			
Quedlinburger Platzester	late flowering	+/+	DEU		x		x
Quinta	late flowering	0/+	DEU	x			
G28	late flowering	+/+	NZL	x	x	x	x
Rasant	late flowering	0/0	DEU		x		x
Recital	early flowering	0/0	DEU	x			
Sarepta	late flowering	+/+	FRA	x	x	x	x
Tenor	early flowering	0/0	FRA	x			
Tomek	late flowering	+/+	POL	x			
Tor	late flowering	0/0	SWE		x		x

Appendix 2: LSM of concentrations of each compound according to genotype. Classes: free amino acids (AA), carbohydrates, polyols, organic acids (CPOA), flavonols (FO), glucosinolates (GSL). Unk.FO1 and Unk.FO2 are two unidentified flavonols.

		Concentrations (nmol.mg^{-1})																		
Compounds	**Classes**	**Akamar**	**Bolko**	**G5**	**Cresus**	**Debruder Dippes**	**G10**	**Grizzly**	**Kombi**	**G14**	**Lembke**	**Lira**	**G18**	**G19**	**Pollux**	**Quedlinburger Platzester**	**G28**	**Rasant**	**Sarepta**	**Tor**
S-methylcysteine sulfoxide (SMCSO)	AA	77.8	96.3	74.4	51.9	58.1	79.1	80.7	42.5	63.7	56.9	65.9	49.6	141.5	41.7	74.8	58.9	67.4	60.2	63.7
4-Aminobutanoic Acid (GABA)	AA	6.5	11	5.5	8.5	6.7	6.9	6.8	7.1	6.8	6	5.8	6	5.9	6.8	6	8.1	7.5	8.7	9.3
Arginine	AA	3.2	3.1	0.8	1.4	4.4	2.4	4.2	1.2	1.1	0.9	1.6	1.3	4.1	3.6	1	1.7	3.7	2	2.6
Asparagine	AA	4.1	4.2	3.2	4.3	9.3	4.7	5.3	2.9	3.8	3.1	3.8	2.3	5.8	5.5	2.2	4.7	5.9	3.7	5.3
Aspartic Acid	AA	8.3	6.6	7.2	7.9	9.5	7.2	8	7.9	9.6	6.7	8.5	6.6	8.4	8.9	5.7	8	8.3	8.7	8.4
Glutamic Acid	AA	11.7	9	14	13.8	14.2	10.2	12.9	13.5	14.5	10.6	14	13.6	13.5	16.7	9.8	13.7	13.7	11.8	12.6
Glutamine	AA	45	49	36	31	96.3	68.4	58.1	28.6	43.2	31.4	48.7	34.6	63.5	39.1	29.3	48.4	52.3	46.4	74.7
Histidine	AA	2.8	2.5	1.3	1.6	3.2	2.2	2.2	1.7	2.1	2.2	1	1.3	3	2.1	2.7	1.5	3	2.5	2.1
Isoleucine	AA	0.7	0.9	0.9	0.8	1.1	0.9	0.8	0.8	0.9	0.7	0.9	0.9	0.9	1.1	0.8	1.1	1	0.9	1.1
Methionine	AA	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.4	0.3
Phenylalanine	AA	0.4	0.6	0.6	0.5	0.9	0.7	0.6	0.6	0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.8	0.8	0.7	0.8
Proline	AA	32.8	34.9	26.2	27.5	56.6	28.4	45.6	18.4	46.5	32.1	26.5	29.9	41.4	30.1	31.8	22.8	43.2	34	24.7
Serine	AA	7.5	8	8.2	8.4	13.1	8.6	9.4	8.4	9.4	8.5	8.5	7.2	9.7	11.7	7.9	11.5	8.8	10.8	11.7
Threonine	AA	2.2	2.7	2.5	3.3	3.7	2.7	3	2.6	2.5	2.2	2.8	3.5	2.9	4	2.5	3.3	3.7	3.3	3.9
Tryptophan	AA	0.2	0.2	0.2	0.3	0.4	0.2	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3
Valine	AA	2.4	2.6	2.8	2.4	3.5	2.9	2.6	3	3	2.5	2.9	2.3	3.2	3.5	2.2	3.4	3.5	3	2.9
α-Alanine	AA	7.6	8.6	8.1	8.2	10	9.2	8.8	8	8.3	8.5	8.9	8.8	7.9	10.9	7.7	10.8	8.8	9.1	9.9
β-Alanine	AA	0.7	0.7	0.9	1.5	1.8	0.7	1.1	0.8	1	1.1	1.2	1	1.1	1.5	1.2	1.2	1.1	1	0.9
Fructose	CPOA	94.9	106.5	96	96.2	74	84.6	101.6	102.2	94.4	78.2	116.8	97	77.4	97.7	130.7	93.2	81.8	91.7	87.2
Glucose	CPOA	77.2	82.5	78	75.8	64.1	66.6	80.6	77.8	84.9	61	96.8	77.2	61.8	74	108.8	64.1	58.7	75.8	68.9
Glyceric Acid	CPOA	3.1	2.8	2.6	2.6	2.4	4.6	2.9	3.5	5.7	3.5	3.3	2.6	2.1	2.6	3.4	7	3.7	3.6	3.1

Appendix 2 (continued)

Compounds	Classes	Concentrations (nmol.mg^{-1})																		
		Akamar	Bolko	G5	Cresus	Debruder Dippes	G10	Grizzly	Kombi	G14	Lembke	Lira	G18	G19	Pollux	Quedlinburger Platzester	G28	Rasant	Sarepta	Tor
Myo-Inositol	CPOA	6.7	7.5	6.3	5.6	4.8	4.6	7.8	7	6.9	9.2	6	7	4.5	6.8	8.8	5.8	5.8	9.4	5.3
Quinic Acid	CPOA	0.8	1.5	2.8	1.2	0.8	0.6	1.1	0.8	1.1	1.1	0.6	0.8	0.8	1.1	0.9	1	0.6	1.1	0.8
Sucrose	CPOA	29	38.5	39.2	37.6	26.3	32	32.2	48	42	38.3	32.5	38.9	26	49.2	41.3	26.9	36.7	32.6	37.3
Unk.FO1	FO	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.6	0.2	0.3	0.3	0.3	0.6	0.4	0.3	0.9	0.4	0.3	0.3
Unk.FO2	FO	0.8	0.9	0.7	0.9	0.7	1	0.8	1.1	1	2.2	0.1	0.6	0.8	1.4	0.6	1.1	0.6	1.1	0.7
Isorhamnetin-Di-Glucoside	FO	40.2	35.6	38.9	40.1	28.2	46.9	40.7	42	38.7	53.1	45	43.7	40.8	38.5	40.5	40.5	24.8	46.1	35.5
Kaempferol-3-O-Sophoroside	FO	0.4	0.4	0.4	0.5	0.4	0.5	0.5	1.6	0.5	1	0.3	0.4	0.6	0.4	0.3	0.5	0.9	0.4	0.4
Quercetin-3-O-Caffeoyl-Sophoroside-7-O-Glucoside	FO	3.4	4	4.5	3.8	2.9	3.2	6.1	3.1	3	3.1	5.4	3.6	3.9	2.7	4.4	2.3	2.7	3.1	3
Quercetin-3-O-Sophoroside	FO	0.4	0.5	0.4	0.7	0.5	0.7	0.5	1	0.9	1.5	0.3	0.4	1	1	0.5	0.9	0.7	0.7	0.4
Quercetin-3-O-Sophoroside-7-O-Glucoside	FO	0.2	0.2	0.7	0.3	0.1	0.2	0.3	0.2	0.2	0.3	0.1	0.1	0.3	0.3	0.2	0.3	0.2	0.1	0.4
Epiprogoitrin	GSL	1.2	0.1	1	1	0.9	0.9	0.9	1.2	0.8	0.8	0.9	0.3	0.2	1.5	1.4	0.8	0.6	0.6	0.8
Glucobrassicin	GSL	0.5	0.2	0.2	0.5	0.2	0.4	0.2	0.6	0.6	0.4	0.1	0.7	0.6	1	0.4	0.9	0.4	0.5	0.4
Gluconapin	GSL	0.7	0.1	0.6	0.8	1.1	0.9	0.9	1.5	0.7	0.8	0.9	0.2	0.1	1.1	1.4	2.1	0.6	0.6	0.7
Gluconasturtiin	GSL	0.2	0	0	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0	0	0.3	0.1	0.2	0.1	0.1	0
Neoglucobrassicin	GSL	0	0	0	0.2	0	0.1	0	0	0.1	0	0	0	0	0.1	0.1	0.3	0	0	0
Progoitrin	GSL	1.3	0.1	1	1.2	1.1	0.9	0.9	1.3	0.9	0.8	1.3	0.5	0.2	1.7	1.5	0.9	0.6	0.7	0.8

Appendix 3: Pearson's correlations of the concentration between different blocks in the field trials.

Compounds	Classes	Mondonville 2017			Liverdy en Brie 2017		
		r blocks B and C	r blocks B and A	r blocks A and C	r blocks B and C	r blocks B and A	r blocks A and C
S-methylcysteine sulfoxide (SMCSO)	AA	0.85	0.81	0.93	0.81	0.65	0.83
γ-Aminobutyric Acid (GABA)	AA	0.48	0.23	0.64	0.26	0.19	0.61
Arginine	AA	0.73	0.54	0.74	0.22	0.36	0.71
Asparagine	AA	0.77	0.59	0.82	0.6	0.74	0.84
Aspartic Acid	AA	0.57	0.69	0.76	0.62	0.54	0.78
Glutamic Acid	AA	0.57	0.5	0.64	0.72	0.71	0.81
Glutamine	AA	0.75	0.65	0.85	0.55	0.81	0.82
Histidine	AA	0.53	0.27	0.62	0.47	0.23	0.57
Isoleucine	AA	0.031	0.13	0.3	0.44	0.41	0.78
Methionine	AA	-0.016	0.047	0.15	0.18	0.18	0.29
Phenylalanine	AA	0.24	0.25	0.49	0.061	0.074	0.55
Proline	AA	0.54	0.58	0.74	0.86	0.79	0.9
Serine	AA	0.34	0.27	0.46	0.66	0.53	0.86
Threonine	AA	0.42	0.28	0.52	0.77	0.63	0.88
Tryptophan	AA	0.39	0.49	0.53	0.07	0.28	0.29
Valine	AA	0.45	0.51	0.51	0.61	0.7	0.75
α-Alanine	AA	0.39	0.21	0.58	0.47	0.47	0.56
β-Alanine	AA	0.76	0.66	0.81	0.62	0.7	0.71
Fructose	CPOA	0.59	0.62	0.79	0.69	0.63	0.78
Glucose	CPOA	0.65	0.7	0.83	0.75	0.68	0.76
Glyceric Acid	CPOA	0.81	0.8	0.93	0.83	0.82	0.92
Malic Acid	CPOA	0.55	0.42	0.56	0.72	0.66	0.78
Myo_inositol	CPOA	0.7	0.59	0.76	0.81	0.82	0.83
Quinic Acid	CPOA	0.41	0.5	0.67	0.64	0.65	0.99
Sucrose	CPOA	0.5	0.49	0.56	0.86	0.87	0.88
Unk.FO1	FO	-0.22	0.07	0.35	0.86	0.74	0.92
Unk.FO2	FO	0.48	0.33	0.75	0.9	0.91	0.94
Isorhamnetin-Di-Glucoside	FO	0.48	0.51	0.53	0.59	0.72	0.77
Kaempferol-3-O-Sophoroside	FO	0.58	0.48	0.77	0.69	0.69	0.83
Quercetin-3-O-Caffeoyl-Sophoroside-7-O-Glucoside	FO	0.5	0.47	0.71	0.6	0.69	0.84
Quercetin-3-O-Sophoroside	FO	0.55	0.33	0.6	0.81	0.68	0.84
Quercetin-3-O-Sophoroside-7-O-Glucoside	FO	0.69	0.58	0.75	0.9	0.91	0.93
Epiprogoitrin	GSL	0.32	0.4	0.83	0.72	0.87	0.91
Glucobrassicin	GSL	0.49	0.63	0.79	0.76	0.74	0.88
Gluconapin	GSL	0.47	0.48	0.65	0.91	0.93	0.95
Gluconasturtiin	GSL	0.67	0.78	0.85	0.6	0.57	0.67
Neoglucobrassicin	GSL	0.14	0.13	0.84	0.69	0.66	0.98
Progoitrin	GSL	0.47	0.5	0.72	0.67	0.87	0.87

Appendix 4: Pearson's correlations of the LSM concentration of each compound between Mondonville and Liverdy en Brie. Significant correlations are presented in bold.

		Correlation between LSM values of different locations	
Compounds	**Classes**	***r***	***P***
S-methylcysteine sulfoxide (SMCSO)	AA	0.88	**0.000**
4-Aminobutanoic Acid (GABA)	AA	0.23	0.351
Arginine	AA	0.27	0.260
Asparagine	AA	0.31	0.196
Aspartic Acid	AA	-0.20	0.409
Glutamic Acid	AA	0.06	0.807
Glutamine	AA	0.40	0.087
Histidine	AA	-0.02	0.937
Isoleucine	AA	0.17	0.489
Methionine	AA	0.14	0.570
Phenylalanine	AA	0.11	0.668
Proline	AA	0.70	**0.001**
Serine	AA	0.61	**0.005**
Threonine	AA	0.43	0.067
Tryptophan	AA	-0.17	0.498
Valine	AA	0.20	0.406
α-Alanine	AA	0.40	0.090
β-Alanine	AA	0.71	**0.001**
Fructose	CPOA	0.55	**0.015**
Glucose	CPOA	0.77	**0.000**
Glyceric Acid	CPOA	0.83	**0.000**
Malic Acid	CPOA	0.34	0.153
Myo-Inositol	CPOA	0.63	**0.004**
Quinic Acid	CPOA	0.84	**0.000**
Sucrose	CPOA	0.29	0.234
Unk.FO1	FO	0.22	0.369
Unk.FO2	FO	0.78	**0.000**
Isorhamnetin-Di-Glucoside	FO	0.84	**0.000**
Kaempferol-3-O-Sophoroside	FO	0.88	**0.000**
Quercetin-3-O-Caffeoyl-Sophoroside-7-O-Glucoside	FO	0.83	**0.000**
Quercetin-3-O-Sophoroside	FO	0.71	**0.001**
Quercetin-3-O-Sophoroside-7-O-Glucoside	FO	0.71	**0.001**
Epiprogoitrin	GSL	0.49	**0.035**
Glucobrassicin	GSL	0.20	0.414
Gluconapin	GSL	0.62	**0.005**
Gluconasturtiin	GSL	0.63	**0.004**
Neoglucobrassicin	GSL	0.54	**0.018**
Progoitrin	GSL	0.68	**0.001**

Appendix 5: Pearson's correlations between the LSM concentration of each compound and number of damage. Significant correlations are presented in bold.

Compounds	**Classes**	***r***	***P***
S-methylcysteine sulfoxide (SMCSO)	AA	-0.01	0.98
4-Aminobutanoic Acid (GABA)	AA	0.15	0.53
Arginine	AA	0.50	**0.03**
Asparagine	AA	0.35	0.15
Aspartic Acid	AA	0.38	0.11
Glutamic Acid	AA	0.17	0.50
Glutamine	AA	0.24	0.32
Histidine	AA	0.24	0.32
Isoleucine	AA	0.00	0.98
Methionine	AA	0.01	0.98
Phenylalanine	AA	-0.06	0.82
Proline	AA	0.10	0.70
Serine	AA	0.30	0.22
Threonine	AA	0.22	0.37
Tryptophan	AA	-0.01	0.95
Valine	AA	0.09	0.72
α-Alanine	AA	0.13	0.60
β-Alanine	AA	0.20	0.40
Fructose	CPOA	0.06	0.82
Glucose	CPOA	0.04	0.86
Glyceric Acid	CPOA	-0.27	0.26
Malic Acid	CPOA	0.15	0.54
Myo-Inositol	CPOA	-0.08	0.74
Quinic Acid	CPOA	-0.51	**0.03**
Sucrose	CPOA	0.03	0.89
Unk.FO1	FO	-0.01	0.98
Unk.FO2	FO	0.01	0.97
Isorhamnetin-Di-Glucoside	FO	-0.09	0.70
Kaempferol-3-O-Sophoroside	FO	0.11	0.65
Quercetin-3-O-Caffeoyl-Sophoroside-7-O-Glucoside	FO	0.15	0.53
Quercetin-3-O-Sophoroside	FO	0.03	0.92
Quercetin-3-O-Sophoroside-7-O-Glucoside	FO	-0.15	0.54
Epiprogoitrin	GSL	0.31	0.20
Glucobrassicin	GSL	0.05	0.85
Gluconapin	GSL	0.14	0.57
Gluconasturtiin	GSL	0.28	0.25
Neoglucobrassicin	GSL	-0.11	0.65
Progoitrin	GSL	0.34	0.16

Short note 1

Exploring the relationship between perianth and floral bud chemistry of oilseed rape to facilitate plant material sampling

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Abstract: Several chemical compounds quantified in the perianth of oilseed rape floral buds have been identified as potential biomarkers of resistance to pollen beetle. To be useful, these biomarkers need to be measured on large genotype collections. Sampling perianths for chemical analyses is currently a long process that strongly constraints the number of genotypes that can be sampled. The purpose of this study is to identify if chemistry of complete green floral buds could be used as a proxy of perianth composition. To do so, perianths and buds were sampled during two years in different locations. Correlations between composition of perianths and complete buds were then computed. Averaging samples over genotypes gave the most reliable estimation of perianth composition. High levels of correlation were found indicating that green bud chemistry could be used as a proxy of perianth composition. However, results were variable according to compounds considered. This study could be used as a base to facilitate plant material sampling during screening for pollen beetle resistance.

Key words: Field, Correlations, Proxy, Biomarkers, Pollen beetle

INTRODUCTION

Chemistry of oilseed rape perianths has been shown to be correlated to pollen beetle feeding damage (*Paper 1*, Hervé et al., 2014). Using identified compounds as biomarkers of resistance to develop marker assisted selection necessitates the collection of a large number of samples. Perianth collection in the field is still a time consuming and labour intensive process. Dissection of floral buds in the field necessitates specific equipment and adequate working conditions (e.g. tent or van) (Fig. 1a). These technical issues prevent screening large sets of genotypes for their chemistry. Alternative sampling methods need to be developed for a faster and easier estimation of the concentration of these compounds.

Perianth represents 30-50 % of green bud fresh biomass in oilseed rape (Fig. 1b; personal observation). Chemical composition of complete floral buds could thus be an effective proxy of perianth chemistry and be used to extrapolate its composition. To test it, perianths and floral buds were collected over two years and two locations to compare their chemistry. Samplings of both organs were made on the same plants or on different plants and chemistry of the sample were averaged at different scales. This experiment will allow identifying the scale at which correlations between chemistry of both organs are the highest and finding if concentrations of potential markers are correlated between buds and perianth.

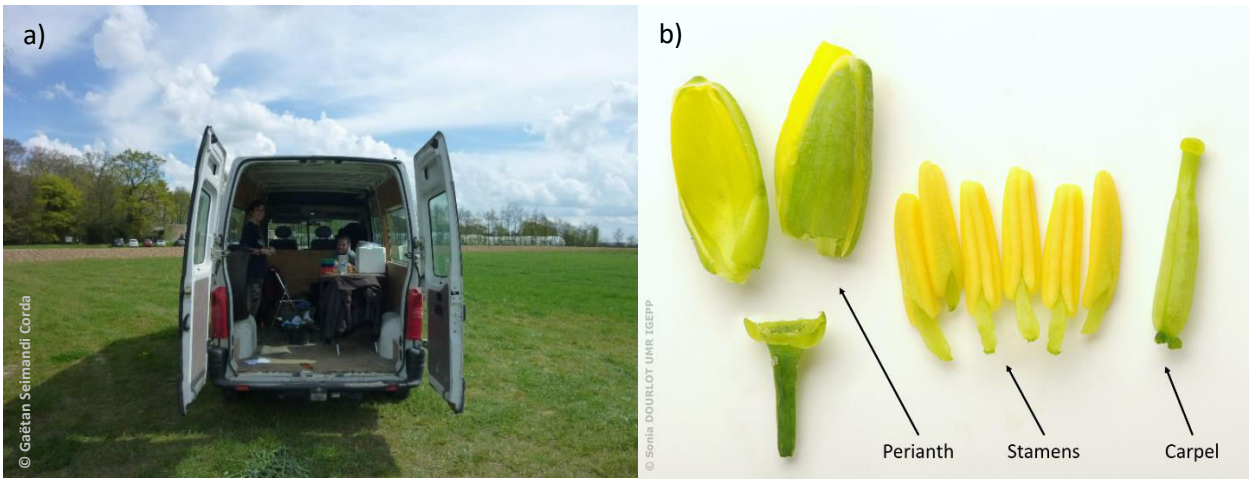

Fig 1. a) Van equipped for plant sampling in the field. b) Dissected floral bud of oilseed rape showing its different parts.

MATERIALS AND METHODS

In September 2014 a field trial was made in Cornebarrieu (Occitanie, France). Ten genotypes were sown at this location (Appendix 1). Two kinds of plots were implanted: small plots with 2 rows of 3 m long and large plots with 6 rows of 3 m long. Small plots were replicated 3 times while large plots were replicated 2 times. These plots were arranged in a randomized complete block design with small and large plots separated. Plots were separated from each other by lines of a mix of the genotypes sown to homogenise plant flowering on the trial. In 2016 two field trials were sown in Mondonville (Occitanie, France) and Liverdy en Brie (Ile de France, France) as described in *Paper 1* (Appendix 1).

Plant material was collected in spring 2015 during oilseed rape flowering, irrespective of plant growth stage. Two samples were prepared in each plot. To complete one sample of perianths and one of buds, four plants were harvested in a plot and 40 buds (length > 3 mm) on the first raceme of each plant were sampled. Twenty of these buds were then dissected to collect perianths and immediately frozen in dry ice. The other twenty buds were directly frozen to complete bud samples. If the first raceme did not

contain enough buds, additional buds were collected on secondary racemes. In 2017, plant material was collected as described in *Paper 1* over 19 genotypes by sampling plants at growth stage BBCH 59. Complete floral buds were also collected by sampling four plants different from those used for perianth analysis. Contrary to perianth samples, bud samples of 2017 contained only 10 buds of each of the four plants harvested as 40 buds where sufficient to collect enough material and complete chemical analysis. Metabolic profiling was performed as described in *Paper 1* for plant material collected in 2015 and 2017.

To compare perianth and bud chemistry, Pearson's correlations between the concentration of compounds in perianth samples and bud samples of the same plant were performed. Similar correlations were also performed between the mean concentration of each compound per plot and per genotype. This led to three different scales to compare chemistry: the *sample scale* where pairs of samples collected on the same plant were compared, *plot scale* where concentration were averaged for each plot in the trial and the *genotype scale* where concentrations were averaged for each genotype in the field. An ANOVA accounting for the scale (sample, plot and genotype), the year (2015 or 2017) and the location (Cornebarrieu, Mondonville and Liverdy en Brie) was carried out to identify which scale gives the best correlations between perianth and bud chemistry. All statistical tests were performed using R software (R Core Team, 2016).

RESULTS AND DISCUSSION

The genotype Akamar was not sampled in 2015 because of heavy phytoplasma infestation that distorted inflorescences. Montego was not included in the analysis because its chemical profile was very different from other genotypes and artificially stretched correlations. Forty two compounds were quantified in perianth and bud samples: 20 free amino acids (AA), 7 flavonols (FO), 7 carbohydrates, polyols and organic acids (CPOA) and 7 glucosinolates (GSL) (Table 1). Glycine was not quantified in 2017 samples because of technical issues during sample preparation. Only two FO were quantified in 2015 and glucobrassicanapin was not found in 2017 samples.

Correlation coefficients were highly variable according to compounds (Fig. 2). They were not different according to the scale of averaging ($F = 1.515$, $df = 2$, $P = 0.222$) and the location ($F = 0.000$, $df = 2$, $P = 0.999$) but they varied according to the sampling year ($F = 7.027$, $df = 2$, $P = 0.009$). Even if no statistical differences were observed mean concentrations by genotype gave slightly better correlations than other scales and could be used as the best scale to estimate the usefulness of complete buds chemistry. Correlations were better in 2017 than in 2015 (Fig. 2). Contrary to 2017, plants collected in 2015 were sampled irrespectively of their growth stage. This could add some noise in the data and reduce the quality of correlations.

Five potential makers of resistance were identified by Hervé et al. (2014). Correlations between average concentrations in the bud and in the perianth per genotype are variable between compounds and sampling (Table 1). Kaempferol-3-O-sophoroside has the lowest correlations varying between 0.34 and 0.30 depending on sampling. These correlations were not significant. Proline, serine and sucrose had low and non-significant correlations in 2015 but correlations were better and significant in 2017 for the two locations. *Paper 1* identified two compounds related to pollen beetle damage: arginine and quinic acid. These compounds had high and significant correlations between the bud and the perianth.

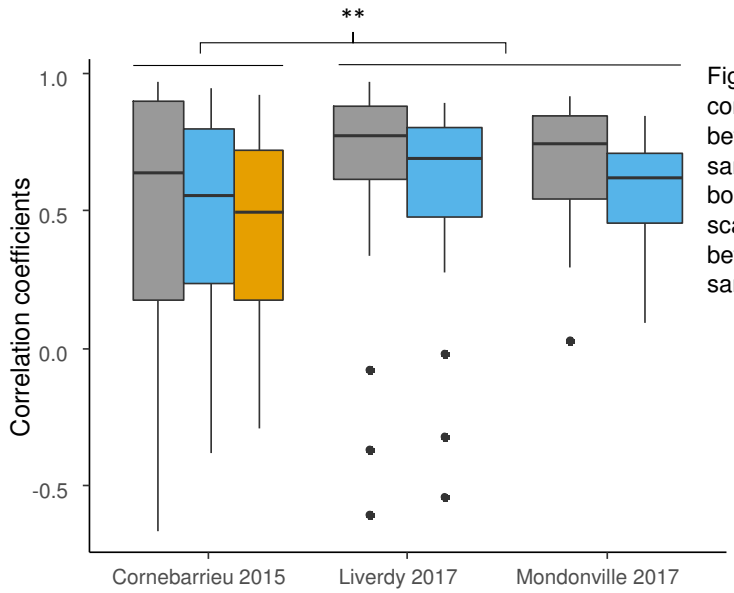

Figure 2: Boxplots of the Pearson's correlation coefficients of the relationship between perianth and bud chemistry for each sampling. Grey boxes: genotype scale, blue boxes: plot scale, yellow boxes: sample scale. Significant differences were found between correlation coefficients of the two sampling years (**: $P < 0.01$).

Overall correlations between bud and perianth chemistry exist. Average concentrations of compounds in buds among different plants are good indicators of the composition of perianths. However, high variability exists between compounds. Consequently, bud chemistry cannot be used to extrapolate chemistry of all the compounds present in the perianth but can be used for some. Bud sampling is quicker, easier and necessitates less equipment than perianth sampling. It could allow the sampling of a large number of genotypes in a reduced time. This large sampling will permit screening large genotype collections if efficient biomarkers of resistance are identified.

Table 1: Correlation coefficients and *P*-value associated to the correlation between bud and perianth composition for each compound. Correlations based on mean concentrations per genotype.

		Cornebarrieu 2015		**Mondonville 2017**		**Liverdy en Brie 2017**	
Compounds	**Classes**	***r***	***P***	***r***	***P***	***r***	***P***
4-Aminobutanoic Acid (GABA)	AA	0.43	0.285	0.59	**0.008**	-0.08	0.750
Arginine	AA	0.91	**0.002**	0.78	**0.000**	0.63	**0.004**
Asparagine	AA	0.73	**0.039**	0.86	**0.000**	0.84	**0.000**
Aspartic Acid	AA	0.93	**0.001**	0.76	**0.000**	0.78	**0.000**
Glutamic Acid	AA	0.95	**0.000**	0.71	**0.001**	0.77	**0.000**
Glutamine	AA	0.87	**0.005**	0.91	**0.000**	0.80	**0.000**
Glycine	AA	0.19	0.652	-	-	-	-
Histidine	AA	0.50	0.204	0.75	**0.000**	0.75	**0.000**
Isoleucine	AA	0.14	0.743	0.30	0.220	0.39	0.096
Leucine	AA	-0.24	0.566	0.03	0.917	-0.61	**0.006**
Methionine	AA	-0.58	0.130	0.46	0.050	-0.37	0.119
Phenylalanine	AA	-0.34	0.415	0.50	**0.030**	0.35	0.141
Proline	AA	-0.66	0.074	0.53	**0.021**	0.56	**0.013**
Serine	AA	0.61	0.111	0.63	**0.004**	0.68	**0.001**
S-methylcysteine sulfoxide (SMCSO)	AA	0.56	0.146	0.86	**0.000**	0.92	**0.000**
Threonine	AA	0.67	0.070	0.52	**0.022**	0.84	**0.000**
Tryptophan	AA	-0.10	0.807	0.31	0.198	0.40	0.093
Valine	AA	0.26	0.537	0.41	0.078	0.65	**0.002**
α-Alanine	AA	0.89	**0.003**	0.57	**0.012**	0.56	**0.012**
β-Alanine	AA	0.75	**0.032**	0.88	**0.000**	0.83	**0.000**
Fructose	CPOA	0.02	0.967	0.79	**0.000**	0.73	**0.000**
Glucose	CPOA	0.08	0.844	0.59	**0.008**	0.80	**0.000**
Glyceric Acid	CPOA	0.59	0.123	0.85	**0.000**	0.89	**0.000**
Malic Acid	CPOA	0.95	**0.000**	0.84	**0.000**	0.74	**0.000**
Myo-Inositol	CPOA	0.91	**0.002**	0.85	**0.000**	0.83	**0.000**
Quinic Acid	CPOA	0.68	0.066	0.90	**0.000**	0.92	**0.000**
Sucrose	CPOA	-0.20	0.634	0.71	**0.001**	0.87	**0.000**
Unk.FO1	FO	-	-	0.30	0.205	0.66	**0.002**
Unk.FO2	FO	-	-	0.78	**0.000**	0.85	**0.000**
Isorhamnetin-Di-Glucoside	FO	-	-	0.72	**0.001**	0.69	**0.001**
Kaempferol-3-O-Sophoroside	FO	0.34	0.417	0.30	0.215	0.34	0.160
Quercetin-3-O-Caffeoyl-Sophoroside-7-O-Glucoside	FO	-	-	0.89	**0.000**	0.66	**0.002**
Quercetin-3-O-Sophoroside	FO	0.27	0.518	0.71	**0.001**	0.60	**0.007**
Quercetin-3-O-Sophoroside-7-O-Glucoside	FO	-	-	0.75	**0.000**	0.92	**0.000**
Epiprogoitrin	GSL	0.90	**0.002**	0.82	**0.000**	0.91	**0.000**
Glucobrassicanapin	GSL	0.95	**0.000**	-	-	-	-
Glucobrassicin	GSL	0.92	**0.001**	0.76	**0.000**	0.90	**0.000**
Gluconapin	GSL	0.83	**0.012**	0.85	**0.000**	0.93	**0.000**
Gluconasturtiin	GSL	0.97	**0.000**	0.90	**0.000**	0.96	**0.000**
Neoglucobrassicin	GSL	0.93	**0.001**	0.75	**0.000**	0.97	**0.000**
Progoitrin	GSL	0.80	**0.017**	0.91	**0.000**	0.90	**0.000**

ACKNOWLEDGEMENTS

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Appendix 1: Information about genotypes used during field trials 2014-2016 and 2016-2017. 0/0: low concentration of erucic acid and glucosinolates in seeds, 0/+ low concentration of erucic acid and high concentration of glucosinolates in seeds, +/+ high concentration of both erucic acid and glucosinolates in seeds. IOC code of the country of origin.

Genotypes	Flowering period	Erucic acid / Glucosinolates	Origin	Field 2014-2015	Field 2016-2017	Sampled in 2015	Sampled in 2017
Akamar	late flowering	NA	FRA	x	x	x	x
Bolko	late flowering	0/0	POL		x		x
G5	late flowering	0/0	FRA		x		x
Cresus	late flowering	+/+	FRA		x		x
Debruder Dippes	late flowering	+/+	NA		x		x
G10	late flowering	+/+	SWE		x		x
Goeland	late flowering	0/0	FRA		x		
Grizzly	late flowering	0/0	FRA	x	x	x	x
Kombi	late flowering	+/+	UKR		x		x
G14	late flowering	+/+	NA		x		x
Lembke	late flowering	+/+	DEU		x		x
Lira	late flowering	0/+	DEU/RUS		x		x
G18	late flowering	0/0	DEU		x		x
G19	late flowering	0/0	DEU		x		x
Montego	early flowering	0/0	GBR	x		x	
Pollux	late flowering	+/+	SWE		x		x
Quedlinburger Platzester	late flowering	+/+	DEU		x		x
G28	late flowering	+/+	NZL		x		x
Rasant	late flowering	0/0	DEU		x		x
Sarepta	late flowering	+/+	FRA	x	x	x	x
Tor	late flowering	0/0	SWE		x		x
Ecrin	medium flowering	NA	NA	x		x	
ES-Alienor	early flowering	NA	NA	x		x	
ES-Antonia	medium flowering	NA	NA	x		x	
ES-Venus	late flowering	NA	NA	x		x	
Pradel	early flowering	NA	NA	x		x	
Tradition	medium flowering	0/0	FRA	x		x	

Short note 2

Effect of host plant genotype on pollen beetle larval development in field conditions

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Abstract: Pollen beetle (*Brassicogethes aeneus*) is one of the main pests damaging oilseed rape in Europe. This insect is a univoltine species, feeding on flowers during both larval and adult stage. It is currently managed through insecticides but populations resistant to pesticides such as pyrethroids are growing. More diverse strategies need to be elaborated to control populations of this insect. Breeding oilseed rape for enhanced resistances to pollen beetle could be a solution. To protect plants, resistances to pollen beetle feeding could be increased but such resistances will not prevent insect development and growth of pest populations. Use of resistance to insect development could be a solution to reduce insect populations and prevent damage. Here we developed a method to monitor larval development and emergence of adults in the field. The number of larvae, the number of adult pollen beetles emerging from plots and their body weight were measured on three oilseed rape genotypes. No difference between genotypes were observed for these traits in our study. However, our sampling method seems to be adequate as the number of larvae and adults emerging from plots were highly correlated. This method paves the way to larger sampling aiming at studying oilseed rape variability for these resistances.

Key words: Resistance, Oilseed rape, Adult emergence, Body weight, Population

INTRODUCTION

Current limitations of pesticides revive the interest for plant breeding for resistances against insect pests. Most studies on plant resistance focus on traits that directly reduce damage caused by insects to plants. Damage reduction can be achieved by decreasing oviposition or insect establishment on the crop through deterrent or adverse effects on adults and larvae (Painter, 1968). These resistances, especially those having negative effects on survival, directly impact insect populations. Limiting growth rate of a pest and its accumulation in the crop is of special interest to control insects completing several generations on the same plant such as aphids (Smith and Chuang, 2014; Aradottir et al., 2016). This strategy is also effective at larger spatial and temporal scales. When implemented through large cropping areas, such resistances could reduce pest population reservoirs and prevent damages in other fields. Limiting the growth rate of pests may be effective even for species achieving only one generation on the plant as it may impact pest population size on the next seasons. Examples of crop resistances having effects on pest populations have already been documented. Introduction of resistant wheat preventing the larval development of *Mayetiola destructor* strongly reduced the population of this fly in the United State during the 40's even in the most favourable locations (Painter, 1958). Other observations have been made following wide use of Bt crops in United State, China and Australia establishing a negative link between surface of Bt crops and pests infestation at regional scale (Carriere et al., 2003; Tabashnik et al., 2005; Wu et al., 2008; Hutchinson et al., 2010; Baker and Tann, 2016). These area-wide reductions of insect populations have reduced yield losses related to insects even on non-Bt crops (Hutchinson et al., 2010). In contrast, introduction of susceptible genotypes has led to pests population increase and thus to larger insecticides use which in turn increased the development of resistance to pesticides (Akbar et al., 2008).

Oilseed rape (OSR, *Brassica napus* L., Brassicacea) is the most cultivated oilseed crop in Europe with 6.46 M ha grown in 2015 (Eurostat, 2015). Its rapid expansion over the last decades has created favourable conditions to increase pest populations of this plant (Hokkanen, 2000). The pollen beetle (*Brassicogethes aeneus*, formerly *Meligethes aeneus*) Fabricius (Coleoptera: Nitidulidae) is one of the most important pests of OSR in Europe. This insect is a univoltine species whose larvae and adults feed on pollen. Adults overwinter in the soil and leaf litter of forests and emerge during early spring (Williams, 2010). They consume pollen of a vast array of plant families but are specialized on Brassicaceous species for oviposition (Kirk-Spriggs, 1996). Adults migrate to OSR fields when plants are at the green bud stage and feed on pollen by destroying flower buds (Williams, 2010). Females lay up to 200 eggs in different buds where larvae develop before reaching open flowers (Scherney, 1953). Once mature, larvae fall to the ground and pupate below the soil surface. The new generation of beetles emerges during the following summer and disperses to feed on flowering plants before seeking overwintering sites (Williams, 2010). Most damage are caused by adult pollen beetles while feeding (Williams and Free, 1978). OSR plants are partly tolerant to pollen beetle attacks and can compensate for most damage but in conditions of heavy infestation (ca. upper 20 insects / plants) yield losses can be high (Ellis and Berry 2012).

Reductions of yield losses related to pollen beetle may be possible through increased resistance to feeding damage. Moderate levels of resistance seem to be present in OSR (Hervé et al., 2014; *Paper 1*) but these resistances may not be strong enough to prevent pollen beetle damage in heavy infestation conditions. High levels of resistance to pollen beetle seem to be present in other Brassicaceous species such as *Sinapis alba* (Ekbom and Borg, 1996; Enzenberg and Ulber, 2016; Seimandi Corda, data not published). However, crossing OSR with *S. alba* to introgress resistances is very challenging (Dosdall and Kott, 2006). Targeting pollen beetle populations by affecting larval development may be an alternative. Evidences of genotypic effect on larval development or adult fitness of pollen beetle are

scarce but development time and larval infestation are known to vary according to genotype (Tölle, 2011; Hervé et al., 2016). These results suggest that some genotypes could be less advantageous for pollen beetle development and help reduce populations at the next generation. The present field experiment investigates how pollen beetle development can be monitored in the field. We tested the potential effect of genotype on larval infestation, emergence and fitness of the following generation of pollen beetle.

MATERIALS AND METHODS

Twelve OSR genotypes with a broad genetic basis were used during this study (see Appendix 1). Plants were sown in a field trial near Le Rheu (Brittany, France) in September 2016. The field trial was designed in a randomized-block design with four blocks. Each genotype was sown in plots of 6 rows of 7.5 m length, spaced by 50 cm. No insecticides were applied and weeds were controlled mechanically. Due to important damages caused by common wood pigeons, only three blocks and three genotypes could be used for the rest of the study (see Appendix 1).

To estimate plant infestation levels by larvae, circular pitfall traps (31 cm in diameter) were used to collect dropping larvae (i.e. larvae that exit flowers to pupate in the soil beneath) between the beginning of petal falling and the end of flowering. Four pitfalls were randomly placed on the ground in each plot below OSR plants. They were filled with water and a few drops of detergent. Pitfall contents were collected once a week between May 11th and May 30th. Plant flowering was monitored during the same period by counting the number of flowering inflorescences above pitfalls.

Pollen beetle emergences were also recorded from June 1st to June 23rd using three photoeclectors (60 cm x 60 cm) randomly placed in each plot. Their collecting jars were filled with water and detergent. Sampling occurred once a day and pollen beetles were kept in 70 % alcohol before being oven dried at 65 °C during 24 hours. Dried pollen beetles were weighted using a microbalance (Mettler Toledo XS 105).

Number of larvae collected over the sampling period was compared among genotypes using a Wald test on a Linear Mixed Model (LMM) with genotype as a fixed factor and block as a random factor. Pairwise comparisons of least squares means were performed using the function “lsmeans” (package “lsmeans”; Lenth, 2016) and the False Discovery Rate correction for P-values (Benjamini and Hochberg, 1995). The same procedure was applied to identify differences between genotypes for the number of new generation pollen beetles collected by photoeclectors. Mortality rate during pupation has been computed for each plot by comparing the mean number of collected larvae and the mean number of adults caught per square meter. Differences in mortality rate between genotypes was tested as above. Insect mass was compared between genotypes using Wald test on LMM. Genotype was used as a fixed factor, block and collection date were considered as random factors. All statistical tests were performed using R software (R Core Team, 2016).

RESULTS

Significant differences in the number of larvae were found according to genotype ($\chi^2 = 6.12$, $df = 2$, $P = 0.047$) but pairwise comparisons between genotypes did not support these differences (Fig. 1a). A positive correlation was found between the number of larvae collected and the number of flowering inflorescences counted over the three sampling dates ($r = 0.53$, $df = 27$, $P = 0.003$) (Fig. 2). No differences in the number of emerging pollen beetles were found between the three genotypes ($\chi^2 = 1.47$, $df = 2$, $P = 0.479$) (Fig. 1b) even when accounting for the number of plants in each cage ($\chi^2 = 1.98$, $df = 2$, $P = 0.372$). Average mortality of pollen beetle during pupation was estimated to be 89 % (SE ± 0.01). No differences were observed between genotypes ($\chi^2 = 1.22$, $df = 2$, $P = 0.461$).

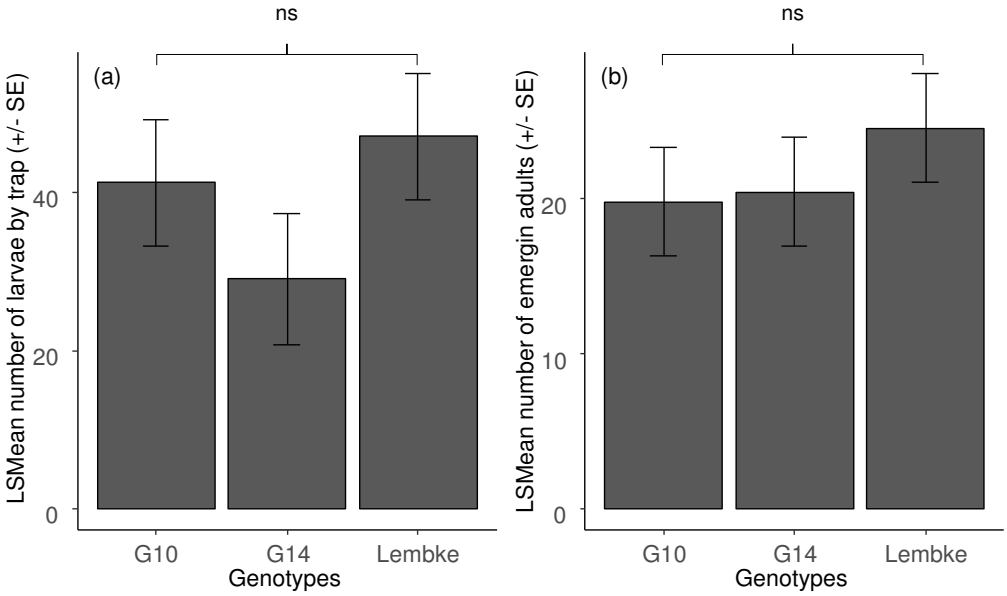

Figure 1: a) Least square means (± SE) of the number of larvae collected on different genotypes. b) Least square means (± SE) of the number of adult pollen beetle collected on different genotypes.

Adult dry mass was measured on 563 insects and even if large variability was present in these data (min = 0.06 mg, max = 0.49 mg), insect dry mass was not influenced by the genotype ($\chi^2 = 0.33$, $df = 2$, $P = 0.847$). Mean insect dry mass was 0.25 mg (SE ± 0.007) for pollen beetles emerging from G10 plants, 0.246 mg (SE ± 0.007) and 0.249 mg (SE ± 0.007) for those emerging from G14 and Lembke plants respectively. Surfaces of pitfall and photoeclectors were used to compute the number of insects collected and emerging per meter square. A high correlation was found between the mean number of larvae collected by plot and the mean number of emerging insects ($r = 0.84$, $df = 7$, $P = 0.004$) (Fig. 3).

DISCUSSION

No differences in number of larvae and emerging adults were found between genotypes. This may be due to the limited sampling (only 4 pitfall traps and 3 photoeclectors / plot) realized in the field which may not have been sufficient to catch the variability in the data. Only three genotypes could be studied here and it does not yield conclusion about absence of variability in OSR for these traits. As explained above, our field trial suffered from heavy damage caused by common wood pigeons which detrimentally affected plant phenotype. Plants were smaller and more ramified than when grown in optimal conditions. Results of the present study showed that larval infestation increased with the number of inflorescences. The reduced size of plants attacked by pigeons may have decreased larval infestations on these genotypes. Similar experiments performed in 2016 with a different set of genotypes showed a higher number of collected larvae and revealed more important differences between genotypes (426 larvae / m² (SE ± 46) for the less infested genotype to 1741 larvae / m² (SE ± 186) for the more infested) (Seimandi Corda, data not published). Adult beetles emerging from OSR genotypes had the same mean body weight. Even if variability seems to be present in the data and that large number of insects were sampled no differences were observed. Previous studies showed that larval diet and host plant quality influence adult body weight (Cook et al., 2004; *Paper 2*). It seems that there is no difference in host plant quality over the three genotypes sampled here.

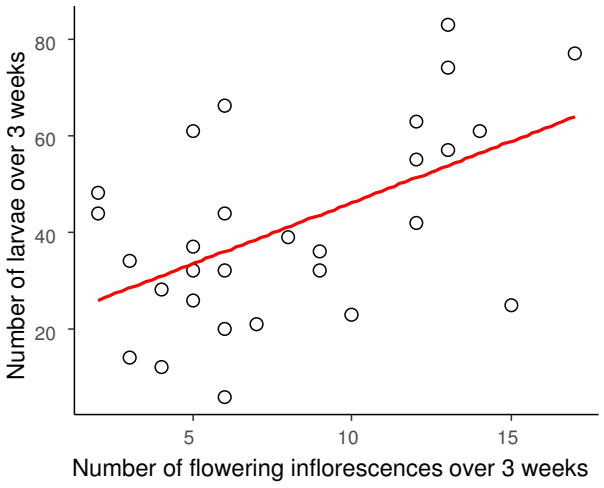

Figure 2: Relationship between the number of larvae collected and the number of flowering inflorescences above pitfalls during the collection period.

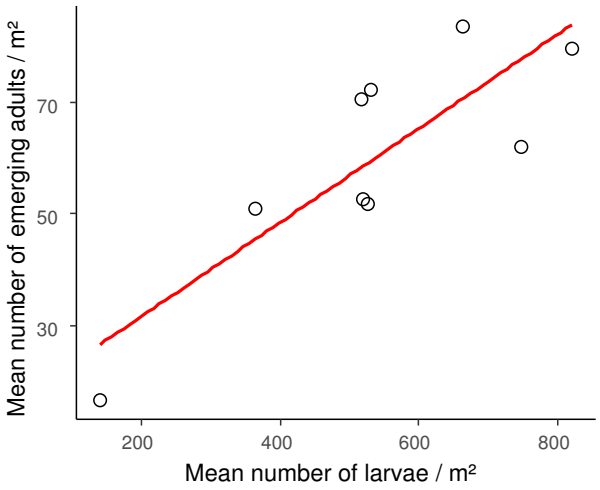

Figure 3: Relationship between the mean number of emerging adults per plot per m² and the mean number of collected larvae per plot per m².

High mortality rate was measured during our experiments. These observations are consistent with others data collected in Sweden that estimated mortality rate at 88 % (Riggi et al., 2017). This high larval mortality may be due to high parasitization rates in our field trial. Larvae sampled over the three first sampling days (May 11th, 12nd, 15th) were inspected for eggs of the parasite *Tersilochus heterocerus* using binoculars. Parasitization rate reached 67 % (SE ± 0.05, n = 104). This is not surprising as *T. heterocerus* is known to have locally high parasitism rate, reaching up to 97 % (Ulber et al., 2010). However, in Riggi et al. (2017), the parasitization rate was much lower (i.e. 16 %) and did not explain this large mortality. Other arthropods such as ground-dwelling predators (i.e. carabids and spider wolfs) may have predated larvae on the ground and reduced pollen beetle populations (Haschek et al., 2011; Öberg et al., 2011). Abiotic factors such as soil conditions (e.g. soil moisture or clay content) could also interact with pupation and impact pollen beetle emergence (Ellis et al., 2004; Riggi et al., 2017).

Although no differences between genotypes were observed, a good correlation between the number larvae collected and the number of emerging pollen beetles was found. This indicates that the sampling method used here was effective. This method paves the way to larger screenings which will help to identify if variability in host plant quality exists in the field. These screening could prevent commercialisation of highly susceptible genotypes leading to heavy utilisation of pesticides. Moreover, even if moderate level of resistance would be found, they could reduce pollen beetle populations to a sufficient extent to be managed with moderate levels of resistance to adult feeding already identified in OSR.

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Appendix 1: Information known about the genotypes used during field trials 2016-2017. 0/0: low concentration of erucic acid and glucosinolates in seeds, 0/+ low concentration of erucic acid and high concentration of glucosinolates in seeds, +/+ high concentration of both erucic acid and glucosinolates in seeds. IOC code of the country of origin.

Genotype	Flowering period	Erucic acid / Glucosinolates	Origin	Sampled in 2017
Akamar	late flowering	NA	FRA	
Bolko	late flowering	0/0	POL	
G5	late flowering	0/0	FRA	
G10	late flowering	+/+	SWE	x
Grizzly	late flowering	0/0	FRA	
G14	late flowering	+/+	NA	x
Lembke	late flowering	+/+	DEU	x
G18	late flowering	0/+	DEU/RUS	
G19	late flowering	0/0	DEU	
G28	late flowering	+/+	NZL	
Rasant	late flowering	0/0	DEU	
Sarepta	late flowering	+/+	FRA	

Chapter 2

Feeding ecology of the pollen beetle

Chapter 2: Feeding ecology of the pollen beetle

Context

Having a good understanding of the biology of phytophagous insects and their interactions with plants is essential to develop new varieties resistant to insect pests. Even if the biology of the pollen beetle is relatively well studied, its feeding behaviour received limited attention. This is surprising as most damage caused by this insect results from its feeding on oilseed rape inflorescences. To identify potential new traits to target for developing oilseed rape varieties resistant to pollen beetle is seems therefore important to better document and understand their feeding ecology. Moreover, to identify new strategies aiming at controlling pollen beetle populations by reducing emergence of new generation adults, a good understanding of larval diet and how plant quality impacts insect development and fitness is also necessary. Development of larvae without pollen has already been demonstrated but how they survive without this food source remains unexplained. Nectar, is a nutrient rich food source also present in flowers and it could be used by larvae during their development. Adult pollen beetles could also use this resource when foraging in flowers and nectar content may affect their preferences. Observations of adult feeding behaviour already pointed out a specific resource partitioning by this insect. Medium size buds are preferentially used for oviposition while feeding occurs mainly on small buds. This pattern seems rather counterintuitive because of the low nutritional quantity of pollen present in these non-mature buds. Understanding costs and benefits associated to feeding on different size of buds such as handling time, exposition to toxic compounds or energetic value of organs may help finding traits potentially allowing to deconstruct this behaviour.

Approach

Three experiments were conducted to study the feeding behaviour of pollen beetle. The first one aimed at disentangling the importance of pollen and nectar on the development of this insect. Pollen beetle larvae were reared on detached flowers containing nectar and/or pollen or neither of both in a laboratory experiment. Effects of these resources on pollen beetle development was investigated.

A second experiment investigated if nectar contained in flowers affects the preferences and the feeding activity of adult insects. This experiment was also conducted on detached flowers in controlled conditions.

The third experiment was focused on adult feeding on plant at green bud stage. The optimal foraging theory was used as a framework to understand how adult pollen beetle chooses between different floral organs on the inflorescence. Insect behaviour was studied using choice tests conducted in the laboratory between different organs (small buds, large buds, anthers and flowers). These experiments were paired with chemical analyses of plant secondary compounds (glucosinolates and flavonols), quantification of energetic metabolites present in insects (proteins, triglycerides and carbohydrates) and performance experiments to estimate cost and benefits associated to feeding behaviour.

Results

These experiments revealed that pollen beetle feed both as larva and adult on nectar. This illustrates that florivorous insects could be more dependent on nectar produced by flowers than previously thought. However, no effect of nectar was observed on larval development or adult preferences. We confirmed that pollen has a detrimental beneficial effect on insect fitness by increasing its weight. However, its

effect on other life history traits such as larval survival or developmental time are less prominent than expected. This indicates that pollen beetle larvae are well adapted to deal with diverse food sources.

Adults were observed feeding more on small buds than medium and large buds on oilseed rape inflorescences. Pollen beetle feeding seems more impacted by the availability and the accessibility of food than its nutrients content or its concentration in secondary compounds. The presence of the perianth and its thickness seems to be an important component of pollen beetle feeding preferences.

Limits

These experiments were a first step to assess the effect of nectar on pollen beetle. They were conduct in Petri dishes with detached flowers and the effect of this source of nutrients may be better estimated under more natural conditions. Field screenings of genotypes already found large variability in nectar production according to genotype. It could be interesting to monitor adult abundance and development of pollen beetle on plants producing contrasted levels of nectar to effectively assess its importance.

Oilseed rape buds' perianth seems to be an important element explaining within-plant pollen beetle feeding behaviour. Several hypothesis have been made to explain its effect such as increase in chemical defences or handling time. However, additional experiments would be necessary before strong conclusions can be made. Indeed, so far we did not demonstrate a direct effect of glucosinolates and flavonols on this insect and we did not prove that increase in thickness resulted in enhanced handling time and reduced energy uptake.

Paper 2

Submitted to Arthropod-Plant Interactions

Impact of flower rewards on phytophagous insects: importance of pollen and nectar for the development of the pollen beetle (*Brassicogethes aeneus)*

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Abstract: Entomophilous plants reward pollinators with provision of nutrient-rich foods such as pollen and nectar. These rewards contain compounds that are essential to insect development and can be used by pollinators as well as herbivorous insects. The pollen beetle (*Brassicogethes aeneus*) whose larvae develop in oilseed rape flowers (*Brassica napus*) is known to feed on pollen. Previous studies already showed the importance of pollen on the development of this insect but it seems that other resource, such as nectar could also be used. The purpose of this study was to assess the respective roles of pollen and nectar on pollen beetle development. We tested them by behavioural and developmental experiments using flowers containing or not pollen and/or nectar. Larvae, irrespective of their instar, fed both on anthers and nectaries. Nectar did not influence larval development or adult survival after development while pollen influenced development by increasing larval and adult weight. However, pollen did not affect larval or adult survival nor development time. These results indicate that pollen beetle larvae are adapted to deal with various diets and can complete their development without pollen or nectar.

Keywords: *Brassicogethes aeneus*, Oilseed rape, Larvae, Nutrition, Insect weight, Survival

INTRODUCTION

Many flowering plants are highly dependent on insect pollinators to ensure their reproduction. To attract flower-visiting insects, plants often reward them through provision of nutrient-rich foods such as pollen and nectar (Kevan and Baker, 1983). Pollen was the primary reward sought by pollinators but during angiosperm evolution, rewarding switched from pollen to nectar provision as consumption of pollen is associated to loss of reproductive potential (Nicholls and Hempel de Ibarra, 2017). Pollen and nectar can strongly impact pollinator fitness as they favour key life history traits like development, fecundity and survival (Burkle and Irwin, 2009). Insects need various nutrients such as amino acids, carbohydrates, lipids, fatty acids, sterols, vitamins and trace elements that can be found in pollen or nectar to complete their development (Schoonhoven et al., 2005). Pollen is recognized as a major source of nitrogen-based compounds such as proteins but it also contains starch, sterols, lipids and vitamins (Roulson and Cane, 2000). Nectar, on the other hand, is mostly an aqueous solution of sugars and also contains minor quantities of amino acids, proteins, lipids and vitamins (Wäckers et al., 2007). These rewards are made available by plants in a way to attract mutualists but antagonists such as herbivorous insects can also benefit from them (Wäckers et al., 2007). Indeed, a reduction of development time or larval mortality has been shown in some herbivorous insects feeding on pollen (Teulon and Penman, 1991; Bauce and Carisey, 1996).

The pollen beetle, *Brassicogethes aeneus* (formerly *Meligethes aeneus*) Fabricius (Coleoptera: Nitidulidae) is a univoltine species and a pest of oilseed rape (OSR) (*Brassica napus* L., Brassicaceae) in Europe (Skellern and Cook, 2017). Larvae and adults of this insect are florivorous and feed on pollen. Adult pollen beetles emerge in the spring and are generalist feeders eating pollen from several plant families but they are specialized on brassicaceous plants for oviposition and larval development (Kirk-Spriggs, 1996; Ouvrard et al., 2016). Two larval instars have been described (Osborne, 1965). The first instar usually develops in closed floral buds while the second instar feeds in open flowers and frequently moves into younger flowers (Williams and Free, 1978). During their development pollen beetle larvae eat OSR pollen which is rich in proteins, starch and free amino acids (Evans et al., 1991; Hervé et al., 2014). Once mature, larvae drop from the plant to the soil and adult emergence occurs during the summer. After emergence, adults feed on flowers before migrating to forests where they overwinter (Williams, 2010).

Based on a comparison between male fertile and male sterile OSR flowers, Cook et al. (2004) showed that pollen consumption has a major effect on pollen beetle development. Survival probability of larvae and weight of larvae as well as adults were significantly enhanced when insects were fed on male fertile flowers. While these results are consistent with current knowledge on pollen chemistry, what was more surprising is that a small number of larvae (6 %) were able to complete their development without any pollen (Cook et al., 2004). This indicates that pollen beetle larvae, generally considered essentially pollinivorous, can use other food sources present in flowers during their development (Cook et al., 2004). OSR flowers produce consistent quantities of nectar which contain high amounts of glucose and fructose (Bertazzini and Forlani, 2016). Nectar could therefore constitute an important alternative source of nutrients for pollen beetle larvae. This food source could be more beneficial to second instar larvae which develop in open flowers than to first instar larvae which develop in closed floral buds that do not produce nectar (Davis et al., 1986; Burquez and Corbet, 1991).

The purpose of the present study is to disentangle the role of pollen and nectar on pollen beetle development. To do so, we tested four hypotheses: (1) larvae feed both on anthers and nectaries, (2) second instar larvae feed more frequently on nectaries than first instars, (3) flowers containing both pollen and nectar allow a better larval development in terms of duration, survival probability and weight than flowers containing only pollen, nectar or neither of those and (4) adult pollen beetles developing on flowers containing both pollen and nectar survive longer and have a higher weight than those developing on flowers containing only pollen, nectar or neither of those.

To test these hypotheses, a first experiment was conducted where feeding behaviour of larvae from the two instars was observed to identify floral parts that are consumed by larvae. To investigate the influence of nectar and pollen on larval development and adult survival, a second experiment was performed using OSR flowers with or without pollen and/or nectar.

MATERIALS AND METHODS

Plants

Seeds of OSR (cv. Express) were sown in individual propagation plugs (75 % peat, 13 % perlite, 12 % vermiculite; pH = 6; Fertiss) and placed in controlled conditions in a climatic room (16:8 L:D, 16 °C) for 3 weeks. Plantlets were then vernalized for at least 8 weeks (8:16 L:D, 4 °C). After this period plantlets were transplanted in individual 2 l-pots (85 % peat, 15 % perlite; pH = 6) and placed again in a climatic room (16:8 L:D, 19 °C) until flower development (ca. 3 to 4 weeks).

Insect sampling and egg production

Pollen beetles were sampled in an unsprayed OSR field by beating inflorescences. Sampling occurred between April and May 2017 near Le Rheu (Brittany, France, 48°06'24.8"N 1°46'43.5"W). Approximately one hundred individuals were placed in a climatic room (16:8 L:D, 19 °C), in a cage with flowering OSR inflorescences (cv. Express) grown as explained above to allow them to feed and oviposit. Inflorescences were checked every day to identify oviposition holes and pollen beetle's eggs were harvested by dissecting floral buds. Groups of 20 eggs were then placed on moistened paper in a Petri dish in the same climatic conditions as the adults until hatching.

Nectar extraction

To produce nectar-free flowers, a protocol adapted from Bosi (1973) and Enkegaard et al. (2016) was used. A group of 10 excised flowers was placed in a 50 ml conical tube with hydrophobic cotton at its bottom. Tubes were then centrifuged (SIGMA 3-16K) during 3 minutes at 1000 g, 2358 rpm and 20 °C to allow nectar to flow through flowers and cotton wool.

To control efficiency of this protocol, a preliminary experiment was conducted. To ensure effectiveness of nectar removal, five samples of 10 flowers were centrifuged as previously explained twice consecutively. Iron beads (5 mm diameter) were added at the bottom of the tube, bellow the cotton wool to allow nectar collection. Nectar accumulated at the bottom of the tube was quantified after each centrifugation using a glass micro-capillary (20 µl). Furthermore, to confirm that excised flowers did not secrete nectar after removal, a second set of 5 samples of 10 flowers were centrifuged as above. After a first centrifugation, flowers were placed individually in Petri dishes lined with moistened filter paper in a controlled climatic room (16:8 L:D, 19 °C). A second centrifugation was performed 24 hours later on the same flowers. Nectar was quantified after each centrifugation as previously described.

Feeding activity of the larvae

To identify flower parts consumed by pollen beetle larvae, 19 first instar larvae and 17 second instar larvae previously fed on fresh cut flowers were observed during one day. One flower was given to each isolated larvae at 09:00 a.m. in a Petri dish (diameter = 3.2 cm) lined with a moistened filter paper. The position of each larva in the flower (stamens, nectaries, other flower parts) and their activity (feeding or not) was recorded every 2 hours between 11:00 a.m. and 05:00 p.m. (ca. at 4 times).

Development and survival

Neonate larvae hatched since less than 24 hours were placed individually on a flower in a Petri dish (diameter = 3.2 cm) with a moist filter paper. Flowers were changed every other day using excised ones opened since less than 48 h. Four different types of diets were given to larvae: (NP) unmodified flowers containing both nectar and pollen, (N) flowers containing nectar but where pollen was removed by cutting anthers, (P) flowers containing pollen and where nectar was removed as previously explained and (O) flowers where both pollen and nectar were removed. Between 40 and 46 neonate larvae were used per treatment. Larval survival and development stage were examined daily. Five stages were used to describe the development (first instar, second instar, prepupa, pupa and adult) as in Cook et al. (2004). To avoid increased mortality due to larval manipulation, immature insects were weighted two times only with a microbalance (Mettler Toledo XS 105): after one week of development and directly after pupation. After emergence, adults were kept in the same conditions as larvae but without food to record survival. Once dead, adults were preserved in alcohol (90 %), oven dried during 24 h at 60 °C and weighted. Adult dry weight is highly correlated with fresh weight ($r = 0.87$, $df = 104$, $P < 0.001$, data not shown).

Statistical analysis

During behavioural observation experiment, larvae were recorded feeding on stamens and nectaries. The number of occurrences of feeding on nectaries during a day was summed for each individual. Similar sums were made for occurrence of feeding on stamens and not feeding. Likelihood ratio tests on Generalized Linear Models (distribution: quasi-poisson, link function: log) were computed to test if sums of observations of the three behaviours were different for first and second larval instars. Likelihood ratio tests on Generalized Linear Mixed Models (distribution: poisson, link function: log) with individual as random factor were computed for each larval stage to test if there was differences in occurrence of the three behaviours. Pairwise comparisons of least squares means were performed using the function "lsmeans" (package "lsmeans"; Lenth, 2016) and the False Discovery Rate correction for P-values (Benjamini and Hochberg 1995).

Differences between treatments for larval survival, adult lifespan and duration of each instar were analysed using a Likelihood ratio test on GLMs (distribution: Gamma, link function: inverse). Effect of treatment on insect weight was tested with ANOVAs at each weighting time. Pairwise comparisons were tested as previously explained. Relations between insect weight, development time and lifespan of adults were investigated using Pearson's correlation test. All statistical tests were performed using R software (R Core Team, 2016).

RESULTS

Nectar extraction

An average of 0.13 µl (SE ± 0.04) of nectar per flower was extracted after the first centrifugation of the two experiments. This quantity is consistent with previous observations (Bertazzini and Forlani, 2016). No nectar was found after the second centrifugation, neither immediately after the first one nor 24 h later.

Feeding activity of the larvae

No difference in the occurrence of feeding on stamens, nectaries and not feeding were found between first and second instar larvae (Table 1). Pairwise comparisons showed that larvae were observed significantly more not-feeding than feeding but no significant differences were observed between occurrence of feeding on stamens or nectaries.

Table 1: Mean number of times ±SE first and second instar larvae were observed feeding on different plant parts. Positions of larvae in the flower and their activity (feeding or not) was recorded every 2 hours between 11:00 a.m. and 05:00 p.m.

Feeding activity	First instar	Second instar	χ^2	*df*	*P*
Feeding on stamens	0.42 ±0.18	0.71 ±0.22	1.00	1	0.32
Feeding on nectaries	0.95 ±0.14	0.82 ±0.21	0.24	1	0.63
Not feeding	2.63 ±0.21	2.47 ±0.27	0.23	1	0.63

Development and survival

Whether pollen or nectar was present or not during development did not influence survival probability of larvae (Fig. 1, $\chi^2 = 1.27$, $df = 3$, $P = 0.74$) neither duration of each instar nor adult survival (Table 2). However, significant differences in larval and pupal weight were observed among treatments ($F = 12.35$, $df = 3$, $P < 0.001$ for larvae and $F = 11.73$, $df = 3$, $P < 0.001$ for pupae). Insects developing on flowers containing pollen were heavier than those developing on flowers without pollen (Fig. 2a). The trend observed for immature weight was consistent with the one observed for adults (Fig. 2b). Adults developing on flowers with both nectar and pollen had the heaviest dry weight and the ones that had none of these sources were the lightest. However, contrary to what was observed for immature insect weights, insects developing on flowers containing one of the resources were not different from other treatments.

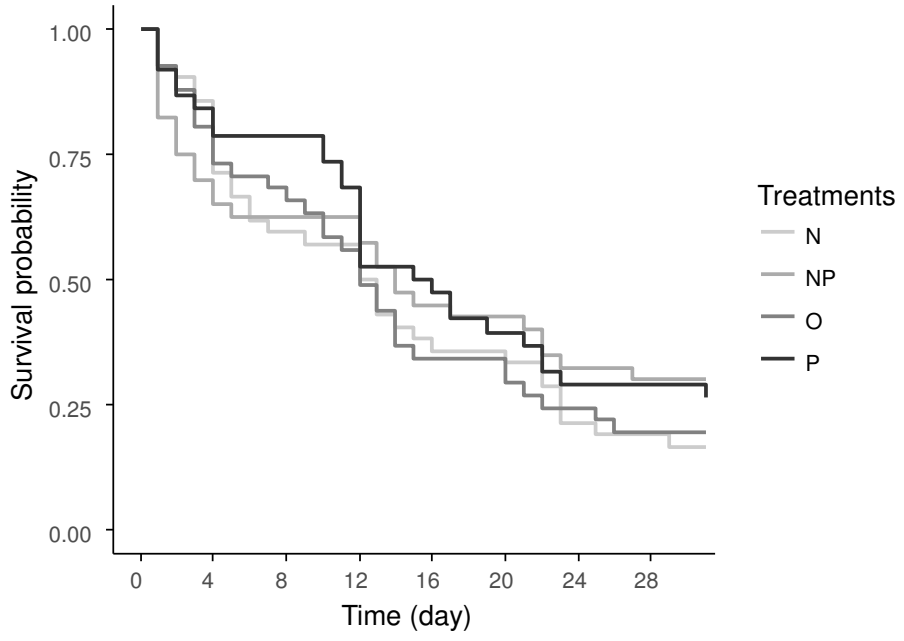

Figure 1: Kaplan–Meier estimates of survivorship function during development in pollen beetles according to four different diets: NP = flowers containing nectar and pollen, N = flowers containing nectar but no pollen, P = flowers containing pollen but no nectar, O = flowers without nectar and pollen

Table 2: Development time (mean number of days ± SE) of the immature stages of *Brassicogethes aeneus* and adult survival for each treatment: NP = flowers containing nectar and pollen, N = flowers containing nectar but no pollen, P = flowers containing pollen but no nectar, O = flowers without nectar and pollen. Number of observations are given between brackets for each stage and each treatment

Instar	NP	N	P	O	χ^2	*df*	*P*
First instar	2.71 ± 0.12 (28)	2.97 ± 0.09 (38)	2.97 ± 0.14 (34)	2.94 ± 0.11 (35)	3.15	3	0.37
Second instar	4.48 ± 0.23 (25)	5.00 ± 0.29 (26)	4.45 ± 0.18 (31)	5.08 ± 0.26 (25)	5.93	3	0.12
Prepupa	4.79 ± 0.25 (19)	4.41 ± 0.24 (17)	4.68 ± 0.22 (19)	4.64 ± 0.29 (14)	1.29	3	0.73
Pupa	9.08 ± 0.31 (12)	9.29 ± 0.36 (7)	8.90 ± 0.38 (10)	8.88 ± 0.30 (8)	0.77	3	0.86
Adult	5.09 ± 0.68 (11)	5.00 ± 1.24 (6)	4.90 ± 0.58 (10)	7.14 ± 0.43 (7)	4.69	3	0.20

Significant correlations were observed between larval and pupal weights ($r = 0.70$, $df = 67$, $P < 0.001$), larval and adult dry weights ($r = 0.50$, $df = 35$, $P = 0.002$) and between pupal and adult dry weights (r = 0.39, $df = 35$, $P = 0.015$). No significant correlation was found between adult survival and measured weights (adult survival / adult dry weight, $r = -0.07$, $df = 32$, P = 0.68; adult survival / pupal weight, $r = -0.16$, $df = 32$, $P = 0.36$; adult survival / larval weight, $r = -0.24$, $df = 32$, $P = 0.17$).

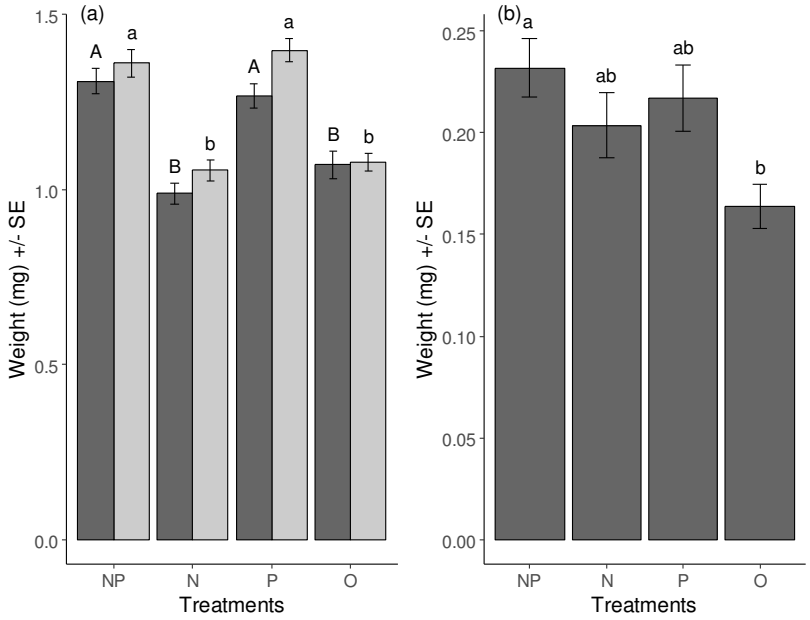

Figure 2: Mean weights of insects fed with different flower treatments: a, Mean weight (mg) ± SE of larvae (dark grey) and pupa (light grey) for each treatment: NP = flowers containing nectar and pollen, N = flowers containing nectar but no pollen, P = flowers containing pollen but no nectar, O = flowers without nectar and pollen. Different letters indicate significant differences in weight among larvae (capital letters) or pupae (lowercase letters). b, mean dry weight (mg) ± SE of adults for each treatment. Different letters indicate significant differences

DISCUSSION

Our behavioural observations reveal that pollen beetle larvae, irrespective of their instar, feed both on anthers and on nectaries of OSR flowers. This indicates that pollen beetle larvae eat nectar and it is one of the rare observations of phytophagous larvae feeding on nectar (Wäckers et al., 2007). Nectar consumption by pollen beetle larvae had already been suspected as flowers containing larvae contain low levels of nectar (Kirk et al., 1995) but it is the first confirmation of this behaviour. Nectar is a sugar rich solution which provides nutrients to larvae but it may have a second function and act on pollen digestion (Roulson and Crane, 2000). Indeed, digestion of pollen grains by enzymes is only known in some species of Collembola and Gelechiidae moths (Scott and Stojanovich, 1963; Luo et al., 2011). One of the most common strategies to extract pollen nutrients is through pollen germination (Roulson and Crane, 2000). Pollen grains of many plant species germinate and release their content in sugary, weakly acid medium (Stanley and Linskens, 1965). Several pollinivorous insects were observed sipping nectar while ingesting pollen and dissection of their gut demonstrated that pollen grains digestion was done by

activating their germination (Gilbert, 1972; Haslett, 1983; Nicolson, 1994). Gut dissections would be necessary to assess if such process also occurs in pollen beetles.

Our experiments did not reveal any effect of nectar on pollen beetle development. Insect survival, development duration and weight of larvae and adults did not differ whether or not nectar was present. While flowers containing nectar were supplied every other day to larvae, the role of nectar may have been slightly minimized in this experiment as preliminary tests we conducted showed that excised flowers stop secreting nectar. Our experiments on the other hand confirmed the importance of pollen for larval development. Flowers containing pollen allow development of heavier larvae and adults which is in line with previous research (Cook et al., 2004). However, contrary to this previous study, survival probability and development time were not affected by pollen presence in our experiments. Differences between the two studies may be due to differences in plant genotypes used. Such an effect has for example been observed for the western flower thrips (*Frankliniella occidentals*) where larval development is much more affected by the presence of pollen when it is reared on resistant cotton plants than on susceptible host plant (Trichilo and Leigh, 1988). The use of a genotype favourable to insect development in the present study (Hervé et al., 2016) may have minimized the effect of pollen absence on larval development.

While pollen seems important, our results confirm that pollen beetles can complete their development in flowers not containing any pollen. Insects developing in these flowers were observed feeding on petals and carpels (personal observations). They were lighter than those growing on flowers bearing pollen but other traits such as survival and development time were not affected. This may indicate that larvae of this species are well adapted to handle various diets. Transcriptomic analyses made on pollen beetle larvae pointed out that numerous genes related to catabolism (such as carbohydrate metabolic process and peptidase activity) are expressed in larvae suggesting that larvae are indeed adapted to feed on diverse diets (Vogel et al., 2014). Moreover, OSR flowers are ephemeral and remain open an average of 3 days (Eisikowitch, 1981), imposing larvae especially second instars to move to open flowers periodically (Williams and Free, 1978). Larvae that do not complete their development before the end of the flowering period have been observed feeding on stalks, pods and stems resulting in “tip drop” damage on plants (Winfield, 1961). This indicates that larval diet is not strictly limited to flowers. The fact that flowers are a transient resource may have selected insects adapted to develop on diverse diets.

Resources consumed by larvae are usually determinant for adult fitness (Awmack and Leather, 2002). Nutrients contained in pollen seem to be responsible for the important weight gain of insects feeding on flowers containing pollen (Patt et al., 2003). On the other hand, in the absence of pollen, larvae feed on flower parts such as carpel and petals that may be less nutritious or contain higher defence compounds and thus do not allow insects to accumulate high quantities of resource (Abdasalamee and Müller, 2015). In our experiment, no relationship between adult body weight and survival was observed. However, environmental conditions can influence the link between body weight and survival. Indeed, pollen beetles are known to suffer important mortality rates during overwintering, and this mortality is related to body weight as lighter insects with low fat reserves do not survive to winter (Hokkanen, 1993; Lehrman et al., 2008). The impact on survival of the weight differences we found here in insects feeding on pollen should be tested under more stressful winter conditions. Furthermore, reduced body weight could also impact female fecundity as positive relationships between female body size and fecundity is a general trend in insects (Honěk, 1993).

The function of nectar for herbivorous insects is increasingly recognized but studies investigating its role in herbivorous larvae remain rare. From the plant perspective, nectar feeding by herbivorous larvae can have detrimental effects on pollinator attraction as it modifies nectar production and quality. OSR flowers containing pollen beetle larvae have three times less nectar than intact flowers and their nectar

contains 29 % less sugar (Kirk et al., 1995). Such a reduction in nectar production due to nectar consumption by phytophagous larvae has been shown to strongly decrease visitation rate of some plants by key pollinators (Missagia and Alves, 2017). Reduction of pollinator visitation leads to increase in autogamy and may impact plant population dynamic (Penet et al., 2009). Whether such interference between nectar feeding by pollen beetle larvae and flower visitation by main pollinators of OSR such as bees exists, remains to be investigated.

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Short note 3

Effect of nectar on preference and feeding behaviour of the pollen beetle (*Brassicogethes aeneus*)

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Abstract: Entomophilous plants use floral rewards to attract pollinators and facilitate their reproduction but these rewards can also attract herbivorous insects. Attraction of herbivorous insects by floral nectar has been mainly observed for lepidopterans whose adults are nectarivorous and larvae are phytophagous. Others insects not specialized on nectar consumption such as florivorours insects could also be attracted and benefit from nectar. We worked on the pollen beetle (*Brassicogethes aeneus*), a florivorous insect that feeds on oilseed rape pollen. We tested the hypothesis that nectar production by oilseed rape flowers attracts pollen beetles. We also tested if pollen beetles have different feeding behaviours when nectar is present in flowers. We found that nectar production did not influence insect choice but insects were observed sipping nectar in flowers. They spent significantly more time foraging on nectaries in flowers containing nectar than feeding on anthers when nectar is present. These results are in line with data collected on other systems signalling that this behaviour is maybe more frequent than thought.

Key words: Choice test, Flower, Oilseed rape, Florivory, Pollen

INTRODUCTION

Flowering plants evolved in close relation with insects, especially pollinators which facilitate their reproduction. To attract these insects, plants reward them with nutrient rich food sources such as nectar and pollen (Kevan and Baker, 1983). These rewards allow insects to increase their fecundity and to survive longer (Burkle and Irwin, 2009). Nectar reward is also attractive to some herbivorous insects that reduce plant fitness (Wäckers et al., 2007). This has for example been demonstrated for hawkmoths which feed as adults on nectar produced by host plants and are attracted to host plants bearing flowers with high quantities of nectar. Females preferentially oviposit on these plants, leading to increased leaf damage by moth larvae (Adler et al., 2004; Kessler and Bronsterin, 2012). Most of the examples of herbivorous insects feeding on nectar are from Lepidopterans specialized in nectar consumption but other insects may also benefit from this reward. Florivorous insects that feed on flower parts could also use nectar as they forage in close vicinity to nectaries.

The pollen beetle (*Brassicogethes aeneus*) is a generalist florivorous insect eating pollen from a vast array of plant families as an adult but its larvae are much more specialized as they can develop only on Brassicaceous inflorescences (Ouvrard et al., 2016). Pollen beetle adults are known to visit oilseed rape flowers (*Brassica napus*) and suspicions about potential feeding on oilseed rape nectar has already been reported (Kirk et al., 1995). Whether this insect feeds on nectar and is attracted by plants according to their nectar content is not known. Using a choice test, the present experiment investigates if adult pollen beetles have a preference for flowers according to their nectar content and if nectar presence impacts its feeding behaviour.

MATERIALS AND METHODS

The influence of nectar presence on flower choice of adult pollen beetles was tested in a choice experiment. Insects were collected in an unsprayed oilseed rape field by beating inflorescences. Sampling occurred between May and June 2017 near Le Rheu (Brittany, France). Insects were isolated without food during 48h hours in a Petri dish (3 cm in diameter) lined with moisten filter paper before the experiments. Flowers were collected on oilseed rape plants grown in a climatic chamber as described in *Paper 2*. Insects were placed individually in Petri dishes (8 cm in diameter) with two excised flowers: one intact flower and one flower from which nectar was removed. To remove nectar without damaging flower tissues and appearance, centrifugation as performed in *Paper 2* was not possible. Consequently, nectar was removed using paper towel pressed directly on nectaries. Flower position of the Petri dish was chosen randomly to avoid potential directional biases. Insects were observed during 30 min, which is enough for them to make a clear choice (personal observations). The flower on which they were feeding and the duration of feeding on anthers or nectaries was recorded. Sixty one individuals were tested and 48 insects made a choice during this experiment.

To identify insect preference, the number of individuals making a choice on flowers with or without nectar was used and analysed with an exact binomial test. Comparisons between times spent in different parts of each flower treatment the proportion of time spent in each flower part was analysed with ANOVAs. The choice made by insects, their sex and the interaction between choice and sex were used as explicative variables. All statistical tests were performed using R software (R Core Team, 2016).

RESULTS AND DISCUSSION

Insects spent an average of 25.17 min (SE ± 0.12) in flowers and only four insects were observed moving from one flower to another in the 30 minutes of observation. Giving insects up to one hour of experimental time did not impact their choices (personal observation). Over the 48 insects making a choice in this experiment, 29 chose flowers without nectar but preference was not significant ($P = 0.19$)

(Fig. 1a). In a previous study, Adler and Bronstein (2004) found that flower attractiveness was enhanced when flowers were supplemented with nectar but that nectar removal did not reduce attractiveness. They advocate that nectar removal may have stimulated nectar production as found in others species (Gill and Conway, 1979; Castellanos et al., 2002). However, *Paper 2* showed that oilseed rape flowers whose nectar was removed did not secrete nectar. Damage caused to the flowers during manipulation may have increased attractiveness of flowers to insects.

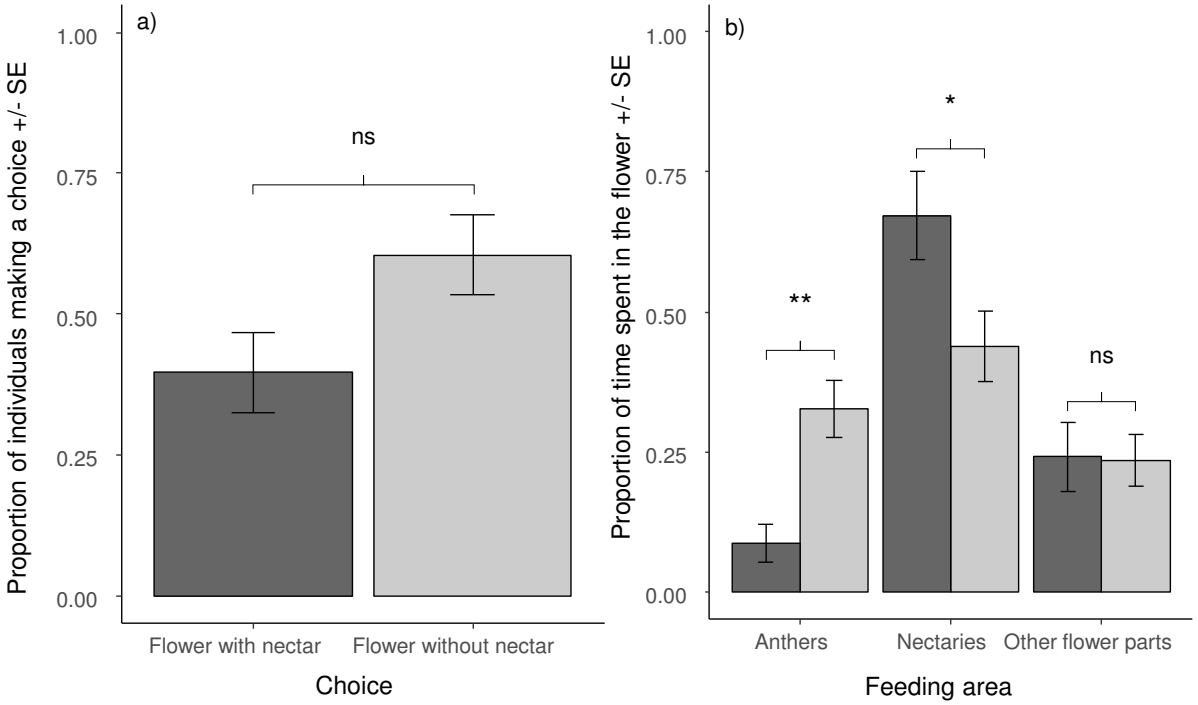

Figure 1: a) Mean proportion of insects (± SE) choosing flowers with or without nectar. b) Mean proportion of time (± SE) spent on different flower parts of flowers with (light grey) or without (dark grey) nectar. Significance of **: $P < 0.01$; *: $P < 0.05$.

Behavioural observations showed that insects did not have the same feeding activity when they fed on flowers where nectar is present of not (Fig. 1b, Table 1) irrespectively of their sex or the interaction between sex and choice (Table 1). Individuals choosing flowers with nectar spent significantly more time in nectaries than those choosing flowers without nectar (Table 1). They also spent less time on anthers but no differences in the proportion of time spent in other plant parts was found (Table 1). These results indicate that adult pollen beetles sip nectar and preferentially feed on nectar than on anthers when nectar is present. Nectar feeding in pollen beetles could be related to nutritional aspects as oilseed rape nectar contains high quantities of fructose and glucose (Bertazzini and Forlani, 2016) which are important for insect survival (Wäckers et al., 2007). It could also be related to digestion of pollen grains. Consumption of nectar by pollinivorous insects has already been observed to facilitate pollen digestion (Roulson and Crane, 2000). Pollen grains are nutritious but they are extremely difficult to digest by insects. One of the most common digestion mechanisms used by pollinivorous animals is to sip nectar from flowers while feeding on pollen (Roulson and Crane, 2000). Pollen grains in sweet and weakly acid mediums germinate and expel their contents in the insect gut (Gilbert, 1972; Haslett, 1983). Pollen beetle is generally considered as pollinivorous but results of the present study and those of *Paper 2* highlight that this insect feeds on more diverse food sources. Pollen beetle adults have already been shown to deter bee pollination (Kirk et al., 1995). Consumption of nectar by pollen beetle could be one of the causes of the reduction in bee visitation. Preferential feeding on nectar over other flower parts has

been observed for sawflies feeding on *Iris bulleyana* and could be a more general phenomenon in florivorous insects (Zhu et al., 2017).

Table 1: Results of ANOVAs on the proportion of time spent in different flower parts.

	Variables	*F*	*df*	*P*
Proportion of time spent in anthers	Choice	11.520	1	**0.002**
	Sex	1.510	1	0.226
	Choice X Sex	0.010	1	0.920
Proportion of time spent in nectaries	Choice	4.768	1	**0.035**
	Sex	0.000	1	0.983
	Choice X Sex	0.484	1	0.420
Proportion of time spent in other flower parts	Choice	0.027	1	0.869
	Sex	1.190	1	0.282
	Choice X Sex	1.047	1	0.312

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Paper 3

In prep. for Oecologia

Effect of availability, quality and accessibility of resources on diet selection by herbivorous insects within a host plant

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Abstract: According to the optimal foraging theory, diet selection by herbivorous insects should result from a trade-off between costs and benefits associated with feeding. Indeed, these insects are expected to optimize their food choice by balancing nutrient intake on the one hand, while minimizing toxicity from specific secondary metabolites in their host plants on the other. Time spent to locate and handle resources can also affect insect behaviour. Plant characteristics such as nutrient content, defence compounds and morphological characteristics vary within-plants and consequently affect insect preferences. The pollen beetle (*Brassicogethes aeneus*) is a florivorous insect that feeds on pollen in oilseed rape inflorescences. This insect has a specific feeding pattern on these inflorescences which is not yet understood. Choice tests were conducted to study preferences between organs. These experiments were paired with analysis of plant secondary compounds, insect energetic metabolites and performance experiments. These experiments showed that nutritional quality of organs is not a determinant factor of pollen beetle within-plant preferences. Accessibility to pollen and, to a lesser extent, availability of resources are important features that determine pollen beetle feeding pattern at the plant scale. Perianth characteristics appear like key mediators of the plant-insect interaction.

Key words: *Brassicogethes aeneus*, *Brassica napus*, Florivory, Optimal foraging, Biochemistry

INTRODUCTION

Optimal foraging theory (OFT) attempts to explain the foraging behaviour of an animal to predict the most efficient strategy that maximizes individual fitness (MacArthur and Pianka, 1966). It has been quickly and successfully adapted to insects like pollinators (e.g. Pyke, 1978) and parasitoids (e.g. Cook and Hubbard, 1977). The theory takes into account the food quality and its availability (Charnov, 1976; Pyke et al., 1977). In case of insect herbivores, the classical prediction is that feeding strategies should be primarily governed by a trade-off between the energetic costs of the feeding behaviour (time needed to locate and handle food) and the rate of energy extraction (Pyke, 1984).

Like any living organism, insects have specific nutrient requirements to develop, grow and reproduce. These broadly correspond to plant primary metabolites: amino acids, carbohydrates, lipids, sterols and vitamins (Behmer, 2009). Some of them must be found in the food source since they are not produced *de novo* by insects. However, plants do not only provide beneficial substances. Indeed, plants allocate part of their energy to secondary metabolites, which have long been recognized to play a defensive role particularly against insect herbivores (Howe and Jander, 2008; Agrawal and Weber, 2015). Plant quality for insects is then the integration of both the benefits provided by nutrients and the negative effect of harmful compounds (Behmer, 2009). A number of studies demonstrated that diet mixing could be the optimal strategy to improve individual fitness (Moreau et al., 2003; Johns and Quiring, 2010), showing that plant chemistry is a strong driver of host plant selection (Waldbauer and Friedman, 1991; Berner et al., 2005). Indeed, insects can adjust to some extent their food intake, tending to approach an optimal diet that is a balance between all nutriments required in specific ratios (Behmer, 2009), but also a compromise between ingesting these nutrients without accumulating an overdose of toxins (Simpson and Raubenheimer, 2001; Behmer et al., 2002).

Nutrients and defensive compounds are known not to be homogeneously distributed in plants. Even for a given organ, its chemical composition can vary depending on its age or according to a spatial pattern. Leaf nutrient composition is known to be dependent on leaf age (Blüthgen and Metzner, 2007), whereas the distribution of defences in the plant is, according to the optimal defence theory, linked with the importance of the organ for plant fitness (McKey, 1974). This fine-scale heterogeneity in plant composition has impacts on herbivores' performance and foraging behaviour (Cates and Rhoades, 1977; Cates, 1980). For example, Müller and Müller (2017) showed that the larval performance of *Phaedon cochleariae* on its host plant depended on the age of the leaf that was fed upon, which was related to nutritional content. Also, Shroff et al. (2008) showed that the feeding pattern of *Helicoverpa armigera* caterpillars on the leaves of *Arabidopsis thaliana* was determined by the spatial distribution of defensive metabolites in leaves.

The pollen beetle, *Brassicogethes aeneus* (formerly *Meligethes aeneus*) Fabricius (Coleoptera: Nitidulidae) is a pollinivorous insect that feeds on floral buds of oilseed rape (*Brassica napus* L., Brassicaceae). This herbivore shows a specific pattern of resource exploitation on oilseed rape inflorescences. Indeed, females preferably lay eggs in middle-size buds (i.e. buds at an intermediate stage of development) (Hervé et al., 2015) while smaller and larger buds (i.e. at an early-development and late- development stage, respectively) are preferably used for nutrition (Hervé, data not published). Moreover, among buds used only for feeding, much more damage is observed on small than on larger ones (Nilsson, 1994; Ferguson et al., 2015). This indicated that there is an intra-plant foraging strategy in this insect but reasons for this strategy are still unknown.

In this study, we tried to explain the feeding pattern of the pollen beetle by addressing the following questions: (i) Is this pattern determined by resource availability? (ii) Is it determined by resource quality? (iii) Is it determined by resource accessibility? We combined whole-plant and small-scale feeding

experiments as well as a performance experiments. These experiments were paired with analyses of energetic metabolites in adults and of secondary metabolites in buds of different sizes and floral organs.

MATERIALS AND METHODS

Plants

For all experiments, winter oilseed rape (cv. Express) was used at the green-yellow bud stage (BBCH 55-57; Lancashire et al., 1991). Plants were grown as described in *Paper 2*. Three classes of bud size were established in the following experiments according to Hervé et al. (2015) who showed that the range 3-5 mm is preferentially used for oviposition: “small” < 3 mm, “medium” 3-5 mm, “large” > 5 mm long.

Insects

Overwintered adult pollen beetles were collected in an unsprayed oilseed rape field near Le Rheu (Brittany, France). Insects were starved prior to all experiments in individual Petri dishes (diameter 35 mm) containing a moistened filter paper. Starvation duration depended on the experiment.

Impact of resource availability on feeding pattern

In order to estimate resource availability for the insect, buds of the main inflorescence of 15 plants were counted, individually and their length measured with a micrometre (0.1 mm precision).

To assess the resource exploitation pattern of the insect, individuals starved for 48 h were individually placed on the main inflorescence of an entire plant as described in Hervé et al. (2014). After a certain feeding period, individuals were removed and sexed following Cook et al. (2006). The total number of buds and the number of damaged buds were counted, and the length of the damaged buds was measured as explained above. Two feeding periods were studied with 10 replicates each: a short term exposition of five hours and a long term exposure of 3 days to test whether feeding pattern is dependent on resources already exploited by insects.

Impact of the type of food and accessibility of resource on insect preferences

Different choice tests were conducted to investigate insect preferences according to food type and accessibility of resource. To test the effect of food accessibility, choice tests were conducted where insects had directly access to the pollen or not. Accessibility was managed by removing perianths or using open flowers. Open flowers allowed to confirm that results obtained were not dependant of bud dissections which could affect organ chemistry.

Before choice tests, insects were starved during 48 h. They were then placed in a Petri dish (diameter 55 mm) with different plant organs for two hours. Six experiments were performed to test preferences for small buds, large buds, anthers of large buds, flowers and anthers of flowers. Insects were given the choice between : Test 1, small and large buds with resource equilibrated between bud size classes according to the number of buds (1 vs. 1); Test 2, small and large buds but resource was equilibrated according to bud weight (8-15 vs. 1); Test 3, small buds and anthers from one large bud with resource equilibrated according to their weight (3-7 vs. 6); Test 4, one large bud and anthers removed from one large bud (1 vs. 6); Test 5, large buds and freshly opened flowers (1 vs. 1); Test 6, anthers of one flower and anthers of one large bud (6 vs. 6).

Whether each group of buds was damaged (binary response) and the sex of the insect was recorded at the end of each experiment. Because pollen clusters were already opened in anthers of flowers, damage were not perceptible for these treatments. Observations were made every 15 min during two hours in

choice tests with flower anthers and insect behaviour (feeding or not) was recorded. Replicates of other treatments in which no damage was observed were discarded and new replicates were performed until reaching 20 replicates with damage.

Impact of nutritional quality on feeding preferences

Quantification of putative plant defence compounds

Buds and different flower parts that can be eaten by pollen beetle were sampled and analysed for their chemical composition. Small buds, large buds, anthers and perianths of large buds and anthers of flowers were collected from the main inflorescence of plants (BBCH 55-57) and directly frozen in dry ice. Anthers and perianths were analysed separately to dissociate their effects. Four small buds, large buds and perianths were sampled on each plant and anthers of four large buds and flowers were collected. Eighteen plants were pooled in one sample to allow quantification of glucosinolates (GSL) and flavonols (FO), two major classes of defensive compounds found in brassicaceous plants. Five samples of each plant part were made. These compounds were analysed as described in *Paper 1*.

Quantification of food-derived energetic metabolites in insects

Nutritional value of plant organs can be assessed through quantification of energetic metabolites in insects. To measure them, insects were starved for four days, then placed in a Petri dish (diameter 55 mm) containing a moistened filter paper and offered a unique but non-limiting pre-weighed food source for 24 h. Four treatments were performed: five small buds, two large buds, two open flowers and a treatment without food served as a negative control. Insects were then frozen in liquid nitrogen six hours after the end of the feeding period to allow metabolization of all of the food ingested. The three main classes of insects' energetic metabolites (triglycerides, carbohydrates, proteins) were then quantified and their energetic equivalents estimated as described in Ruhland et al. (2016). Seven replicates were performed per treatment, each consisting of a pool of three unsexed individuals. An additional treatment was performed with insects frozen immediately after their starvation period (t0).

Influence of the food on insect performance

The setup was identical to the energetic metabolites quantification experiment, but after exposition to a food source, insects were starved and individual survival was checked daily. Individual sex was determined after death. Forty-two replicates were made for each treatment.

Statistical analysis

The software R (R development Core Team, 2016) was used for statistical analyses. When performing multiple comparisons, the Least Squared Means (LSM) method (R package "lsmeans"; Lenth, 2016) was used and P-values systematically adjusted using the FDR correction (Benjamini and Hochberg, 1995).

Cramér-von Mises tests (R package "RVAideMemoire"; Hervé, 2017) were used to compare the distribution, in terms of bud size of (i) available buds, (ii) buds damaged in three days and (iii) buds damaged in five hours. The proportion of each class was used to compare availability with feeding exploitation using χ^2 tests.

The probability of being damaged in choice tests was compared between the two groups of organs using Cochran's Q test.

To test if global composition in secondary metabolites (GSL and FO) of buds and organs was different, a Powered PLS-DA (Liland and Indahl, 2009) (R package "pls"; Mevik et al., 2016) was performed

using centred and scaled values of concentrations. A permutation test based on cross validation was made following pairwise comparisons between organs (R package "RVAideMemoire"; Hervé, 2017) to test if there were differences in the dataset. ANOVAs were also computed for each compound to compare different organs. They were followed by multiple comparisons.

ANOVAs were used to compare the different treatments for concentrations in energetic metabolites (one test per class of metabolites) and energy equivalents (one test per class of metabolites and for total energy measured).

Insect survival was compared between treatments, sexes and their interaction using a Wald test applied on a Generalized Linear Mixed Model (distribution: gamma; link: inverse). The date at which insects were starved was included in the model as random factor.

RESULTS

Impact of resource availability on insect preferences

The distribution of buds damaged over 5 hours and 3 days significantly differed from the distribution of buds available (T = 6.11, P = 0.007 and T = 18.38, P < 0.001, respectively) (Fig. 1a). However, the distribution of buds damaged did not differ between the two time exposures (T = 0.78, P = 0.523). The proportion of small buds was significantly higher in damaged buds than in available buds in both treatments (Fig. 1b). The opposite was true for medium buds whereas no difference was observed for large buds (Fig. 1b).

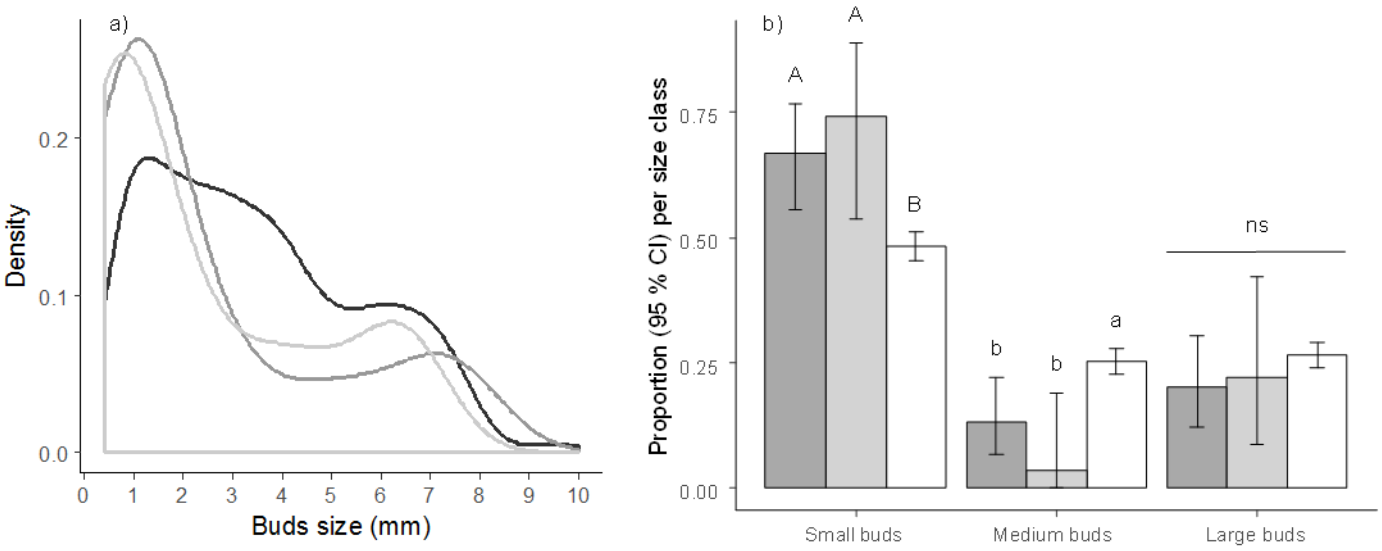

Figure 1: a) Comparison of the distributions of available buds (black line), buds damaged over 5 hours (dark grey line) and buds damaged over 3 days (grey line). b) Proportions (± 95 % CI) of available buds (white), buds damaged over 5 hours (grey) or 3 days (dark grey). Different letters indicate significant differences among small buds (capital letters) or medium buds (lowercase letters).

Impact of the type of food and accessibility of resource on insect preferences

Preferences toward one of the choice given were observed for all dual choices except for Test 2 (Table 2). Insects preferred large buds to small buds when they were in equal numbers but not when they were in equal masses. They preferred buds anthers to small and large buds, and preferred flowers to large buds and flower anthers to bud anthers.

Table 2: Results of choice tests between different plant organs. Significant *P*-values are indicated in bold.

Tests	Choices	Proportion of insects making a choice (± SE)	*Q*	*df*	*P*
Test 1	Large bud (equal number)	0.83 ± 0.06	11.84	1	**< 0.001**
	Small bud (equal number)	0.20 ± 0.06			
Test 2	Large bud (equal masse)	0.55 ± 0.11	0.25	1	0.617
	Small bud (equal masse)	0.65 ± 0.11			
Test 3	Bud anthers	0.96 ± 0.04	12.25	1	**< 0.001**
	Small bud	0.24 ± 0.09			
Test 4	Bud anthers	0.96 ± 0.04	18.00	1	**< 0.001**
	Large bud	0.11 ± 0.06			
Test 5	Flower	1.00 ± 0.00	20.00	1	**< 0.001**
	Large bud	0.00 ± 0.00			
Test 6	Flower anthers	0.74 ± 0.09	5.55	1	**0.018**
	Bud anthers	0.33 ± 0.09			

Impact of nutritional quality on feeding preferences

Quantification of plant secondary metabolites

Five GSL and seven FO were identified in floral organs. Concentration of secondary metabolites (GSL and FO) differed globally among organs (PLS-DA: classification error = 23 %, $P = 0.001$; Appendix 1a and b). All pairwise comparisons between organs were significant (Appendix 1c). Significant differences between organs were also observed for each compound individually except for quercetine-3-O-sophoroside-7-O-glucoside (Appendix 2, Fig. 3). Progoitrin, isorhamnetin-di-glucoside and quercetin-3-O-caffeoyl-sophoroside-7-O-glucoside were more concentrated in perianths of large buds than in other organs (Fig. 3). Concentrations of gluconasturtin, unknown flavonol 1 (Unk. FO1), quercetin-3-O-sophoroside and kaempferol-3-O-sophoroside were higher in bud and flower anthers than in other organs (Fig. 3). However, except Unk. FO1 and Unk. FO2 that are respectively less and more concentrated in small buds than other organs, no major differences between small buds, large buds or their anthers were found.

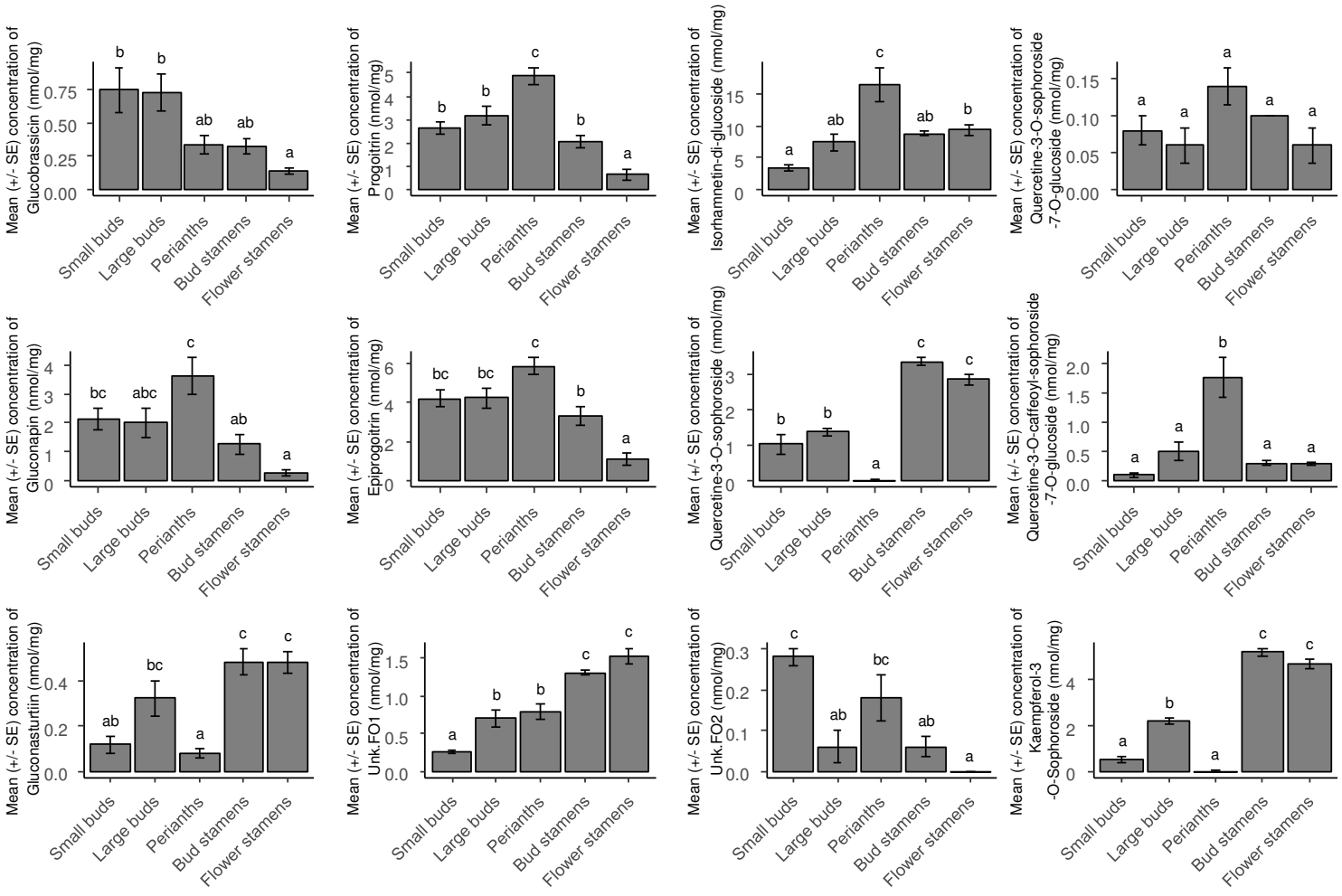

Figure 3: Mean concentrations (± SE) (nmol.mg^{-1}) of secondary metabolites according to floral organs of oilseed rape. Different letters indicate significant differences.

Quantification of food-derived energetic metabolites in insects

The different food sources had a significant influence on the concentrations of proteins, triglycerides and carbohydrates in insects (Table 1). For proteins, only the "t0" and "no food" treatments were different from each other. Triglycerides concentration was the highest for large buds and flowers, the lowest for the "no food" treatment and intermediate for "t0" and small buds. Carbohydrates concentration was particularly high in flower-fed insects but no difference was observed between other treatments.

The different diets provided significantly different total energy to insects ($F = 15.59$, $df = 5$, $P < 0.001$) (Fig. 4, Appendix 3). The most energetic diet was flowers whereas "no food" was the least energetic one. Large buds and anthers provided more energy than "no food" treatment but they were not different from "t0" and "anthers".

Table 1: mean concentrations (µg/mg) ± SE of energetic metabolites (proteins, triglycerides and carbohydrates) found in insects fed, or not with different food sources (before starvation (t0), starved insects (No food), small buds, large buds, bud anthers, flowers) and results of ANOVAs. Different letters indicate significant differences among treatments for each class of metabolite. Significant *P*-values are indicated in bold.

Treatments	**Proteins**		**Triglycerids**		**Carbohydrates**	
t0	354.71 ± 35.93	b	126.45 ± 21.13	abc	84.08 ± 11.36	a
No food	215.56 ± 14.91	a	70.63 ± 4.59	a	86.43 ± 10.75	a
Small buds	274.23 ± 17.75	ab	107.91 ± 11.91	ab	114.39 ± 9.51	a
Anthers	327.35 ± 29.05	b	154.07 ± 5.93	bcd	109.81 ± 17.73	a
Large buds	266.57 ± 16.31	ab	184.96 ± 25.22	cd	149.82 ± 9.17	a
Flowers	280.06 ± 32.24	ab	193.31 ± 8.53	d	377.68 ± 56.12	b
Anova						
F	3.602		9.832		19.479	
df (*df* residual)	5 (34)		5 (34)		5 (34)	
P	**0.01**		**< 0.001**		**< 0.001**	

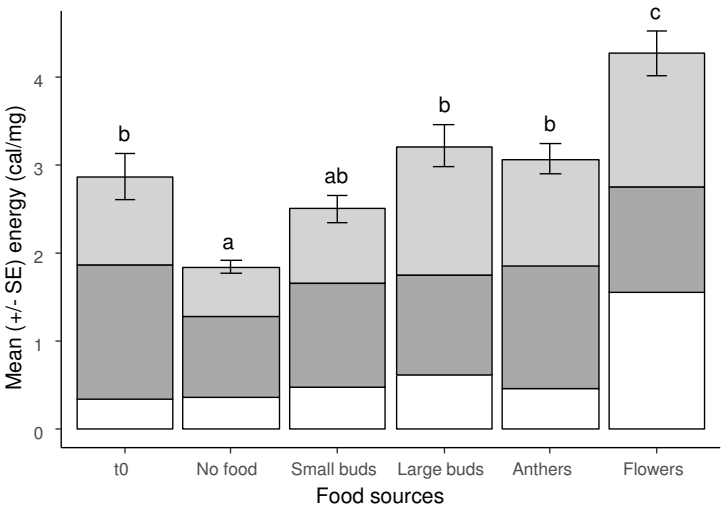

Figure 4: Mean total energy (± SE) per mg of insect according to food sources (before starvation (t0), starved insects (No food), small buds, large buds, bud anthers, flowers). Contributions of each 3 classes of energetic metabolites are represented inside bars (carbohydrate: white, proteins: dark grey, triglycerides: grey). Different letters indicate significant differences among food sources.

Influence of the food on insect performance

Insect diet and sex significantly influenced insect survival time ($\chi^2 = 15.57$, $df = 4$, $P = 0.004$ and $\chi^2 = 5.32$, $df = 1$, $P = 0.019$ respectively), whereas the interaction between sex and food source had no effect ($\chi^2 = 1.81$, $df = 4$, $P = 0.771$). Survival time was longer when insects were given flowers than when they were fed on anthers or small buds (Fig. 5). Survival of insects fed on large buds or without food did not differ from insects given other food sources. Males survived longer than females (mean ± SE days, 6.66 ± 0.75 and 5.41 ± 0.56 respectively).

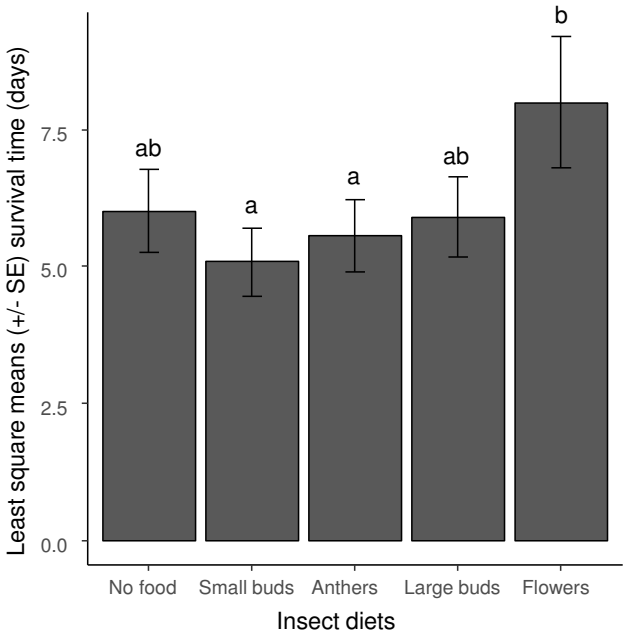

Figure 5: LSM (± SE) of survival time of pollen beetles according to their diet. Different letters indicate significant differences among diets.

DISCUSSION

Our study confirmed a strong resource partitioning of oilseed rape flower buds by the pollen beetle at the scale of the inflorescence. Differences in nutritional quality between buds seems not to explain insect feeding preferences as no differences between insect performances were observed between insects fed with different sizes of buds. Availability of resource and its accessibility seem to be key features. These results are in line with the OFT which assumes that animal preferences are explained not only by nutritional quality but also by costs associated to feeding such as handling time or exposition to toxic compounds (Pyke, 1984).

Our experiments showed that small buds were more used for feeding than medium and large buds which is in accordance with previous observations (Ferguson et al., 2015). Small buds were more abundant on oilseed rape inflorescences and they were over-exploited compared to their availability. On the other hand, medium buds were under-exploited and large buds use was balanced with their availability. Under-exploitation of medium buds could be related to oviposition as these buds are preferentially used for oviposition by the pollen beetle (Hervé et al., 2015). Availability seemed to have an impact on insect feeding preferences but it did not completely explain the pattern observed. Interestingly, the resource partitioning was not affected by the duration of the feeding periods as results obtained with feeding period of five hours or three days on the inflorescence led to the same feeding pattern. This indicates that pollen beetles did not feed first on a specific size of buds and then moved to another class because its nutritional needs or the availability of resource changed.

This resource partitioning does not implicate that pollen beetles have preferences for small buds. If insects had preference toward small buds, they should always be preferred over large ones (Pyke, 1977; Goss-Custard, 1977). However, choice tests confronting small and large buds gave different results. When insects could choose between small and large buds in the “one vs. one” situation, they preferred to feed on large buds. This could be explained by differences in detectability between buds in our choice tests. In a Petri dish, small buds could be less detectable visually because they are smaller and/or they could emit less volatile organic compounds. However, visual and olfactory detectability of buds should

be equivalent in situation where resources were balanced according to their weight. In these tests, no preferences were observed which illustrates that small buds are not systematically preferred over large buds. Increase in feeding preferences for less valuable food sources because of their increased availability has already been demonstrated in other animals (Goss-Custard, 1977; Turner, 1982; Stemberger, 1985). Availability could partly explain the feeding behaviour of the pollen beetle but observations made on complete plants showed that this feature alone is not sufficient to explain the resource partitioning observed on entire plants.

Comparing results obtained in choice tests between small and large buds at equal weight (Test 2) and those with small buds and anthers of large buds (Test 3), indicates that accessibility to pollen is probably an important driver of pollen beetle feeding behaviour. Access to resource (i.e. pollen) is obstructed by the presence of the perianth enclosing pollen. Once removed, pollen beetle clearly preferred to feed on anthers than small buds. Choice tests comparing preferences between large buds and flowers gave the same result (Test 5) with insects preferring to feed on stamens from open flowers than attacking closed buds. Perianth could act as a physical barrier against pollen beetles since adults need to dig though the bud to access the pollen. Moreover, perianth thickness increase with bud size (Hervé, not published) and pollen beetle could spend more time accessing feeding resource in large buds than in small buds. This increase in thickness could protect large buds. Similar results have been reported for the maize weevil (*Sitophilus zeamais*) where plant with thicker seed coats were less damaged (Oloyede-Kamiyo and Adetumbi, 2017). Perianth could also have chemical protection against insects. Three GSL (gluconapin, progoitrin and epiprogoitrin) and two FO (isorhamnetin-di-glucoside and quercetin-3-O-caffeoyl-sophoroside-7- O-glucoside) are more concentrated in the perianth of large buds than in anthers of these buds. These classes of compounds are known to be involved in defence mechanisms against insects (Bones and Rossiter, 1996; Simmonds, 2001). Oilseed rape could protect pollen from insects by increasing constitutive defences in the perianth to avoid bud destruction. This strategy could be more suitable for plants than enhancing defences in the pollen as increase in defence compounds in the pollen could also affect pollinators (Arnold et al., 2014). According to the optimal defence theory, plants should invest more defences in the most valuable organs (McKey, 1974; McCall and Fordyce, 2010). At the scale of an inflorescence, large buds could be more interesting to defend as plants already allocate resources to their growth and to pollen maturation. When comparing the concentration in secondary metabolites from small buds and perianths from large buds, only three compounds were found to be more concentrated in the perianth than in small buds (i.e. progoitrin, isorhamnetin-di-glucoside and quercetin-3-O-caffeoyl-sophoroside-7- O-glucoside). These compounds are among the most concentrated secondary compounds quantified. They could defend large buds and explain why small buds are more used for feeding. Previous experiments conducted in no-choice situation and comparing feeding damage in different genotypes did not find correlations between the concentrations of these compounds on the feeding behaviour of the pollen beetle (Hervé et al., 2014). However, effect of these molecules could act at a smaller scale such as differences between organs in an inflorescence. Glucosinolates are defence compounds produced by brassicaceous plants (Fahey et al., 2001), they negatively act on generalist insects but insects specialised on this plant family are adapted to deal with them (Wittstock et al., 2003). The specificity of the pollen beetle is problematic. This insect is specialised on brassicaceous plants during its development but adults feed on a vast array of plant families (Ouvrard et al., 2016). Transcriptomic analyses performed on pollen beetles revealed differential expression of genes in larvae and adults with genes associated with digestion and detoxification more expressed in larvae than in adults (Vogel et al., 2014). This indicates that adult pollen beetles could be less adapted to deal with defence compounds from brassicaceous plants and partly susceptible to these compounds.

Nutritional quality of resources was measured through analyses of major energetic metabolites in adult pollen beetles (i.e. triglycerides, proteins and carbohydrates) and combined with performance experiments to confirm that differences in energetic rate have an effect on their fitness. No differences in total energetic contents of insects fed on small buds, large buds or anthers were observed. No differences were neither observed for the concentrations of proteins or carbohydrates in insects. However, triglycerides were more concentrated in insects fed on large buds than those fed on small buds. This is not surprising as triglycerides content of pollen increases during bud development (Piffanelli et al., 1997). Triglycerides are used by insects to store lipids, carbohydrates and proteins (Arrese and Soulages, 2010). They increase survival during starvation periods and overwintering and support long flight and thus are important for insect fitness (Arrese and Soulages, 2010; Laparie et al., 2012; Sinclair, 2015). However, in our experiments, this increase in triglycerides did not lead to increased survival. This lack of differences between the different feeding treatments may be related to the method used. Insects were starved during four days but pollen beetles can survive to longer periods of starvation during overwintering (Lehrman et al., 2008). This relatively short period of starvation in favourable experimental conditions (stable humidity and temperature) may have not been strict enough to lead to differences.

Interestingly, flowers used during choice tests were clearly preferred over buds (100 % ± 0 of insects preferred flowers to buds). This is in line with previous observations made at the plant scale where pollen beetles were attracted to flowering plants more than non-flowering ones (Cook et al., 2007; Hervé et al., 2017). Attraction of pollen beetles to yellow colour could explain this preferences (Döring et al., 2012). However, pollen beetle also preferred anthers form flowers to those detached form buds (Test 6) and insects fed on flowers have higher energetic content (especially in carbohydrates) and survive longer than insects fed with other organs. This demonstrates a clear benefit for pollen beetles to feed in flowers. Such benefit may also be related to nectar consumption in flowers (*Short note 3*, Kirk et al., 1995). Flower feeding would clearly fit predictions from the OFT in this insect where it could be the best compromise between energy gained from resource and energy spent to access the resource.

These experiments demonstrated that accessibility to pollen and to a lesser extent availability of resource are important features that determine pollen beetle feeding pattern at the plant scale. Perianth seems to have an important function for oilseed rape but how exactly it constraints pollen beetle behaviour remains to be investigated.

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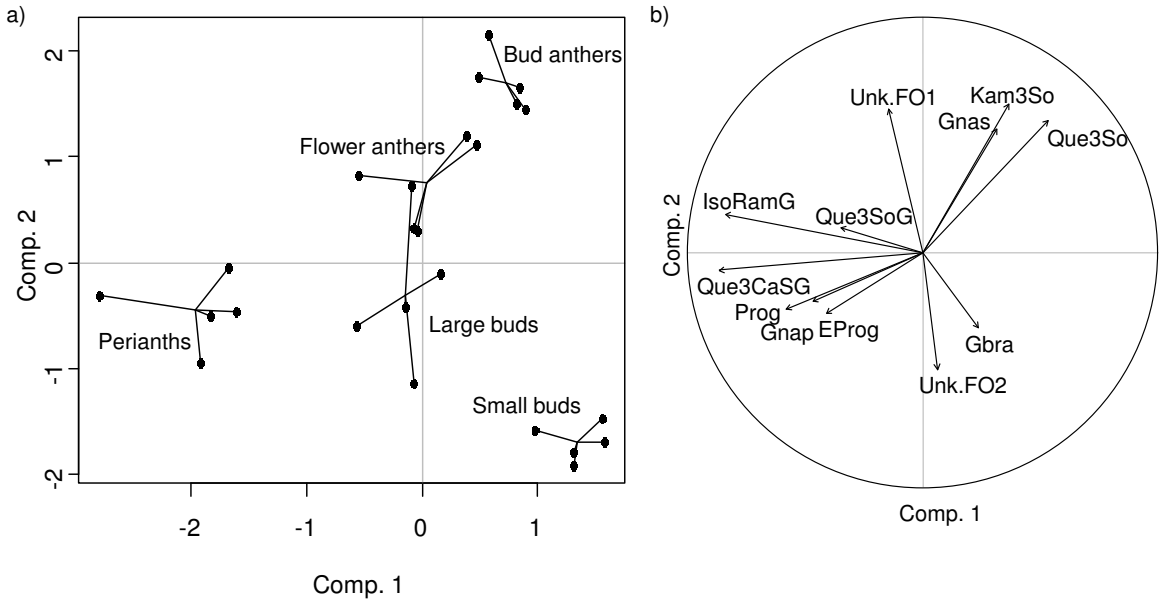

c)

	Flower anthers	Bud anthers	Complete large buds	Perianth of large buds
Bud anthers	0.027	-	-	-
Complete large buds	0.018	0.018	-	-
Perianth of large buds	0.018	0.019	0.019	-
Small buds	0.018	0.018	0.037	0.018

Appendix 1: Illustration of the PLS-DA performed on the chemical composition in secondary metabolites (GSL and FO) of different plant organs (Small buds, large buds, perianths, flowers anthers and bud anthers). a) Score plot (components 1 and 2). b) Loading plots of metabolites. c) P-values associated to pairwise comparisons between floral organs.

Appendix 2: Results of ANOVAs comparing the concentration of secondary metabolites between different organs. Significant *P*-values are indicated in bold.

Compounds	Classes	*df*	*F*	*P*
Isorhamnetin-di-glucoside (IsoRamG)	FO	4	11.50	**0.001**
Kaempferol-3-O-Sophoroside (Kam3So)	FO	4	271.00	**< 0.001**
Quercetin-3-O-caffeoyl-sophoroside-7-O-glucoside (Que3CaSG)	FO	4	15.54	**< 0.001**
Quercetin-3-O-sophoroside (Que3So)	FO	4	76.56	**< 0.001**
Quercetin-3-O-sophoroside-7-O-glucoside (Que3SoG)	FO	4	2.55	0.857
Unk.FO1	FO	4	35.58	**< 0.001**
Unk.FO2	FO	4	10.57	**0.001**
Epiprogoitrin (EProg)	GSL	4	15.81	**< 0.001**
Glucobrassicin (Gbra)	GSL	4	6.46	**0.020**
Gluconapin (Gnap)	GSL	4	8.20	**0.005**
Gluconasturtiin (Gnas)	GSL	4	13.03	**< 0.001**
Progoitrin (Prog)	GSL	4	24.61	**< 0.001**

Appendix 3: Mean energetic content (cal.mg^{-1}) ± SE of energetic metabolites (proteins, triglycerides and carbohydrates) found in insects fed with different treatments (time before starvation (t0), starved insects (No food), small buds, large buds, bud anthers, flowers) and results of ANOVAs. Different letters indicate statistically different treatments. Significant *P*-values are indicated in bold.

Treatments	**Proteines**		**Triglycerids**		**Carbohydrates**	
t0	1.53 ± 0.15	b	1.00 ± 0.17	abc	0.34 ± 0.05	a
No food	0.93 ± 0.06	a	0.56 ± 0.04	a	0.35 ± 0.04	a
Small buds	1.18 ± 0.08	ab	0.85 ± 0.09	ab	0.47 ± 0.04	a
Anthers	1.41 ± 0.12	b	1.22 ± 0.05	bcd	0.45 ± 0.07	a
Large buds	1.15 ± 0.07	ab	1.46 ± 0.20	cd	0.61 ± 0.04	a
Flowers	1.20 ± 0.14	ab	1.53 ± 0.07	d	1.55 ± 0.23	b
Anova						
F	3.602		9.832		19.479	
df (*df* residuals)	5 (34)		5 (34)		5 (34)	
P	**0.01**		**< 0.001**		**< 0.001**	

General discussion

General discussion

The main objectives of this PhD were to develop methods enabling to screen resistance against the pollen beetle in the field and identify biochemical biomarkers of this resistance but also to better understand the interaction between the insect and its host plant to identify potential new target traits for resistance. Here we will try to give our feedback about field screening possibilities in this crop/pest system and discuss how major biases can be managed. We will also discuss the limitations of approaches based on biochemical biomarkers and identify different applied perspectives to this work. To discuss these points, conclusions from data presented in the manuscript will be completed with original data also collected during this PhD.

1. Screening for resistance of oilseed rape against the pollen beetle in the field

During three years of experimentations a total of 12 field trials were conducted in different locations to screen OSR genotypes for resistance against the pollen beetle. These field trials gave us a better understanding of potential biases related to such experimentations and how they can be managed. This knowledge of constraints related to field trials has allowed us to develop an efficient method to screen OSR for resistance against the pollen beetle. How these resistance traits could be used in breeding programs and which further ecological data are needed is discussed.

Adequacy between plant and insect phenology

As explained before (see *Introduction*), variation in plant phenology is a major constraint when screening large numbers of genotypes for resistance to insects. Most plants are susceptible to insect pests at a specific developmental stage but genotypes vary in their development speed. Therefore, the susceptibility period may not occur at the same time for all genotypes tested in a trial. Moreover, date of insect arrival can vary between seasons and consequently insects will affect genotypes at the susceptible stage more than others. Variability of both plant and insect can challenge relevance of results obtained in field trials.

The pollen beetle is assumed to prefer early flowering plants but contrary to expectations, they do not necessarily cause more damage on these plants. Additional results obtained from a field trial conducted in spring 2015 at Le Rheu (Bretagne, France) illustrate this relationship. Podless stalks resulting from bud abortion caused by pollen beetles were counted in this field trial where a set of seven genotypes with diverse flowering periods was sown. Early flowering genotypes had fewer podless stalks and thus were less damaged than late flowering ones (Fig. 1a). This could be explained by early flowering plants being at the green bud stage before or at the beginning of insect infestation. Once these genotypes began to blossom, they attracted pollen beetles but these insects fed on flowers and did not damage buds (Fig. 1b). Late flowering genotypes on the other hand, were at green bud stage when insect infestation was the highest which generated a lot of damage (Fig. 1b).

To manage issues related to variation of plant phenology, genotypes screened can be selected with limited phenological variations (Agrawal and House, 1982; Hardie et al., 1995; Pinto et al., 2010). We used this strategy to study plant resistance present in oilseed rape against pollen beetle (*Paper 1*). This method allowed to make more reliable comparisons between genotypes. However, screenings were limited to late flowering plants because early flowering genotypes can escape from pollen beetle infestation by flowering before insect colonisation (as observed during the spring 2016). This restriction strongly limits the diversity of genotypes that can be screened using this method. Caged plot experiments

could be an interesting alternative as insects can be kept on a specific genotype, but such experiments require large quantities of equipment to screen a large set of genotypes. Moreover microclimate in the cage may not reflect field conditions and lead to other biases.

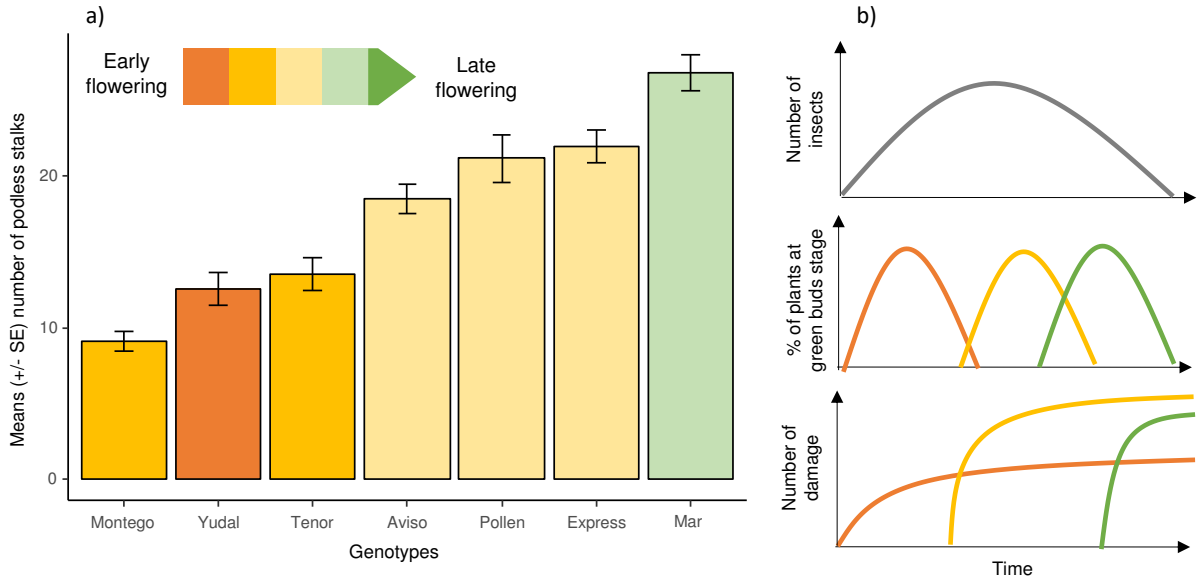

Figure 1: a) Mean (± SE) number of podless stalks per genotype sampled in spring 2015 at Le Rheu. Twenty four plants were sampled in each plot and each genotype was replicated two times. Differences in the number of podless stalks between genotypes was tested using a Wald χ^2 tests on a Linear Mixed Model (LMM) and the genotype had a significant effect ($F = 13.67$, $df = 6$, $P = 0.034$). b) Potential mismatch between insect abundance and percentage of plants at the susceptible stage leading to biased estimations of damage on early (orange), intermediate (yellow) and late (green) flowering phenotypes.

Variability of insect infestation

To develop a breeding program aiming at increasing resistance to insects, a first step is to identify an appropriate damage level allowing to dissociate resistant from susceptible genotypes. In conditions where insect infestations are low, differences between genotypes are difficult to observe and resistance cannot be assessed. On the contrary, if infestation is too high, moderate level of resistance will not be identified (Dahm, 1972; Dhillon and Wehner, 1991). Once such level identified, suitable locations where environmental conditions are favourable to stable pest levels and infestations (i.e. not dependant on the year or the season) need to be found.

By comparing differences in a set of genotypes sampled during different years and locations where pollen beetle infestations were different, it was possible to identify an appropriate damage level allowing to dissociate genotypes that are susceptible or resistant to pollen beetles. The genotype Grizzly, one of the most susceptible genotype was used as reference. Only in our site in Occitanie did Grizzly display a consistent contrast with other genotypes (Fig. 2). The average damage level of this susceptible genotype varied between 30 and 40 buds damaged on this site. This pressure could be an appropriate level to screen resistance to the pollen beetle.

During our experiments, we observed strong variability in infestation levels between years and locations. Uncertainty of pollen beetle infestation is a major issue as differences in susceptibility between genotypes increased with insect pressure (Fig. 2). Damage observed near Mondonville (Occitanie, France) were high on two consecutive years. This location could be considered as a hotspot for future screening (Fig. 2). On the other hand, field trials conducted in Le Rheu or Louville-la-Chenard (Centre-

Val de Loire, France) had low infestation levels in springs 2015 and 2016 and may not be the most appropriate locations to conduct this kind of experiments.

To circumvent problems related to variability in insect infestation levels, no-choice experiments were conducted in the field in spring 2015 and 2016 at Le Rheu and Cornebarrieu (Occitanie, France). Insects were maintained on the inflorescence as in Hervé et al. (2014) except that we did it in the field. We first used self-fertilization bags and then plastic pots to enclose insects but technical difficulties (high moisture inside the bags or pots, insect escape…) challenged this work. Experiments using larger cages filled with wild insects like in Schaefer et al. (2017) could be an interesting alternative to maintain a suitable population of insects on specific plots.

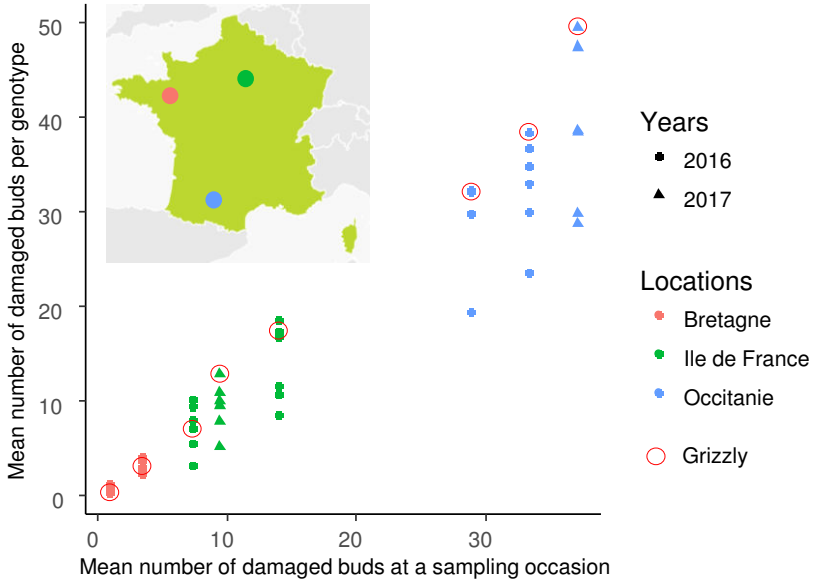

Figure 2: Mean number of damaged buds per genotypes according to mean level of damage per sampling occasion. These results were obtained over 6 identical genotypes sampled during 2 years and on 3 locations. In 2016, 2 samplings were performed on the same trial at 2 different dates. Grizzly is the most susceptible plant and is used as control.

Resistances to pollen beetle in oilseed rape

We observed strong and reliable differences in numbers of feeding attacks among late flowering genotypes. Mechanisms behind this gradient are not known and compounds correlated with resistance or susceptibility (i.e. quinic acid and arginine) seem not to be directly related to plant defence mechanisms. Moderate levels of resistance were observed in some genotypes such as G5 and G10. These genotypes could bring interesting resistances but their ability to maintain yields during important pest attacks needs to be validated before investigating the mechanisms behind these resistances. Furthermore, the screening method used during these experiments and the good correlations between perianth and complete bud chemistry (*Short note 1*) could allow screening more genotypes. These future screenings could permit to identify new and stronger resistances.

No differences in larval infestation or adult beetle emergence was observed between genotypes tested in *Short note 2*. This experiment was conducted on only three genotypes heavily damaged by pigeons and did not reflect the potential variability of the resistance present in oilseed rape. The method presented in this experiment open doors to larger screenings to identify variability in resistances during pollen beetle development. These experiments could also be completed with development experiments in controlled conditions to identify important plant traits that affect larval development. Laboratory experiments showed that pollen is a major source of nutrients for pollen beetle larvae and increases insect fitness (*Paper 2*; Cook et al., 2004). Pollen is a nutrient rich food and its starch content seems to favour pollen beetle development (Hervé et al., 2016). Nectar on the other hand is used by larvae but no effect of its presence on larval development could be observed in our laboratory experiments (*Paper 2*).

However, these experiments were performed under controlled conditions on detached flower and may not reflect real influence of nectar on development of the pollen beetle in field situations. Large differences in nectar production and composition between genotypes have already been observed in the field (Pierre et al., 1999; Bertazzini and Forlani, 2016) and insect development could be monitored in the field on genotypes producing high and low levels of nectar to assess its effect on pollen beetle development. While pollen composition or nectar production could be interesting traits to target to manage pollen beetle damage on OSR, they should be manipulated with extreme caution. Indeed, any modification of these traits could strongly affect pollinator insects and OSR pollination.

If reduction of pollen beetle populations becomes a new target for breeding, data on the ecology of this insect need to be collected. Mortality of the pollen beetle throughout its life cycle should be more accurately estimated. In the field trial conducted in *Short note 2*, a high level of mortality was observed during pupation (about 89 %). This is in line with previous field observations and indicates that most larvae fail to complete their development (e.g. Nielsen and Axelsen, 1988; Büchi, 2002; Riggi et al., 2017). High mortality of adults having completed their development was also observed during overwintering (e.g. 85-98 % in Hokkanen (1993) and 98 % in Lehrman et al. (2008)). If other factors such as climatic conditions or natural enemies are responsible for this important insect mortality, acting at the level of the plant may not be the best option to reduce pollen beetle populations. However, data on pollen beetle mortality are rare. Overwintering mortality for example was only investigated in Sweden and Finland but results obtained do not necessarily reflect results that could be obtained in other countries where climatic conditions could be quite different. Similar experiments should be performed in countries further south to accurately estimate this mortality in less harsh climatic conditions.

Pollen beetles are good dispersers, able to fly up to 10 km (Taimr et al., 1967) but most of the time they seem to disperse only 1-2 km at the end of the overwintering or when moving to overwintering sites (Stechmann and Schütte, 1976; Juhel et al., 2017). To effectively reduce pollen beetle populations, in a metapopulation context, genotypes having a negative effect on insect development need to be implemented at the landscape scale. Insect dispersal in the landscape and long distance dispersal needs to be better understood to identify the scale at which these resistance could have an effect on pollen beetle populations.

2. Linking plant resistance against insects to plant chemistry

Utilisations of biochemical biomarkers of resistances against insects

A large number of studies tried to understand mechanisms behind resistances against insects and identify plant morphological or chemical traits related to these resistances. Stoner reviewed 705 papers dealing with resistances to arthropods in vegetables and found 174 studies investigating such relationships between traits and plant defence (Stoner, 1992). However, even if such relationships have been identified, little is known about their utilisation in commercial breeding programs.

A few of these relationships seem to have been used to some extent in breeding programs and among them some chemical compounds. Leptinine, a glycoalkaloid produced by a wild potato species (*Solanum chacoense*) and giving resistance to the Colorado potato beetle (*Leptinotarsa decemlineata*) has been used as marker during breeding programs (Hutvager et al., 2001). This resistance was introgressed in cultivated potatoes and resistance of progenies resulting from a cross between *Solanum chacoense* and *Solanum tuberosum* was further followed through monitoring plant concentration in leptinine (Sagredo et al., 2009). In tomatoes, high concentrations of zingiberene and acylsugar in trichomes were also used as markers of plant resistance to several insects and mites (*Bemisia* spp., *Tuta absoluta* and *Tetranychus urticae*). These compounds were brought to cultivated lines with crosses between cultivated tomatoes

and wild species (i.e *Solanum habrochaites* var. *hirsutum* for zingiberene and *S. pennelli* for acylsugar) (Baulth et al., 1998; Freitas et al., 2002; de Azevedo et al., 2003; Maluf et al., 2010; Baier et al., 2015; Lima et al., 2015; Lima et al., 2016). One of the most advanced example of such approach is the case of the maysin, a flavone C-glycoside produced by maize silks which is toxic to larvae of the corn earworm (*Helicoverpa zea*) (Elliger et al., 1980). Once its effect on the insect was identified, large screenings of maize collections were made and accessions containing high level of maysin were identified (Widstrom et al., 1983; Snook et al., 1993). The genetic basis of the production and the heritability of the concentration of this compound was also investigated (Byne et al., 1996). Recurrent selection was carried out to multiply by four the maysin content in maize populations a level eight time above the minimum toxicity threshold (Widstrom and Snook, 2001). While promising, these results did not necessarily lead to further commercial developments.

Limits of this approach

Even if breeding approaches based on biochemical biomarkers are tempting, they have several limitations. The maysin example is a good illustration of the difficulties of the understanding of plant defence mechanisms. Indeed, the defensive effect of this compound was demonstrated in the laboratory but field experiments using high-maysin maize populations failed to confirm the effect of maysin concentrations on the corn earworm (Rector et al., 2002). Authors hypothesized that larvae behave differentially in the laboratory and in the field and thus may not be exposed to the toxic effect of maysin present in the silks (Rector et al., 2002). Other factors such as morphology of husk seem to explain most of the difference in damage observed in the field (Rector et al., 2002). Other studies also reported that compounds having an effect on insect in the laboratory explained only a small part of the variation in plant resistances in field situations (Nishizawa et al., 2007; Sagredo et al., 2009). This indicates that resistances against insects often result from a combination of chemical and morphological traits making them inherently complex and difficult to study and implement in resistant varieties.

Experiments conducted in *Paper 1* showed that potential biochemical biomarkers could be highly variable depending on environmental conditions. Most of the variation in perianth chemistry we observed was related to the environment or the interaction between the environment and the genotype. This is in line with other studies reporting great variations in plant chemistry according to the environment (Lee et al., 2003; Davies et al., 2010; Beleggia et al., 2013; Chen et al., 2016; Matros et al., 2017). These variations are not surprising as plant chemistry is known to be influenced by several environmental factors such as light condition, temperature, water regime and attack by other insect pests or pathogens (López-Gresa, 2010; Jänkänpää et al., 2012; Marti et al., 2013; Sprenger et al., 2016; Sun et al., 2016). This variability greatly affects the utilisation of these biomarkers in breeding processes (Guo et al., 2016). Most experiments seeking a relationship between resistance to insects and plant chemistry have been conducted under controlled conditions (Wang et al., 2005; Omoloye et al., 2007; Leiss et al., 2009; Elek et al., 2013; Wang et al., 2017). However, it is essential to account for the environmental variability of the plant chemistry to develop an approach based on reliable biochemical biomarkers of resistances.

Biochemical biomarkers in the OSR-pollen beetle system

In the OSR-pollen beetle system, five compounds present in the perianth have been identified as potential biomarkers of resistance during experiments performed under controlled conditions (Hervé et al., 2014). Relationships between concentrations of these biomarkers and damage levels of genotypes in the field showed that these compounds were not reliable biomarkers under field conditions (*Paper 1*). Low levels of correlations between damage level and metabolites present in the perianth (free amino acids, carbohydrates, polyols, organic acids, glucosinolates and flavonols) were identified in the field

(*Paper 1*). Two compounds (quinic acid and arginine) had significant correlations with damage level but their usefulness as biomarkers need to be confirmed on another set of genotypes. Furthermore, due to lack of previous information, we used genotypes with unknown resistance levels in our trials and most of them turned out to have a low resistance level. To go further and look for additional biomarkers, it could be interesting to use our field screening method to screen for a larger number of genotypes and find highly resistant genotypes (if they exist). If highly contrasting genotypes are found, plant traits explaining this contrast could then be sought and biomarkers identified as was done in previous studies (Uawisetwathana et al., 2015; Wang et al., 2017).

Lack of correlation between perianth chemistry and damage level may indicate that the resistance observed is not related to the perianth or that other classes of compounds and/or morphological traits could be involved in the resistance. Moreover, in *Paper 3*, we found that pollen beetles cause most damage on the plant by feeding on small buds but large buds (>3 mm) were sampled for chemical analyses in our experiments. These buds were chosen because they allowed to collect enough material for chemical analyses. Stronger correlations could be found by comparing small buds chemistry to damage levels. Although tempting, this approach may be quite challenging to achieve in the field due to large quantities of plant material needed (as an example, as many as eighteen plants were needed to complete one sample in *Paper 3*).

3. Perspectives

Exploring beyond Brassica napus

Even if we identified interesting variability in OSR for feeding resistance to pollen beetle, these resistances are partial and may not be strong enough to prevent yield losses in conditions of heavy infestation. Other resistances, present in related plant species could exist. White mustard (*Sinapis alba*) has already been shown to be resistant to pollen beetle feeding (Enzenberg and Ulber 2016; Ekbom and Borg, 1996), to limit oviposition (Ekbom and Borg, 1996, Veroman et al., 2014, Hopkins and Ekbom, 1996) and to reduce egg production (Hopkins and Ekbom, 1996) and larval development (Ekbom, 1998, Kaasik et al., 2014, Veroman et al., 2014). Several field trials with camelina (*Camelina sativa*) also reported that pollen beetle does not attack this plant (Crowley and Frohlich, 1998; Henriksen et al., 2009). These species could be used as new sources of resistance to reduce damage caused by feeding or pollen beetle population by decreasing oviposition and larval development.

In spring 2016, we conducted a preliminary experiment at Le Rheu to screen resistance to pollen beetle over 42 accessions of white mustards and camelina. Two spring OSR genotypes were also placed in this trial as control plants but they were too damaged by insects to be sampled. Some of the accessions of white mustard and camelina were sampled for abundance and we observed that white mustard was more attractive than camelina (Fig. 3a). This is not surprising as camelina is much smaller, produces less numerous and smaller flowers than white mustard and may consequently be less attractive. Estimations of poldless stalks due to abortion of buds caused by pollen beetle attacks gave interesting results as large differences were observed between accessions ($F = 44.78$, $df = 19$, $P < 0.001$). Camelina accessions were less damaged than mustard accessions but variability existed in these species (Fig. 3b). Both plant species were more resistant than OSR which was completely destroyed by insects.

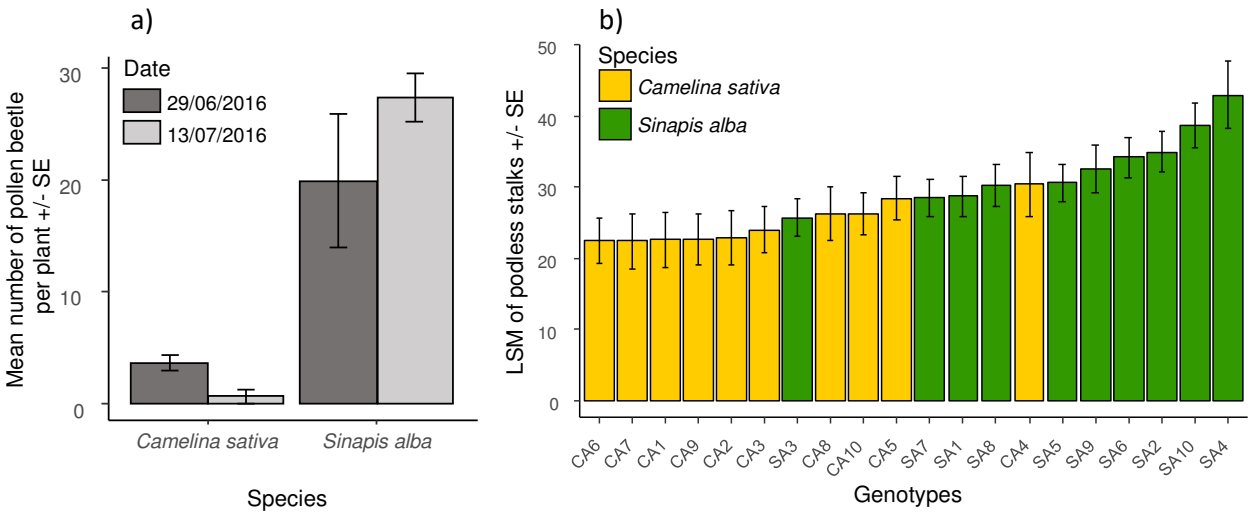

Figure 3. a) Abundance of adult pollen beetles sampled on different accessions of camelina and white mustard. 29/06/2016: 27 plants of white mustards and 9 plants of camelina. 13/07/16: 20 plants of white mustard and 6 plants of camelina. At the second date almost all camelina plants finished their blossom and no pollen beetles were observed on these plants. Differences in the number of insects per plant between the two species for each date were tested using Wilcoxon rank sum tests. 29/06/2016: $W = 6.5$, $P < 0.001$, 13/07/16: $W = 10.5$, $P = 0.003$. b) Least Square Mean (LSM) number of podless stalks (± SE) on the first raceme per accessions. LSM were obtained from a LMM where the number of podless stalks was explained by the total number of stalks and aborted stalks on the inflorescence and the accession, the plot was used as random factor. Between 7 and 32 plants were sampled per accession.

The basis of these resistances is not known and could arise from chemical as well as morphological factors. White mustard chemistry is quite different from that of OSR (Cole, 1976; Tollsten and Bergstrom, 1988). One of the most concentrated glucosinolate in white mustard is sinalbin, a compound mainly found in species of the *Sinapis* genus (Agerbirk et al., 2008). However, this compound did not seem to have an effect on pollen beetle oviposition (Hopkins et al., 1998) nor on other insect species specialised on brassicaceous plants such as flea beetles (*Phyllotreta cruciferae*) or aphids (*Brevicoryne brassicae*) (Bodnaryk, 1997; Hopkins et al., 1998). Camelina on the other hand is more distant from OSR and white mustard and contains specific phytoalexines (Rauhut and Glawishnig, 2009), glucosinolates (Fahey et al., 2001) and even some flavonoids known to have an effect on the flea beetle (*P. cruciferae*) (Onyilagha et al., 2012). Compounds present in these two species and allowing them to resist to pollen beetle attacks are still unknown.

The pollen beetle feeding pattern could give some indications about resistance of white mustard. No-choice experiments were also performed in a greenhouse in spring 2016 where four insects were caged on OSR and white mustard inflorescences of different genotypes. The feeding pattern observed in *Paper 3* was clearly present on OSR but not on white mustard (Fig. 4). Small buds of white mustard were clearly less damaged that those of OSR (Fig. 4). These results are preliminary as few mustard plants were sampled but it seems that differences in resistance level between the two species originate from differences in susceptibility of small buds. Morphological or chemical differences between small buds of these two species that could explain this contrast should to be further investigated.

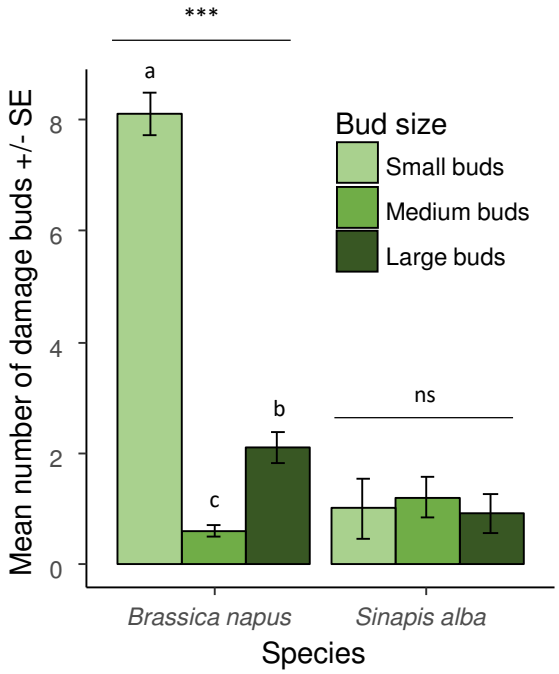

Figure 4. Mean number of damaged buds per size classes (small buds: < 2 mm, medium buds: 2-3 mm, large buds: > 3 mm) according to plant species (*B. napus* and *S. alba*). Four insects were caged on each plant and left three days for feeding. Fifty six and nine plants of OSR and white mustard were sampled respectively. Differences in the number of buds attacked between bud sizes was tested with Wald χ^2 test on LMM. The number of damaged buds was explained by the bud size and plant sampled was accounted as a random factor. Multiple comparisons were performed using LSM. Wald χ^2 for OSR: χ^2 = 389.65, df = 2, P < 0.001. Wald χ^2 for white mustard: χ^2 = 0.31, df = 2, P = 0.857. Different letters indicate significant differences. ***: P < 0.001.

These resistances could be interesting for breeding but introgressing them in OSR is challenging, especially for camelina (Vollmann and Eynck, 2015). Crosses between white mustard and OSR have already been realised to implement for resistance to insects (Dosdall and Kott, 2006; Malchev et al., 2010) in high yield genotypes. Resistance to pollen beetle seems to be heritable but variable. Enzenberg and Ulber (2016) for example used crosses between OSR and white mustard and found variability in the resistance level of progenies to pollen beetle.

White mustard is also resistant to other insect pests of OSR such as *Delia radicum* (Dosdall et al., 1994; Dosdall et al., 2000), *P. crucifera* and *P. striata* (Bodnaryk and Lamb, 1991; Galoski et al., 2000; Soroka et al., 2013) and *Ceutorhynchus obstrictus* (McCaffrey et al., 2004). This kind of broad resistance is rare but some genotypes resistant to several pests in other crops have already been found (Hatchett et al., 1979; Vosman et al., 2018). Such multiple resistances could be highly interesting as they could allow to protect OSR from important damaging pests.

Choosing an adapted strategy

Strong levels of resistance seem to be present in white mustard and could be introgressed in OSR. These resistances could allow to reduce feeding damage but also decrease pollen beetle populations by negatively affecting oviposition and larval development. Introgression of resistances to pollen beetle from white mustard may also be effective against other insect pests. However, a major limitation of such strategy is that introgressing resistance from white mustard to OSR is potentially a long term goal, complex and will probably require numerous backcrosses. Furthermore, strong resistances are prone to apparition of resistant biotypes in insects because they exert an important selection pressure. This is especially true for monogenic resistances that can be easily overrode by insects (Stuart, 2015). For example, resistance against the brown planthopper (*Nilaparvata lugens*) in rice was broken in 2-3 years only for one genotype and 8 years for two others (Heinrichs, 1986). Polygenic resistances are theoretically more difficult to override (Smith and Clement, 2012) but the genetic basis of white mustard resistance remains unknown. Even if this resistance has a polygenic basis, monitoring pest populations for the apparition of resistant biotypes is essential to ensure its durability.

As pointed previously, introgression of resistance from another species has benefits but also important drawbacks. Using moderate levels of resistance to pollen beetle feeding as the ones we observed in OSR could be a more efficient strategy. Theoretically such resistances should be easier to introgress in high yield genotypes than resistance originating from other species. However, moderate levels of resistance to feeding may not be enough to resist to heavy pollen beetle infestations and cannot be used alone. These resistances need to be paired with resistance traits diminishing pollen beetle populations by negatively affecting larval development for example. Pairing traits reducing feeding to traits reducing larval development could allow to lower the frequency of heavy infestations while enabling plants to resist to moderate levels of attack. Moreover, OSR is very tolerant to damage caused by the pollen beetle and can compensate or even over-compensate most of bud abortion by producing new inflorescences and elongating the flowering period (Williams and Free, 1979; Tatchell, 1983; Pinet et al., 2015). Physiological mechanisms behind OSR tolerance capacity and genetic variability of tolerance are not known. Experiments conducted on three genotypes failed to identify differences in tolerance between these genotypes (Pinet et al., 2015) but this trait should be more deeply investigated.

To decide which level of resistance is the most appropriate, we need a precise evaluation of the pollen beetle harmfulness and to forecast how its incidence on OSR yield will evolve in a context of climate change and pesticide reduction. The most recent estimation of pollen beetle damage threshold was made in the United Kingdom and identified a threshold of 20 insects per plant (Ellis and Berry, 2012). Pollen beetle populations exceeding this threshold in the United Kingdom remain rare and most of the time pollen beetles do not cause important yield losses. In this context, moderate level of resistance could be effective to manage the pollen beetle.

However, the frequency of pollen beetle outbreaks could change with the reduction of pesticide applications on the one hand and global warming on the other hand. Indeed, pesticides sprays partly control larval populations of the pollen beetle in OSR (Nilsson, 1994) and reduction of their use could lead to an increase of pollen beetle populations. However, this reduction could also allow the maintenance of more important populations of natural enemies that partly regulate pollen beetle populations. In a context of climatic change an increase of mean temperature during the spring could affect pollen beetle populations. Climatic forecasting predicts that arrival date of pollen beetle on OSR will be earlier in the future (Junk et al., 2015) but how this could affect population sizes remains unknown.

Moderate levels of resistance could thus be adequate to control yield losses related to pollen beetle but how pollen beetle populations will develop in the future and whether moderate resistance will always be effective remains difficult to predict.

Integrating resistance to insects with other management practices in agrosystems

Interest about plant resistance to insects as an alternative to control pest insects has recently grown (Broekgaarden et al., 2011; VanDoom and de Vos, 2013; Stenberg et al., 2015; Tamiru et al., 2015; Fatouros et al., 2016; Mitchell et al., 2016). However, it is important to keep in mind that plant resistances are not a panacea and should be viewed as a strategy complementary to other management practices. Except for some classical examples, resistant cultivars have rarely been used as a primary method of control (Wiseman, 1994; Teetes, 1993). Most of the times, resistances identified were not strong enough to control pest populations but as we saw, moderate levels of resistance can also be useful. To be efficient in controlling pest insects these resistances need to be combined with other management practices (pesticides, biocontrol, preventive measures, etc.). If they are found, strong resistances also need to be completed with other management practices to divert selection pressures and avoid development of resistant populations. Plant resistances consequently need to be replaced in a broader

agrosystem context and should be viewed as a basis to insect pest management. If highly susceptible genotypes are used, efficient control level can only be achieved using high quantities of pesticides (Akbar et al., 2008). On the other hand, if resistant genotypes are used, less pesticides could be necessary and their use could be compatible with more environmentally sound management practices.

The use of resistant genotypes has been combined with other practices to increase efficiency and reduce development of resistant biotypes. For the Hessian fly (*Mayetiola destructor*) in the United States for example, the resistance was combined with delaying wheat plantation until the pest-free date to allow wheat seedlings to partly escape from infestation by this insect (Foster et al., 1991). For the pink bollworm (*Pectinophora gossypiella*) on cotton in the United States, the use of resistant varieties was coupled with destruction of stalks soon after harvest and followed by a ploughing that killed the remnant insects (Adkisson and Gaines, 1960). One of the most integrated management is the one against the sorghum midge (*Stenodiplosis sorghicola*) in Australia. The use of varieties resistant to this insect has been combined with a reduction of pesticide sprays to increase natural enemy populations, an early planting date to avoid high midge populations and the elimination of alternative host plants (Franzmann et al., 2008). These selected examples illustrate four main management practices compatible with utilisation of resistant varieties: the temporal escape, the mechanical destruction of the pest on the field, the increase of biological control and the destruction of pest reservoirs.

Concerning the pollen beetle, the mechanical destruction of the pest in the field and the destruction of alternative host plants do not seem appropriate as insects leave OSR fields before the harvest and overwinter in natural habitats. Temporal escape may be possible through the use of genotypes with a relatively early flowering period that could escape from most damage. This practice was one of the first proposed to avoid pollen beetle damage (Kleine, 1921) but it is not efficient every year and genotypes with very early flowering period could also suffer from damage related to frost. Reduction of pesticide applications could also have beneficial effects on natural enemies such as parasitoids and ground-dwelling predators and reduce pollen beetle populations (Skellern and Cook, 2017). A reduced plant density could also be interesting as it increases the number of inflorescences and flowers produced by OSR making plants more tolerant to pollen beetle damage (Ellis and Berry, 2012).

Conclusion

Plant breeding has long been focused on the increase of yields in optimal cropping conditions where insects were managed with pesticides. Plant resistances against insects often remained in the background and have mainly been developed in agrosystems where insecticides were not profitable (e.g. extensive cultures) or difficult to access (e.g. developing countries). Today, the social and political contexts are evolving and demands to develop a low input agriculture less damageable to human health and the environment are increasing. These conditions revitalise researches on plant resistance against insects.

Creating genotypes resistant to insects remains difficult, especially during the plant screening process. New approaches are thus necessary to overcome this problem. The present work aimed at contributing to this essential step by developing a screening approach based on plant biochemistry to facilitate the identification of resistant genotypes. Although some progress has been achieved, more studies are necessary to have a better understanding of insect ecology and identify robust traits to target.

The relatively recent interest about plant resistance to insects must not neglect previous studies. One of the conclusion that can be retrieved from this knowledge is that plant resistances against insects alone will not be sufficient to manage insect pests. These resistances need to be integrated in the global management of agrosystems and combined with cultural practices. Only such integration will enable resistances to be efficient and durable.

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French summary

French summary / Résumé en français

Le contrôle des insectes ravageurs, de l'écologie à de nouvelles méthodes de lutte

Les insectes phytophages sont responsables de 13 à 16 % des pertes de rendement annuelles des principales cultures dans le monde. A l'heure actuelle, ces insectes sont principalement contrôlés par des pesticides. Ces molécules ont l'avantage d'être faciles à utiliser, d'avoir un prix relativement bas et un effet curatif rapide. Toutefois, les pollutions engendrées par l'utilisation de ces pesticides ont un effet néfaste sur la santé humaine et l'environnement. De plus, l'utilisation systématique d'insecticides a provoqué l'apparition de populations d'insectes pouvant résister à ces molécules, diminuant ainsi leur efficacité. Dans certains pays, notamment au sein de l'Union Européenne, la législation tend à restreindre l'utilisation d'insecticides mais les solutions de substitution restent toutefois limitées. De nouvelles stratégies de contrôle des ravageurs doivent donc être identifiées et mises en place. Ces stratégies doivent prendre en compte les demandes des agriculteurs et celles de la société en étant à la fois faciles d'utilisation, efficaces et relativement économes tout en permettant la production de produits sains sans impact important sur l'environnement. L'étude des interactions entre les insectes ravageurs et leur plantes hôtes et en particulier l'étude des mécanismes de défense pourrait permettre d'élaborer ce type de stratégie.

Les plantes et les insectes phytophages sont en interaction depuis plus de 400 millions d'années et cette interaction prolongée a abouti à une grande diversité d'utilisation des plantes par l'insecte ainsi que des mécanismes de défenses complexes du côté de la plante lui permettant d'éviter ou de supporter les attaques d'insectes. Ces mécanismes de défenses peuvent être dissociés en deux grandes catégories. D'un côté, la *résistance* qui correspond aux traits des plantes qui vont réduire les blessures causées par l'insecte sur la plante. La présence de molécules toxiques ou des protections physiques comme l'augmentation de la dureté des tissus peuvent être considérés comme des mécanismes de résistance. D'un autre côté, la *tolérance* correspond aux traits et aux processus physiologiques qui réduisent la quantité de dégâts résultant de la phytophagie. Il s'agit par exemple du développement de méristèmes secondaires ou l'utilisation d'organes de réserve. Les résistances peuvent être également subdivisées en différentes catégories. On peut dissocier les mécanismes de résistance *constitutifs*, c'est-à-dire produits en permanence par la plante ou *induits*, dans ce cas ils ne sont produits ou augmentés qu'après l'attaque d'un insecte. On peut également séparer les mécanismes *directs*, avec un effet non médié par un autre organisme sur l'insecte et *indirects*, c'est-à-dire dépendants de l'action d'un ennemi naturel comme un parasitoïde ou un prédateur. Ces mécanismes de défense vont avoir un effet important sur l'insecte en affectant sa survie ou ses choix alimentaires et/ou un effet sur la fitness de la plante. Ces connaissances des interactions entre plantes et insectes ont permis de développer des stratégies de lutte contre les

ravageurs. Un bon exemple de ceci est la mise en place de cultures pièges. Dans ces systèmes, une plante attractive pour le ravageur est implantée à proximité des parcelles de culture d'intérêt et va permettre de détourner l'insecte de la culture. Ces méthodes peuvent être efficaces mais elles restent majoritairement utilisées dans des systèmes de culture de taille relativement petite (maraîchage ou arboriculture) et peuvent difficilement être mises en place pour des grandes cultures.

Une autre approche serait d'augmenter les défenses des plantes par la sélection de plantes plus résistantes. Cette stratégie à l'avantage d'être facile à mettre en place (une fois que les plantes résistantes sont disponibles), relativement économe et compatible avec d'autres méthodes de lutte contre les ravageurs. L'idée de sélectionner des génotypes plus résistants aux insectes est ancienne et la commercialisation de variétés résistantes a débutée dans les années quarante aux USA avec une variété de blé résistante à la mouche de Hess (*Mayetiola destructor*). Depuis le milieu du 20ème siècle, de nombreux criblages de variétés ont été effectués pour identifier des variétés résistantes aux insectes et ont abouti à l'identification de nombreux génotypes résistants chez la plupart des cultures d'importance économique. Entre 1975 et 1999, plus de 500 génotypes résistants aux insectes ont ainsi été commercialisés aux USA. L'intérêt pour ces variétés résistantes a toutefois diminué avec l'apparition de cultures OGM tels que les cultures Bt mais les contraintes en termes de réglementation, le cout élevé de développement de ces variétés, l'émergence d'insectes résistants aux cultures Bt et l'inefficacité de ce type de technologie contre certains insectes, (notamment les homoptères) ravivent l'intérêt des chercheurs et des sélectionneurs pour ce type de sélection.

Sélectionner des cultures pour leur résistance envers les insectes reste un processus difficile pour plusieurs raisons. Tout d'abord du fait que ces résistances sont rares. On estime qu'entre moins d'1 % et 10 % des accessions présentes dans la plupart des collections peuvent être considérées comme résistantes. Un grand nombre d'accessions doit donc être criblé pour identifier des résistances intéressantes. De plus, des résistances suffisamment fortes pour limiter les dégâts liés aux insectes peuvent ne pas être connues chez une espèce cultivée. Dans ces cas, il devient intéressant de regarder du côté d'espèces proches ou d'ancêtres non domestiqués pour intégrer leurs résistances dans des variétés à fort rendement mais la plupart des résistances ont une base polygénique et sont donc difficiles à introgresser. Au cours du processus de sélection, il est donc nécessaire de phénotyper un grand nombre de plantes pour leur résistance aux insectes et c'est justement cette phase de phénotypage qui peut être particulièrement contraignante. De plus l'estimation de la résistance peut être très difficile dans la mesure où certains insectes causent des dégâts qui ne sont pas facilement quantifiables ou visibles et que de nombreux facteurs peuvent biaiser les expérimentations.

Le développement récent de technologies comme l'analyse d'image ou la démocratisation d'outils d'analyses biochimiques pourraient permettre d'éviter ces difficultés. En effet, l'analyse automatisée d'images pour quantifier des dégâts ou un nombre d'insecte peut permettre d'augmenter la vitesse et le

nombre de plantes phénotyper. D'un autre côté, l'analyse biochimique des tissus de la plante peut permettre d'identifier des molécules dont la concentration est corrélée au niveau de résistance des génotypes. Ces molécules peuvent être simplement corrélées à l'expression des défenses ou directement impliquées dans les mécanismes de résistance aux insectes. Une fois ces molécules identifiées, il est possible de déterminer le niveau de résistance d'un génotype sur la base de sa composition biochimique et sans passer par une confrontation avec l'insecte. Ce type de phénotypage indirect, basé sur des biomarqueurs biochimiques pourrait ainsi grandement faciliter le travail de phénotypage et permettre de cribler un grand nombre de génotypes.

L'interaction colza - méligèthe

Dans cette thèse, nous nous sommes focalisés sur l'interaction entre le colza *(Brassica napus*) et un de ses ravageurs, le méligèthe (*Brassicogethes aeneus*).

Le colza est une culture oléagineuse issue d'un croisement entre la navette (*Brassica rapa*) et le chou (*B. oleracea*). C'est une culture économiquement importante : elle est la seconde culture oléagineuse dans le monde mais aussi la plus cultivée au sein de l'Union Européenne. Elle est principalement cultivée en Europe, au Canada, en Chine et en Inde. En Europe, la France et l'Allemagne sont les deux principaux pays producteurs de colza avec respectivement 1,5 et 1,28 millions d'hectares cultivés. Différentes cultures de colza existent mais le plus cultivé en Europe est le colza d'hiver qui est semé entre août et septembre et est récolté entre juin et août. C'est une culture longue qui est soumisse à de nombreux insectes ravageurs, principalement au cours de l'automne et du printemps. De nombreuses applications de pesticides sont généralement nécessaires à la conduite de cette culture. Toutefois, l'utilisation d'insecticides chez le colza devient de plus en plus problématique car un nombre grandissant de résistances à ces molécules sont observées chez ses ravageurs.

Le méligèthe est l'un des principaux ravageurs du colza. Au début du printemps, lorsque les températures moyennes excèdent les 10°C, il sort de sa diapause hivernale dans la litière des forêts et migre vers les parcelles de colza. C'est un insecte dont l'adulte se nourrit de pollen issu de plantes très diverses alors que la larve se développe uniquement sur les Brassicaceae. Les adultes s'alimentent et pondent sur les fleurs et les boutons floraux des plantes de colza. La larve du méligèthe se développe dans les boutons et les fleurs ouvertes, en consommant leur pollen. A la fin de son développement, la larve tombe au sol et s'enterre à faible profondeur avant de nymphoser. Un adulte de la seconde génération émerge ensuite des parcelles de colza au début de l'été et s'alimente sur les plantes en fleurs disponibles avant de repartir vers des sites de diapause au cours de l'automne. Les dégâts sont principalement causés par l'adulte. Au moment de l'arrivé de l'insecte sur les parcelles de colza, la plupart des plantes sont encore au stade bouton que l'insecte doit percer pour pouvoir s'alimenter sur les anthères, causant ainsi l'avortement des boutons floraux. Le méligèthe peut donc causer des dégâts importants aux cultures de colza. Il est principalement contrôlé au moyen d'insecticides mais des

résistances, en particulier vis-à-vis des pyréthrinoïdes sont très présentes en Europe. D'autres stratégies de lutte telles que la lutte biologique ou l'utilisation de cultures pièges ont été testées mais elles manquent d'efficacité pour contrôler cet insecte.

Sélectionner des génotypes de colza plus résistants aux méligèthes pourrait être une solution. Cinq cibles prioritaires ont été précédemment identifiés pour favoriser le contrôle de cet insecte : réduire l'attractivité des plantes, la survie des adultes, la ponte, l'alimentation de l'adulte et affecter le développement larvaire. Parmi ces cinq cibles, les deux dernières semblent les plus prometteuses car (i) la majorité des pertes en rendement sont associées à l'alimentation de l'adulte et (ii) affecter le développement des larves peut permettre de réduire les populations de méligèthes et donc indirectement de réduire leurs dommages.

Toutefois, sélectionner des génotypes de colza résistants aux méligèthes est complexe. Cet insecte ne peut être élevé et le criblage des génotypes dépend donc complètement d'insectes collectés en champ, où ils ne sont présents que 4 à 5 mois par an. De plus, il a été démontré que l'utilisation de parties de plantes détachées (comme des boutons floraux) n'est pas représentative des expériences faites sur des plantes entières. Mettre en place des essais au champ est là aussi particulièrement complexe. En plus de certains biais caractéristiques de ce type d'expérimentations, la phénologie des plantes peut grandement affecter l'estimation des résistances. Le méligèthes est attiré par les plantes en fleurs et l'utilisation de variétés avec des périodes de floraison différentes peut rendre les résultats difficilement comparables sur des génotypes qui ne sont pas soumis aux mêmes pressions. Pour contourner ces restrictions, il a été proposé d'utiliser une méthode indirecte de phénotypage de la résistance. Celle-ci est basée sur la compréhension des mécanismes de résistance des plantes au méligèthe et plus particulièrement sur la biochimie des plantes. Une précédente étude menée en conditions contrôlées a ainsi pu montrer sur un groupe restreint de génotypes de colza que les différences de résistances étaient corrélées à la concentration en cinq composés présents dans le périanthe des boutons floraux. Parmi eux figurent deux acides aminés libres (la proline et la sérine) et un glucide (le saccharose) dont la concentration est corrélée négativement au niveau de résistance ainsi que deux flavonoïdes (la quercétine-3-O-sophoroside et le kaempférol-3-O-sophoroside) pour lesquels la concentration est plus forte chez les plantes résistantes. Ces composés pourraient donc être utilisés comme biomarqueurs biochimiques de la résistance du colza aux attaques alimentaires de méligèthes. Cependant, ces résultats ont été obtenus en conditions contrôlées de laboratoire sur un groupe limité de génotypes et doivent être confirmés sur un panel plus large dans des conditions de culture plus réalistes.

Afin de sélectionner des plantes pour leur résistance au méligèthe, il est également important de recueillir davantage d'informations sur le développement des larves, le comportement alimentaire du méligèthe et leurs relations avec la fitness de l'insecte. Ces informations sont essentielles pour comprendre les mécanismes de résistance et identifier de nouvelles cibles pour la sélection.

Les objectifs de cette thèse sont donc 1) de développer une méthode de criblage des génotypes de colza au champ qui permettra de valider ou non les biomarqueurs biochimiques de résistance potentiels et 2) de mieux comprendre le comportement et les besoins alimentaires du méligèthe.

Chapitre 1 : Criblage des variétés de colza pour la résistance au méligèthe

Article 1 : Identification de biomarqueurs biochimiques au champ pour cribler les plantes selon leur résistance aux insectes : application à l'interaction entre le méligèthe et le colza.

Dans cet article, des expérimentations de terrain ont été conduites afin de (i) valider les biomarqueurs biochimiques potentiels reliés à la résistance de certains génotypes de colza aux attaques alimentaires du méligèthe précédemment identifiés, (ii) identifier de nouveaux biomarqueurs et (iii) évaluer leur variabilité environnementale. Pour cela, des essais en champ ont été réalisés durant deux années dans deux régions de France. Vingt génotypes représentant une forte diversité génétique ont été implantés sur des parcelles expérimentales. Afin d'éviter de potentiels biais liés à la phénologie de floraison, ces génotypes ont été sélectionnés en fonction de leur période de floraison. Au cours de la saison 2015-2016, deux groupes de génotypes ont été semés : précoces et tardifs, alors qu'en 2016-2017 seul des génotypes tardifs ont été semés. Le nombre de boutons détruits sur chaque inflorescence a été compté pour estimer le niveau de résistance des plantes et les périanthes des boutons ont été prélevés dans le champ au cours de la seconde année. La composition des périanthes en acides aminés libres, carbohydrates, polyols, acides organiques, glucosinolates et flavonoïdes a été analysée.

Six et 19 génotypes ont pu être échantillonnés respectivement en 2016 et 2017. De fortes différences ont été observées entre les différents génotypes, les génotypes les plus sensibles ayant reçu deux fois plus de dégâts que les génotypes les plus résistants. Les gradients d'attaque observés dans différents lieux et années sont fortement corrélés ce qui montre que les différences observées entre génotypes sont robustes. Des différences de concentration entre génotypes ont aussi été observées pour la plupart des composés biochimiques. Les composés identifiés en conditions contrôlées comme étant corrélés au niveau de résistance n'ont pas été retrouvés significativement corrélés aux attaques observées en champ. Les coefficients de corrélation pour ces cinq composés s'élevent à 0,03 pour la quercétine-3-O-sophoroside, à 0,11 pour le kaempférol-3-O-sophoroside, à 0,10 pour la proline, à 0,30 pour la sérine et à 0,03 pour le saccharose. Deux composés avec une relation significative avec les niveaux d'attaques ont été identifiés : le quinate (–0,51) et l'arginine (0,50). Ces résultats montrent que les cinq composés identifiés en conditions contrôlées comme des biomarqueurs potentiels ne permettent pas de prédire le niveau de résistance des plantes au champ mais que d'autres composés pourraient être intéressants. Il est à noter que la biochimie des génotypes est fortement variable selon les sites échantillonnés. L'environnement explique en moyenne 44 % de la variabilité de la composition biochimique et son interaction avec le génotype explique en moyenne 15 % de cette variabilité. Le génotype quant à lui n'explique que 19 % de la variabilité de la biochimie. Ces résultats sont en accord avec d'autres études

conduites sur d'autres cultures qui ont montré que la biochimie des plantes était fortement variable selon l'environnement. Ces résultats montrent les limites de ce type d'approche basée sur des biomarqueurs biochimiques mais certains génotypes présentant des niveaux de résistance modérés aux attaques alimentaires de méligèthes ont tout de même été identifiés. Ces génotypes pourraient être utilisés en sélection mais leur résistance doit être confirmée sur d'autres essais.

Brève 1 : Explorer la relation entre la chimie du périanthe et celle des boutons floraux pour faciliter l'échantillonnage du matériel végétal

De précédents travaux ainsi que l'*Article 1* ont montré une relation entre la biochimie du périanthe des plantes de colza et le niveau de résistance des génotypes aux attaques de méligèthes. Cependant, l'échantillonnage des périanthes de boutons floraux de colza est un processus long nécessitant un équipement particulier. Les contraintes liées à l'échantillonnage de ces périanthes limitent fortement le nombre de génotypes pouvant être échantillonné au cours d'une saison car les plantes de colza restent au stade de bouton quelques semaines seulement. Pour utiliser la biochimie des boutons floraux comme biomarqueur des résistances du colza aux attaques de méligèthes, un grand nombre de plantes devra être échantillonné. Il est donc essentiel de développer une méthode d'échantillonnage plus rapide et nécessitant moins de main d'œuvre.

Le périanthe représente une part conséquente de la biomasse des boutons floraux (30 à 50 %). La composition chimique des boutons entiers pourrait donc être un bon proxy de la chimie du périanthe. L'échantillonnage des boutons entiers est plus facile et rapide que celle des périanthes qui nécessite la dissection de chaque bouton échantillonné et pourrait permettre de prélever un grand nombre de génotypes.

Afin d'évaluer la relation entre la chimie des boutons entiers et celle de leurs périanthes, des périanthes et des boutons floraux ont été échantillonnés durant plusieurs années sur différents essais en champ. En 2015, des échantillons de périanthes et de boutons ont été prélevés sur les mêmes plantes en prélevant autant de périanthes que de boutons entiers sur ces plantes. Dix génotypes ont été échantillonnés sur un lieu au cours de cette saison. En 2017, des échantillons de périanthes et de boutons entiers ont été prélevés sur différentes plantes provenant d'une même parcelle. Ces prélèvements ont été réalisés sur les essais présentés dans l'*Article 1* et 19 génotypes ont été prélevés. Les corrélations entre la chimie des boutons entiers et des périanthes ont été calculées pour chaque composé. Pour les données de 2015 où les deux types d'échantillons ont été prélevés sur les mêmes plantes il a été possible de comparer leur chimie par paire d'échantillons (*échelle de l'échantillon*) mais aussi en faisant la moyenne des concentrations obtenues par parcelle (*échelle de la parcelle*) ou par génotype (*échelle du génotype*). Pour les données de 2017, seules les moyennes à l'échelle de la parcelle et celle du génotype ont pu être

calculées car les échantillons ont été prélevés sur des plantes distinctes. Ces moyennes ont été mesurées pour les deux sites échantillonnés séparément.

Les moyennes des corrélations pour l'ensemble des composés étaient assez fortes pour l'ensemble des prélèvements, montrant que la chimie du bouton entier pouvait être utilisée comme proxy de la composition du périanthe. C'est à l'échelle du génotype que les corrélations étaient les meilleures. Cependant, les coefficients de corrélation varient d'un composé à l'autre. Les deux flavonoïdes identifiés comme étant de potentiels biomarqueurs en conditions contrôlées avaient des corrélations non significatives. Les trois autres composés identifiés en conditions contrôlées (proline, sérine et saccharose) ainsi que les deux composés identifiés comme potentiels biomarqueurs dans l'*Article 1* (arginine et quinate) avaient des corrélations fortes et significatives. Ces résultats montrent que la chimie des boutons entiers de colza est un assez bon proxy de la composition du périanthe mais cette méthode d'échantillonnage n'est pas utilisable pour estimer la concentration de tous les composés car les corrélations sont assez variables selon les composés.

Brève 2 : Effet du génotype de la plante hôte sur le développement larvaire du méligèthe au champ

Réduire directement le nombre de dégâts alimentaires causés par le méligèthe est la cible principale pour diminuer les pertes de rendement liées à cet insecte, mais affecter le développement larvaire du ravageur serait également utile. Diminuer la survie des larves pour réduire le nombre de ravageurs a été utilisé dans différents systèmes et a permis de contrôler leurs populations. Dans le cas du méligèthe, cette diminution ne va pas avoir un effet direct sur les rendements dans la mesure où les larves ne causent pas l'avortement des fleurs mais ceci pourrait réduire la population de méligèthes et avoir un effet sur les saisons suivantes et les parcelles alentour. De plus, les niveaux de résistances observés chez le colza ne permettent pas de maintenir des rendements en situation de forte infestation, diminuer la taille des populations pourrait donc permettre de gérer cet insecte avec des niveaux de résistance modérés.

Pour cela, un essai en champ a été conduit en 2017. Le nombre de larves ainsi que le nombre d'adultes de la seconde génération émergeant de ces parcelles ont été estimés. Les larves ont été récoltées avant la nymphose à l'aide de pièges posés au sol au moment de leur chute des plantes de colza alors que les adultes ont été récoltés grâce à des tentes à émergence placées sur les parcelles des différents génotypes. La masse sèche des adultes a également été mesurée car elle semble être corrélée à la capacité de ces insectes à survivre à leur diapause hivernale.

Seuls trois génotypes ont pu être échantillonnés au cours de cet essai du fait d'importants dégâts causés par des pigeons. Aucune différence dans le nombre total de larves collectées, le nombre d'adultes collectés ou la masse des adultes selon le génotype n'a été observée. Toutefois, une forte corrélation entre le nombre de larves et le nombre d'adultes récoltés par m² a été observée.

L'expérience conduite sur cette parcelle n'a pas montré de différences entre génotypes mais seuls trois d'entre eux ont pu être échantillonnés. Une plus grande variabilité pourrait exister si davantage de génotypes étaient testés. La forte corrélation entre le nombre de larves et le nombre d'adultes montre que la méthode d'échantillonnage utilisée était correcte et qu'elle pourrait être utilisée sur un panel plus large.

Chapitre 2 : Ecologie alimentaire du méligèthe

Article 2 : Impact des récompenses florales sur les insectes phytophages : importance du pollen et du nectar pour le développement du méligèthe.

Les plantes à fleurs sont fortement dépendantes des insectes pollinisateurs pour leur reproduction. Pour les attirer, elles mettent à disposition des récompenses sous forme d'aliments riches en nutriments comme le pollen et le nectar. Toutefois, des insectes phytophages peuvent également profiter de ces ressources et être attirés par elles.

Le méligèthe se développe dans les boutons et les fleurs de colza, il est connu pour en consommer le pollen. D'après une étude antérieure, le pollen semble avoir un rôle important sur le développement de cet insecte en augmentant la probabilité de survie et le poids des larves tout en diminuant leur temps de développement. De façon plus surprenante, les larves de méligèthes peuvent se développer sans apport de pollen, ce qui montre que ces larves peuvent utiliser d'autres sources de nutriments présentes dans les fleurs. Le nectar, qui est une solution aqueuse riche en sucres produite par les fleurs de colza ouvertes, pourrait être une ressource complémentaire utilisée par l'insecte au cours de son développement.

Dans cet article, les objectifs étaient de savoir d'une part si les larves se nourrissaient de nectar et si les différents stades larvaires étaient plus ou moins dépendants envers cette ressource. D'autre part, l'effet du nectar et du pollen sur le développement des larves ainsi que la fitness des adultes a été étudié.

Pour cela, des observations ont été faites pour savoir sur quels types de ressources les larves s'alimentaient aux deux stades larvaires du méligèthe. L'effet du nectar et du pollen sur le développement des larves a quant à lui été testé en alimentant des larves en conditions contrôlées sur des fleurs dont le nectar et/ou le pollen avaient été retirés. Pour retirer le pollen, les anthères étaient sectionnées alors que le nectar était retiré par centrifugation des fleurs.

Les larves ont bel et bien été observées s'alimentant au niveau des nectaires ainsi que sur les anthères mais aucune différence n'a été observée entre les stades larvaires. Les fleurs privées de pollen ou de nectar n'ont modifié ni la probabilité de survie ni le temps de développement des différents stades larvaires, ni la survie des adultes. Par contre, la masse des larves et des adultes a été fortement affectée

par la présence de pollen (les insectes ayant accès au pollen étaient significativement plus lourds) mais pas par la présence de nectar.

Cette étude a montré que les larves de méligèthes consommaient du nectar mais l'effet de cette ressource sur l'insecte n'a pas été observé. Le pollen a par contre un effet important sur le développement mais il reste moindre que ce qui a déjà pu être observé. Les larves de méligèthes parviennent donc à se développer correctement sans pollen ni nectar, ce qui montre qu'elles sont en mesure de s'alimenter sur des sources de nutriments assez diverses.

Brève 3 : L'effet du nectar sur les préférences et le comportement alimentaire du méligèthe

Des études antérieures ont montré que la présence de nectar dans les fleurs pouvait attirer certains insectes ravageurs mais ceci n'a été démontré que chez des lépidoptères dont l'adulte est nectarivore et la larve est phytophage. Les insectes florivores qui consomment les fleurs pourraient également utiliser cette ressource et donc être davantage attirés par les plantes produisant plus de nectar.

Dans cette étude, l'effet de la présence de nectar dans les fleurs sur les choix alimentaires du méligèthe et son comportement ont été étudiés. Une précédente étude laisse penser que les méligèthes adultes pourraient eux aussi consommer du nectar lorsqu'ils s'alimentent sur les fleurs mais ceci reste à démontrer.

Des tests de choix en conditions contrôlées avec des fleurs contenant ou non du nectar ont été effectués. Les choix des insectes ainsi que le temps passé sur différentes parties de la fleur (anthères, nectaires, autres parties de la fleur) ont également été mesurés.

Aucune préférence entre les fleurs contenant ou non du nectar n'a été observée. Par contre, dans les fleurs contenant du nectar, l'insecte passe significativement plus de temps à s'alimenter sur les nectaires et moins de temps à consommer les anthères que dans les fleurs sans nectar. Ceci montre que les insectes testés ont une préférence pour le nectar lorsqu'ils s'alimentent dans les fleurs mais que ceci n'affecte pas leur choix dans les conditions expérimentales utilisées. Des résultats proches démontrant une préférence pour la consommation du nectar ont pu être obtenus chez d'autres insectes florivores ce qui laisse penser que ce phénomène pourrait être courant chez ce type d'insectes.

Article 3 : Effets de la disponibilité, de la qualité et de l'accessibilité des ressources sur les choix alimentaires d'un insecte phytophage au sein d'une plante hôte.

Les préférences alimentaires des insectes phytophages peuvent être le résusltat de compromis entre les bénéfices (absorption de nutriments) et les coûts (exposition à des toxines, temps de manipulation) associés à la consommation de certaines parties de la plante. Ces coûts et bénéfices peuvent varier entre

plantes mais aussi à plus petite échelle au sein même d'une plante. Il a ainsi été démontré que la concentration en métabolites secondaires toxiques pour l'insecte ou en nutriments peut varier selon le type et l'âge de différents organes d'une même plante. Cette variabilité peut donc être à l'origine de patrons d'alimentation spécifiques sur une plante.

Le méligèthe semble avoir un patron d'alimentation particulier sur les inflorescences de colza. Les boutons de taille moyenne sont principalement utilisés pour la ponte alors que l'alimentation se concentre sur les boutons jeunes de petite taille ou les boutons matures de grande taille. Parmi ces deux dernières classes de boutons, la plupart des dégâts sont observés sur les boutons de petite taille. Ceci est particulièrement contre-intuitif dans la mesure où ces boutons contiennent très peu de pollen et donc moins de ressources à première vue. L'objectif de cet article est de comprendre cette préférence. L'accessibilité, la qualité et la disponibilité des ressources sur l'inflorescence de colza pourraient expliquer ce patron d'alimentation.

Pour réaliser ces objectifs, des méligèthes ont été déposés sur des inflorescences de colza et leur patron d'alimentation relevé au bout une période d'exposition courte (5 heures) ou longue (3 jours). Des tests de choix confrontant l'insecte à des boutons de différentes catégories de tailles présentes en différentes proportions ont aussi été effectués pour déterminer si la disponibilité des ressources affectait les préférences de l'insecte. L'effet de l'accessibilité de la ressource a également été testé en retirant ou non le périanthe protégeant le pollen dans les boutons. La qualité des organes floraux (petits boutons, gros boutons, anthères de gros boutons, périanthes de gros boutons et fleurs) a été évaluée en analysant leur composition en métabolites secondaires (flavonoïdes et glucosinolates) et en quantifiant les métabolites énergétiques (protéines, triglycérides, glucides) chez des insectes alimentés avec différents organes (petits boutons, gros boutons, anthères de gros boutons et fleurs). Ces analyses ont été complétées par des tests de survie effectués avec des insectes nourris au préalable avec ces mêmes organes.

Un patron d'alimentation très clair a ainsi pu être observé au cours de ces expériences avec les boutons de petite taille nettement surexploités par rapport à leur distribution sur la plante, les boutons de taille moyenne étaient quant à eux sous-exploités. La proportion de gros boutons attaqués était conforme à leur disponibilité. La durée d'exposition, longue ou courte ne semble pas affecter la distribution des attaques. Les tests de choix ont montré un fort effet de la disponibilité des ressources sur les attaques de l'insecte. En situation où l'insecte a le choix entre un seul gros bouton et un seul petit bouton, le bouton de grande taille est préféré. Par contre, dans une situation ou l'insecte a le choix entre deux masses égales de ces deux catégories de boutons, aucune préférence n'est observée. Lorsque les anthères sont directement accessibles, une très nette préférence pour ces anthères est observée vis-à-vis des boutons de grande ou petite taille. Douze métabolites secondaires ont été quantifiés dans les organes floraux du colza, des différences de composition entre ces organes ont été observées mais les boutons de petite et de grande taille ne semblent pas présenter de différences majeures. La quantification des métabolites

énergétiques dans les insectes a permis de quantifier les apports nutritionnels des différentes ressources. Des différences entre traitements pour les trois composés quantifiés ont été observées mais les boutons de petite et de grande taille ne diffèrent pas dans leur composition. L'expérience de survie réalisée avec ces mêmes ressources n'a pas permis d'observer de différences entre les traitements à l'exception des insectes nourris sur des fleurs, qui ont survécu plus longtemps.

Ces expériences montrent que le méligèthe a un patron d'attaque prédéfini et stable sur l'inflorescence de colza et s'alimente majoritairement sur les boutons de petite taille. La disponibilité des ressources semble avoir un effet sur ce patron d'attaque dans la mesure où les boutons de petite taille sont plus nombreux sur l'inflorescence et sont préférés pour l'alimentation. De plus, la comparaison des résultats obtenus dans les différents tests de choix montre un effet de cette disponibilité. Cette dernière n'est toutefois pas suffisante pour expliquer le patron d'attaque. L'accès à la ressource semble également joueur un rôle important sur les préférences de l'insecte dans la mesure où l'insecte montre une préférence forte pour les anthères des boutons de grande taille mais que ces préférences disparaissent lorsque le périanthe est présent. L'épaisseur du périanthe semble augmenter avec le développement du bouton et pourrait contraindre l'insecte durant son alimentation sur les boutons de grande taille. De plus, certains métabolites secondaires ont été retrouvés en fortes concentrations dans le périanthe des gros boutons ce qui indique que cet organe pourrait être bien défendu et protéger le contenu du bouton. La qualité nutritionnelle des boutons ne semble pas affecter les choix de l'insecte au vu des faibles différences observées dans les concentrations en métabolites énergétiques et dans les tests de survie entre les boutons de grande et petite taille.

Discussion générale

Cribler des génotypes de colza pour leur résistance au méligèthe en champ

Les expérimentations réalisées durant cette thèse ont mis en évidence une variabilité importante de la résistance du colza aux attaques du méligèthe. Les mécanismes à l'origine de cette variabilité ne sont toutefois pas connus. Parmi les génotypes criblés au champ, certains avaient un niveau de résistance modéré dans différents lieux et pourraient être des sources de résistance intéressantes. L'effet de ces résistances sur les rendements de ces génotypes en situation de forte infestation doit tout de même être confirmé avant leur utilisation dans un programme de sélection. La méthode utilisée pour cribler les génotypes de colza dans cette thèse pourrait être étendue à davantage de génotypes ce qui permettrait d'identifier de nouveaux génotypes résistants. De plus, la composition biochimique des périanthes semble pouvoir être estimée par l'analyse des boutons de colza entiers. Ceci permettra d'échantillonner un grand nombre de génotypes et de mieux identifier des biomarqueurs biochimiques potentiels.

Les expériences conduites dans cette thèse n'ont pas permis d'identifier de génotypes affectant le développement des larves de méligèthes au champ mais elles ont été réalisées sur un groupe très restreint de génotypes et ne sont pas représentatives de toute la diversité présente chez le colza. La méthode utilisée au cours de ces expériences semble être efficace pour estimer le nombre de larves et d'adultes émergeant par parcelle. Des cribles plus larges pourraient donc être réalisés afin d'identifier des plantes ayant un certain niveau de résistance. Toutefois, mieux connaître l'écologie du méligèthe est nécessaire pour évaluer l'efficacité et la faisabilité d'une stratégie de lutte se basant sur une réduction de la population de cet insecte. En effet, la mortalité du méligèthe durant son développement mais également au cours de l'hiver semble être forte. Il faut donc étudier davantage en quoi la plante hôte affecte la fitness des individus au cours de leur vie. Les capacités de dispersion de cet insecte doivent également être prises en compte pour déployer efficacement ce type de stratégie à l'échelle du paysage.

Faire le lien entre la résistance des plantes aux insectes et leur biochimie

Plusieurs études conduites sur des cultures et des insectes différents ont déjà identifié des composés biochimiques dont les concentrations étaient corrélées à l'expression d'une résistance et ont permis de proposer d'utiliser ces molécules comme des biomarqueurs de résistance aux insectes. Toutefois, il reste difficile d'estimer si ce type de biomarqueurs a véritablement pu être utilisé lors de programmes de sélection.

Plusieurs limites contraignent l'utilisation de ces biomarqueurs. Tout d'abord, il est difficile de dissocier l'effet de différentes molécules pour comprendre les mécanismes de défense. Certaines molécules identifiées à plusieurs reprises comme étant à l'origine de résistances dans d'autres systèmes se sont révélées n'expliquer d'une petite partie de la résistance observée au champ. Il est aussi important de noter la forte variabilité environnementale de ces composés qui peut compromettre leur utilisation dans le cadre d'un programme de sélection.

Dans le cas du méligèthe, les composés identifiés en conditions contrôlées ne semblent pas être corrélés au gradient d'attaque observé au champ. De plus, les deux composés corrélés au gradient d'attaque identifiés au champ ne semblent pas être directement impliqués dans la résistance de ces génotypes. De meilleurs résultats pourraient être obtenus en analysant spécifiquement la biochimie des boutons de petite taille qui constituent la majorité des boutons consommés par le méligèthe.

Perspectives

Cette thèse, s'est concentrée sur l'étude de la variabilité aux attaques de méligèthe présente dans le colza mais des résistances peuvent également être présentes chez d'autres espèces proches. D'après la

littérature, la moutarde blanche (*Sinapis alba*) semble être une source importante de résistances au méligèthe. Cette plante est résistante aux attaques alimentaires de cet insecte mais réduit également la ponte et le développement larvaire du méligèthe. La cameline (*Camelina sativa*) pourrait elle aussi être une source de résistances dans la mesure où plusieurs études indiquent que cette plante n'est pas ou peu attaquée par le méligèthe en champ.

Des essais préliminaires en champ ont été conduits au printemps 2016 pour savoir si de la variabilité face aux attaques de méligèthes était présente chez ces deux espèces. Ces résultats préliminaires indiquent que la cameline est moins attaquée que la moutarde blanche. Il semble que cette plante soit moins attractive que la moutarde blanche, ce qui n'est pas surprenant car elle est nettement plus petite et porte moins de fleurs que la moutarde blanche. De la variabilité dans la résistance au méligèthe au sein de ces deux espèces a été observée indiquant qu'elle pourrait être utilisée pour de la sélection.

L'origine de ces résistances n'est pas connu mais une expérience préliminaire réalisée au cours de cette thèse sur la moutarde blanche semble indiquer que la majeure partie des différences d'attaques entre la moutarde blanche et le colza pourrait être liée au fait que le méligèthe ne consomme pas les petits boutons sur les inflorescences de moutarde blanche alors qu'il attaque principalement ces boutons chez le colza. Une composition biochimique ou une morphologie particulière de ces petits boutons pourrait donc être à l'origine de la résistance de la moutarde blanche.

La résistance présente chez *S. alba* pourrait être particulièrement intéressante à introgresser chez le colza puisque la moutarde blanche est résistante à différents ravageurs du colza dont la mouche du chou (*Delia radicum*) et le charançon des siliques du colza (*Ceutorhynchus obstrictus*). Introgresser des résistances depuis la moutarde blanche dans le colza reste un processus long et complexe et il est nécessaire de savoir au préalable si un niveau de résistance aussi important sera nécessaire pour éviter les pertes de rendements liées aux attaques de méligèthes.

Les niveaux de résistance observés dans le colza ne semblent pas suffisants pour résister à de fortes infestations de méligèthes mais elles pourraient être complétées avec des résistances réduisant les populations de méligèthes. Implantée à large échelle, cette seconde résistance permettrait d'éviter les années de forte infestation. De plus, le colza est connu pour avoir une forte tolérance aux attaques de méligèthes mais la variabilité de ce trait au sein du colza n'est pas connue. Il pourrait donc être intéressant d'avoir également des plantes en mesure de tolérer partiellement les attaques de méligèthes.

La diminution des traitements insecticides sur le colza ainsi que les changements climatiques dans les années à venir pourraient fortement affecter les populations de méligèthes. Il reste toutefois difficile de prédire si celles-ci vont augmenter ou diminuer. Au vu des problèmes agronomiques causés actuellement par le méligèthe, un niveau de résistance modéré pourrait être suffisant pour résister à la plupart des attaques. Si les populations de ce ravageur ont tendance à augmenter, il sera alors plus intéressant de choisir une stratégie basée sur des résistances plus fortes.

L'utilisation de génotypes résistants doit également être intégrée à un ensemble de pratiques. En effet, la plupart des résistances aux insectes utilisées par le passé n'ont pu être utilisées seules comme moyen de contrôle des ravageurs. Elles doivent être complétées avec d'autres pratiques pour parvenir à gérer efficacement l'insecte. Ceci est vrai pour les résistances modérées qui ne sont pas suffisantes pour permettre un contrôle total du ravageur mais aussi pour les résistances fortes qui peuvent rapidement aboutir au contournement de la résistance par l'insecte. Pour les résistances fortes, il est donc important de diminuer la pression de sélection exercée par la résistance sur l'insecte en la complétant avec d'autres pratiques.

Dans le cas du méligèthe, différentes pratiques culturales pourraient venir compléter l'utilisation de résistances. Choisir des génotypes ayant une période de sensibilité décalée par rapport au pic d'abondance de l'insecte en semant des génotypes à floraison précoce pourrait permettre d'éviter les principaux dégâts causés par ce ravageur. La réduction de l'utilisation de pesticides pourrait également avoir un effet bénéfique sur les ennemis naturels comme les parasitoïdes et les prédateurs de larves de méligèthes et ainsi permettre de réduire les populations de ravageurs. Enfin, la densité de semis semble aussi fortement jouer sur la tolérance de la plante. Réduire la densité de semis permettrait ainsi d'augmenter la tolérance au méligèthe.

Conclusion

La sélection des cultures s'est longtemps focalisée sur l'augmentation des rendements en conditions de culture optimale où les insectes étaient contrôlés par des pesticides. A l'heure actuelle, la demande sociétale pour réduire l'utilisation de ces produits chimiques augmente et pousse à chercher de nouvelles stratégies pour gérer les insectes, augmentant l'intérêt pour la sélection de plantes résistantes. De nombreux problèmes techniques limitent le développement de ce type d'approche, en particulier durant les phases de phénotypage. Le travail présenté ici s'inscrit dans ce cadre pour identifier de nouvelles méthodes permettant de phénotyper plus facilement un grand nombre de plantes. Il est également important de noter que pour identifier des cibles de résistances ou évaluer la pertinence de certaines stratégies, il est essentiel d'avoir une connaissance approfondie de l'écologie du ravageur.

Scientific communicaions

Scientific communications

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