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## Original Article

# Responsiveness of the broad bean weevil, *Bruchus rufimanus*, to *Vicia faba* genotypes

Denise Dalbosco Dell'Aglio\* & Nadim Tayeh

Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

\*Correspondence: Denise Dalbosco Dell'Aglio, E-mail: denise.ddd3@gmail.com

**Running head:** BRUCHUS RESPONSE TO *VICIA FABAE* GENOTYPES

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## Abstract

The broad bean weevil, *Bruchus rufimanus* Boh. (Coleoptera: Chrysomelidae), also called bruchid beetle, is a major pest of *Vicia faba* L. (Fabaceae) given that larvae develop inside the seeds causing direct quality losses in agricultural products. Recurrent chemical applications are inappropriate because they represent a serious threat to pollinators in general. In addition, biological control approaches that have been attempted so far showed limited effectiveness, which makes studies of methods to control bruchid populations extremely important.

Breeding resistant cultivars is the most adequate approach to achieve efficient levels of pest resistance and promote sustainable agriculture. To explore the mechanisms underlying bruchid resistance, we explored *B. rufimanus* behavior at different life stages and preference in plant feeding, oviposition, and larval survival into adulthood on a range of susceptible and resistant broad bean cultivars (genotypes), both in greenhouse experiments and in the field. Our greenhouse results showed that females, compared to males, prefer flowers from some genotypes over others, although the choices made did not correspond with egg-laying preference. Egg-laying preference was also affected by cultivars. This attraction for egg-laying was associated with the number of seeds per pod produced by the genotypes. In the field flowering date influenced bruchid infestation. Also the survival of larvae into adulthood was greater in certain genotypes over others. The results of our study lay the groundwork for further analyses to finely dissect *V. faba* resistance towards bruchids and pave the way for the development of methods to predict potential resistant genotypes in research and breeding programs.

## Graphical abstract text

*Bruchus rufimanus* (Coleoptera: Chrysomelidae) is a major pest of *Vicia faba* (Fabaceae). Chemical applications are a threat to pollinators and biological control has shown limited effectiveness. Therefore, we studied *B. rufimanus* behavior on a range of susceptible and resistant cultivars of *V. faba*. Results showed that females prefer flowers of some genotypes and also egg-laying preference and larval survival were significantly affected by cultivars. Our study lays the groundwork for further analysis of *V. faba* resistance towards bruchids.

## Introduction

*Vicia faba* L. (Fabaceae), commonly referred to as broad bean or faba bean, is a grain legume crop which provides seeds with high content of proteins, starch, and micronutrients that are widely used as food resource for humans and feed for livestock (Crépon et al., 2010). Faba bean also brings environmental and agricultural benefits due to its ability to improve soil fertility by fixing nitrogen and increasing crop yields when used in crop rotations with cereals (Köpke & Nemecek, 2010). Nevertheless, it is sensitive to various abiotic and biotic stresses such as frost, drought, fungal diseases, and insect pests, which reduce crop productivity (Torres et al., 2006). A common pest worldwide is the broad bean weevil, *Bruchus rufimanus* Boheman (Coleoptera: Chrysomelidae, Bruchinae), also called bruchid beetle, which infests seeds of leguminous plants. The larvae of bruchids develop in forming seeds of faba bean, causing direct quality losses to the seed industry. Therefore, the study of methods to control bruchid populations is of great economic importance (Segers et al., 2021).

The *B. rufimanus* life cycle strongly depends on host plant phenology. Adults hibernate during winter, hiding under the bark of trees or leaf litter before coming out to feed on flowering bean crops (Dupont, 1990; Tran et al., 1993). During spring under natural conditions, the signal to terminate diapause is when the photoperiod reaches 16 h per day and the diurnal temperature reaches on average 16 °C (Tran & Huignard, 1992; Roubinet, 2016; Segers et al., 2022). Males and females colonize faba bean crops and consume their pollen, essential for females to acquire sexual maturation (Tran et al., 1993). After mating, females lay eggs on the surface of developing pods during the flowering period, and are able to deposit up to 100 eggs while they are sexually active. The emerging larvae burrow into the underlying seeds, devouring the seed endosperm. After pupation in the seeds, the young adult beetles emerge through a hole that they make in the seed before or after harvest, thereby completing the life cycle (Hamani & Medjdoub-Bensaad, 2015; Roubinet, 2016; Hamidi et al., 2021; Segers et al., 2021). These holes are the main damage to the crop, as they affect the dry seed weight and germination rate (Titouhi et al., 2015; Ward, 2018). Export quality standards are strict; seed lots with >3% and >10% infested seeds are not acceptable for human consumption and for animal feed, respectively (Institut du Végétal Arvalis, 2014).

Previous efforts on the management of broad bean weevils depended on the use of chemical insecticides. However, many of these have been banned, as they have a negative impact on the environment and pollinators. Biological control methods have been attempted but their application is still limited (Sabbour & E-Abd-El-Aziz, 2007; Titouhi et al., 2017).

Kairomones transmitted from faba bean plants and pheromones from broad bean weevil males have also been studied (Bruce et al., 2011; Johansson, 2022) but totally efficient semiochemical traps have not been reported to date. In this context, breeding resistant cultivars is the most appropriate approach to achieve effective levels of resistance and promote sustainable agriculture.

In faba bean, mechanisms of bruchid resistance have not yet been identified. Although bruchids are known to be attracted by flower chemicals (Bruce et al., 2011), their preferences and the general behavior in response to various plant attractants are poorly understood. From previous studies, some accessions showed more resistance to bruchids than others but the reason for that remains unknown (Carrillo-Perdomo et al., 2019; Segers et al., 2022). A biparental population of *V. faba* resulting from a cross between a partially resistant genotype (Nova Gradiska) and a susceptible winter variety (Hiverna) was developed and contrasted recombinant inbred lines for their resistance or susceptibility to bruchids in the field could be identified (Carrillo-Perdomo et al., 2020).

As a starting point to explore the mechanisms behind the plant resistance against bruchids and to address key gaps in the current knowledge of this study system, we observed *B. rufimanus* behavior at different life stages to identify plant preference in (1) feeding, (2) oviposition, and (3) larval survival into adulthood. In the feeding experiment, the role of volatile stimuli from faba bean flowers in the mechanism of plant choice by *B. rufimanus* was explored in both males and females in a Y-tube apparatus. A multiple-choice oviposition experiment was performed, under glasshouse conditions, to identify plant preference by egg-laying females in order to understand the high infestation observed in the seeds of some genotypes in past research, and consequently egg-to-adult survival was also considered. In parallel, we also performed a field experiment using multiple genotypes to access different phenotypic traits impacting bruchid infestation. Our aim was to understand the mechanisms of resistance and susceptibility in faba bean that would help the future management of bruchids.

## **Material and methods**

### **Insect culture and experimental plants**

*Bruchus rufimanus* adults were collected from infested seeds harvested during the 2020 season in Escacena del Campo, Spain (37°27'17"N, 6°21'46"W). Males and females were stored at 4 °C to keep them in cold diapause for 8 months until the experiment. In order to break the overwinter diapause, insects were left at room temperature for 1–2 h until they were

actively moving and looking for food. Sex determination was based on the presence of a small spine on the tibia of the middle leg in male individuals (Yus-Ramos et al., 2014). Males and females were kept separately in a climate chamber (20 °C, 60% r.h., and L16:D8). Fresh faba bean flowers and water were provided 3× a week for 15 days to allow males and females to sexually mature, as the end of reproductive diapause results from the interaction between a 16-h photoperiod and the ingestion of pollen from the host (Tran & Huignard, 1992). To avoid feeding bias, bruchids were fed with pollen of all genotypes at the same time.

Five faba bean accessions showing different levels of *B. rufimanus* infestation in previous years were selected. The accession ‘Nova Gradiska’, hereafter referred to as ‘159B’, is a landrace originating from Croatia and presenting partial resistance to the attack of bruchids (Carrillo-Perdomo et al., 2019). ‘Hiverna’ is a winter German cultivar susceptible to bruchids. The two recombinant inbred lines, POP1-R1 and POP1-S1, are from the population POP1 derived from the cross between 159B and Hiverna (Carrillo-Perdomo et al., 2020). Those two lines previously showed a contrasted response to bruchid infestation in field conditions: POP1.R1 (resistant) was one of the less attacked, in contrast to POP1.S1 (susceptible) (E Carrillo-Perdomo, unpubl.) The cultivar ‘Acequia’, producing large seeds and demonstrated to have a high infestation rate, was selected as a susceptible accession. Infestation results for 159B, Hiverna, and Acequia were confirmed through a field study conducted in seven localities across Europe by the ERA-NET SusCrop project PROFABA in 2020 (E Carrillo-Perdomo, unpubl.). Plants were grown in the greenhouse under controlled conditions in two separate experiments. The first group of plants was used as pollen supply for adult insects and provided flowers for the flower attractiveness behavioral experiment. The second batch of plants was grown inside a cage (see below). To ensure a sufficient supply of fresh flowers and pods at a required stage, accessions were sown at various dates. Plants were drip irrigated with a nutrient solution (Liquoplant FD34; Plantin, Courthézon, France) until full saturation, and no pesticides were applied.

## **Experiments under controlled conditions**

### *Flower attractiveness assay*

The attractiveness of the faba bean flowers was tested using a Y-tube glass olfactometer (Figure S1A). This apparatus was inspired by previous studies on preference of beetles to odor sources in dual-choice tests (Bartlett et al., 1993; Salisbury et al., 2012). The experiments were divided in one-flower and two-flower choices. In a one-flower choice assay, faba bean flowers were tested vs. clean air. Naïve adults were tested in order to explore whether insects

were willing to move towards the plant and/or had a plant preference before being sexually matured. In a two-flowers choice assay, four faba bean genotypes had their flowers tested against each other (159B, Hiverna, POP1.S1, and POP1.R1) and, because of short flower availability, Acequia was tested only against 159B (see Table S1 for details). Both reproductive states were tested, naïve and mature adults. Naïve adults were tested as soon as they were removed from the cold condition and therefore were still not fed with pollen. Individuals were tested once as naïve and once as mature in random comparisons, resulting in two releases for each comparison (see Table S1 for details).

Three fresh flowers of each faba bean genotype were cut and placed inside the container at the end of each side of the olfactometer (Figure S1). These containers were open at both sides and the openings were protected by a white mesh, preventing bruchids to reach the flowers and ensuring the air flow. The position of all apparatus and genotypes were switched every test to avoid biases due the positional effects. Also, the apparatus was cleaned and rinsed with distilled water and 70% ethanol to avoid chemical trails. For each experiment, two olfactometers were used at the same time, one with males and the other with females. The experiment was performed at room temperature (20–24 °C) and the apparatus was covered with a green mesh to exclude visual stimuli and to avoid potential influence of light. Ten individuals were inserted at the base of the apparatus and left for 1 h to choose one arm of the tube. At the end of the arm, there was a funnel which let the individuals go in, but prevented them from going back (Figure S1B). At the end, we registered the total number of individuals at each arm of the tube (Table S1).

Flower attractiveness was analyzed using Pearson's  $\chi^2$  test by comparing the observed frequencies against 0.5 (expected frequency of random choice, no preference). A generalized linear mixed-effects model (glmer), package *lme4* (Bates et al., 2015), was used to analyze the influence of sex (female/male) and status (naïve/matured) in the choices taken (observed frequencies, binomial test) using 'compared genotype pair' as random factor and adding w (weights), followed by ANOVA Type II  $\chi^2$  test.

#### *Oviposition preference and larval success into adulthood assay*

The plants were grown inside one cage (6 × 4 × 2 m) covered with an insect-proof white mesh. In the same cage, there were three replicates for the five genotypes, each consisting of one 4-l pot with three plants of the same genotype, totaling 15 pots inside the cage. The cage was under optimal conditions for bruchids (24 °C day, 18 °C night, 60-70% r.h., and L16:D8 photoperiod). Inside the cage, flowers were manually fertilized and labelled at the moment of

fertilization to have the age of pods in days. At the start of the experiment, all five genotypes had flowers and pods at early seed filling stage (ca. 10 days since hand pollination). Sexually matured adult bruchids (30 females and 15 males) were released inside the cage to feed, mate, and oviposit freely. Adults were released in three batches of 10 females and five males every week to maintain the population size and to control for mortality. The cage was monitored daily to assess the number of new eggs laid (per pod, per genotype, and per replicate). New eggs are bright yellow when recently laid, whereas older eggs are opaque white. After larvae penetrate inside the pod, empty transparent shells are left together with dark holes (Segers et al., 2021).

At the end of the experiment (after 25 days), no more active bruchids were found in the cage. Labelled pods were manually harvested when they reached maturity (dry pods). Pods were stored at room conditions (25 °C, 60–70% r.h., and natural light cycle) for approximately 3 months to favor a homogeneous development of the larval stage into pupae and adults. Thereafter, pods were carefully opened and healthy and damaged seeds were quantified and weighed. A seed was considered damaged if it showed one or more circular emergence holes caused by an adult bruchid (Carrillo-Perdomo et al., 2019; Segers et al., 2021), otherwise the seed was considered healthy.

Whether pod age (days since hand pollination) influenced the laying of new eggs, a glmer model (package *lme4*) was used with a binary category (pods with or without new eggs) as response factor, pod age as predictor factor, and genotype and replicate as random factors (family binomial). To analyze the difference in eggs laid between genotypes, a linear mixed-effects model (*lmer*, package *lme4*) was performed for each week of the experiment using number of eggs as response factor, genotype as predictor factor, and replicate as random factor, followed by post-hoc Tukey tests.

To check the influence of seeds on egg laying and adult survival, we used *lmer* models (package *lme4*) using replicate as random factor followed by an ANOVA to analyze whether (1) the number of laid eggs was influenced by the genotype or by the number of seeds inside the pod, (2) the number of damaged seeds was influenced by the number of eggs on the pod or by the genotype, and (3) the number of emergence holes depended on the weight (g) of the seed or on the genotype.

### **Field experiment**

Faba bean genotypes showing different levels of bruchid infestation in previous years (Carrillo-Perdomo et al., 2019) were selected essentially from the collection available at the

National Research Institute for Agriculture, Food and Environment (INRAE) at Dijon, France, and from the material composing the diversity panel of the PROFABA project. Fifteen susceptible genotypes and 15 genotypes less damaged by bruchids were selected, including the genotypes evaluated previously in the greenhouse: 159B, Hiverna, and Acequia. We also added the two recombinant inbred lines from the POP1 population, POP1.S1 and POP1.R1. The experiment was conducted at the INRAE experimental farm of Epoisses in Bretenière, France (47°24'10"N, 5°11'40"E). Seeds were sown in two replicates, at 1 month apart (sowing 1: 24 February 2021, sowing 2: 23 March 2021). The field plan was carried out with a randomized design. For each accession, 20 seeds were sown in two consecutive rows, with intra- and inter-row distances of 40 cm and 1 m, respectively. As the experimental farm has a known history of seed weevil infestation, we relied on natural infestation. No fungicides were sprayed on the experimental plot or surrounding fields during the experiments and a single application of an insecticide (Karate K, active substances: pyrimicarb (100 g l<sup>-1</sup>) and lambda-cyhalothrin (5 g l<sup>-1</sup>), at 1 ml l<sup>-1</sup>; Syngenta Agro, Basel, Switzerland) was performed on 12 May 2021 to manage an infestation of pea aphid, *Acyrtosiphon pisum* Harris. In the field, plants were monitored daily for presence of bruchids and phenology traits such as flowering time (days since sowing), flowering duration, and pod setting period. At maturity, plant height was measured and pods were manually harvested (20 July 2021–2 August 2021). Harvested pods were kept at room temperature (25 °C) to favor a homogeneous development of the weevils. One-hundred seeds of each genotype and replicate were randomly selected and weighed for the quantification of healthy seeds, following the same methodology described previously. A seed was considered infested if it showed a circular emergence hole caused by an adult bruchid and/or a hole caused by adults of parasitoid wasps (Braconidae) that develop within the larvae of the seed weevil (Carrillo-Perdomo et al., 2019; Segers et al., 2021).

Finlay-Wilkinson mixed model analysis with the package *statgenGxE* (van Rossum, 2021) was used to calculate the effects of environment (two sowing dates) and genotypes on the percentage of healthy seeds. A general linear model (GLM) followed by an ANOVA was performed to analyze the influence of phenological traits, such as flowering time, plant height, and 100-seed weight, on bruchid infestation levels. All statistical analyses were done using R (R Core Team, 2021) and graphs were made using the package *ggplot2* (Wickham, 2016).

## Results

### Flower attractiveness

Naïve and sexually mature bruchids were evaluated for their preference of faba bean flowers using Y-tube olfactometers in two sets of experiments. First, we tested the attractiveness of faba bean flowers vs. clean air using only unfed naïve adults. Flowers from five genotypes were considered, separately. Only flowers from one genotype (POP1.R1) significantly attracted females ( $\chi^2 = 7.36$ , d.f. = 1,  $P = 0.006$ ; Figure 1A). No particular preference was seen in male bruchids when the flowers were presented (Figure 1B). On the other hand, we found that flowers from Hiverna conferred aversion to naïve adult females ( $\chi^2 = 6.25$ ,  $P = 0.012$ ; Figure 1A) and Acequia to naïve adult males ( $\chi^2 = 5.4$ ,  $P = 0.02$ , both d.f. = 1; Figure 1B). Interestingly, some bruchids did not choose any arm of the Y-tube (159B: 45%, Hiverna: 20%, POP1.R1: 47%, and Acequia: 17%; Table S1), apart from POP1.S1 in which all individuals made a choice.

In the two-flowers choice experiment, naïve and sexually mature individuals were tested. All flowers were attractive to some degree, as most of the individuals ended up in one arm of the Y-tube in all trials (Table S1, Figure 1C,D). Acequia was preferred over 159B by insects of both sexes ( $\chi^2 = 4.5$ , d.f. = 1,  $P = 0.034$ ). For females, 159B was preferred over Hiverna ( $\chi^2 = 10.8$ ,  $P = 0.001$ ) and POP1.R1 ( $\chi^2 = 6.2$ ,  $P = 0.013$ , both d.f. = 1). Also, females preferred POP1.R1 over POP1.S1 ( $\chi^2 = 8.0$ , d.f. = 1,  $P = 0.005$ ). The choices differed between sexes but not between statuses (sex:  $\chi^2 = 12.4$ ,  $P = 0.0004$ ; status:  $\chi^2 = 0.62$ ,  $P = 0.62$ ; sex\*status:  $\chi^2 = 2.8$ ,  $P = 0.09$ , all d.f. = 1), with the females being choosier than the males.

### Oviposition preference

In a monitored bruchid infestation inside the cage, female adults freely laid eggs in the five selected genotypes, to estimate oviposition preference. After 25 days, 250 pods and 1276 eggs were observed. On average, 42.5 eggs were laid per female, assuming all were actively fertile. In this multiple-choice test, bruchids had pods from all genotypes at seed filling stages available to oviposit. The pod stage (days since pollination) had influence on the laying of new eggs ( $z = -7.86$ ,  $P < 0.001$ ), although it varied depending on the genotype (Figure 2A).

In the 1st week of releasing bruchids, females laid more eggs on pods from Acequia (week 1:  $\chi^2 = 22.75$ , d.f. = 4,  $P < 0.001$ ; Figure 2B). At the end of the experiment, POP1.S1 elicited significantly more *B. rufinamus* females to lay eggs than any other genotype inside the greenhouse cage (week 4:  $\chi^2 = 42.93$ , d.f. = 4,  $P < 0.001$ ; Figure 2B). The number of eggs on 159B and Acequia was not different from the number on the other genotypes and the fewest eggs were laid on Hiverna and POP1.R1 (Figure 2B, Table 1).

### **Egg-to-adult survival**

At maturity, and following the monitored bruchid infestation inside a cage in the greenhouse, pods were harvested to assess larval survival rate difference between faba bean genotypes. Some pods collected did not produce seeds, therefore we analyzed 225 pods. A total of 313 larvae successfully finished their development into adults inside the seeds, representing an egg-to-adult survival rate of 25% (Table 1). The genotype with the highest percentage of healthy seeds (98%), and thus exhibiting the lowest egg-to-adult survival rate, was 159B. Acequia was the most damaged with only 16% healthy seeds having also the highest number of emergence holes (Table 1).

We observed more eggs laid on POP1.S1 pods than on any other genotype. The number of laid eggs is likely influenced by the number of seeds inside each pod, and not by the genotype (seeds:  $\chi^2 = 21.8$ , d.f. = 1,  $P < 0.001$ ; genotype:  $\chi^2 = 1.1$ , d.f. = 4,  $P = 0.88$ ). POP1.S1 is the genotype which produced the highest number of seeds and pods in our experiment (Table 1). The number of damaged seeds was influenced by both the number of eggs on the pod and the genotype (eggs:  $\chi^2 = 13.3$ , d.f. = 1; genotype:  $\chi^2 = 99.3$ , d.f. = 4, both  $P < 0.001$ ). The number of eggs had a positive influence on the seed infestation rate in genotypes Acequia, Hiverna, and POP1.S1, but did not influence POP1.R1 and 159B (Figure 3A). The number of emergence holes per seed was higher in Acequia (Table 1). The number of emergence holes could be predicted by the weight of the seed, but not by genotype (weight:  $\chi^2 = 8.7$ , d.f. = 1,  $P = 0.003$ ; genotype:  $\chi^2 = 8.9$ , d.f. = 4,  $P = 0.063$ ; Figure 3B).

### **Phenological factors influencing seed weevil infestation in the field**

Thirty-two faba bean accessions were evaluated for bruchid resistance in the field in Bretenière, France, in the spring of 2021, including the five genotypes studied in the greenhouse experiments. Two sowing dates were considered. Bruchids were observed on the flowers and pods during a period of 23 days (3 June 2021–25 June 2021). We observed strong associations between phenological traits and bruchid infestation: early flowering and short accessions with large seeds exhibited the highest bruchid infestation rates (Table 2). The influence of flowering time is more pronounced when each sowing is considered. As the second sowing had its flowering time later in the year – start of flowering time on 10 May 2021 (sowing 1) and on 24 May 2021 (sowing 2) – seeds harvested from the second sowing date had a lower infestation rate than seeds from the first sowing (68.3% healthy seeds from sowing 1, 82.4% from sowing 2; ANOVA:  $F_{1,63} = 5.14$ ,  $P = 0.027$ ), confirming again that flowering time and bruchid infestation are strongly positively correlated (Figure 4). The

accessions varied in their response to bruchid infestation (Table S3). The results showed a significant genotypic main effect on seed infestation in this field experiment (Table 3). Genotype explained 58.1% of the variance observed and the heritability estimate was high (0.83).

## Discussion

The objective of this study was to answer three main questions: (1) do bruchids have preference for flowers from particular faba bean genotypes? (2) Do female bruchids have preference for laying eggs on pods of particular genotypes? And (3) does egg-to-adult survival rate in *B. rufimanus* differ between faba bean genotypes? A group of five faba bean genotypes, known from previous field experiments to vary in the percentage of seed infestation by bruchids, was considered. These genotypes were phenotypically evaluated both in the field and under greenhouse conditions. The list of genotypes was extended for the field trial. Results clearly showed that females, compared to males, have preferences for some flowers over others although the choices made did not correspond with the laying preference. Females also laid more eggs in some genotypes compared to others. This attraction matched the number of seeds produced by the genotypes. Finally, the survival of larvae into adults was higher in certain genotypes over others. Whereas the same strategies identified under greenhouse conditions might apply in the field and contribute to lower infestation rates in some genotypes, flowering time had an important impact on bruchid infestation rate in the field.

### **Do bruchids have preferences for flowers from specific faba bean genotypes?**

The reproduction of *B. rufimanus* is synchronized with the flowering and fructification cycles in faba bean (Medjdoub-Bensaad et al., 2007; Hamidi et al., 2021). Therefore, the colonization of faba bean cultures strongly depends on plant flower availability, as pollen consumption provides the insect with nutrition and induces females into reproduction (Segers et al., 2021). However, we found inconclusive results testing flower attraction. In fact, we could not observe a strong flower preference for a particular genotype by the males, and the female choice results did not correspond with the oviposition preference. In our experiment, floral odor differences might not have been strong enough or the methodology used was not adequate for individuals to have a clear attraction.

Previous experiments in semiochemical attractants with *B. rufimanus* showed that

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host-plant floral odors were attractive to both sexes (Bruce et al., 2011; Johansson, 2022). Here, naïve and mature females were choosier than males, suggesting that they might have a higher sensibility when searching for plants. Moreover, the reproductive state of the adult bruchid was not a significant factor influencing the choice of the flowers, whereas in an experiment with lily beetles, the beetle state (in diapause vs. pre-diapause) was important in moving towards the host plant (Salisbury et al., 2012). It is also unknown whether host-specific oviposition is driven by larval dietary or female feeding preference (Thompson, 1988) and bruchids are known to use a diversity of pollen in their diet (Hamidi et al., 2021). From our results, flower attractants do not seem to be an important cue for ovipositing females and thus might not explain the difference in bruchid infestation rate between genotypes.

### **Do female bruchids have preference for laying eggs on pods from particular faba bean genotypes?**

Our results showed that some genotypes were significantly more attractive to egg-laying *B. rufimanus* females than others. Pods from all genotypes and at all development stages were available for adults during oviposition in our experimental design but females laid more eggs on POP1.S1 and fewer on Hiverna and POP1.R1. This partly matched the number of seeds per pod but the genotype POP1.S1 produced also more pods per plant in comparison to other genotypes. This indicates that females might be attracted by some chemical cues elicited by seeds or pods on the plant, strongly signaling that the plant is suitable for egg-laying – the number of seeds or pods on the plant could then influence the strength of the signal.

Bruchid oviposition repellence or attraction to pods might be due to mechanical or chemical defense mechanisms. Secondary metabolites such as volatiles or plant defense responses to insect presence could interfere in oviposition. A study demonstrated that volatiles released by *Pisum sativum* L. at different phenological stages elicited behavioral responses from both males and females of the pea weevil, *Bruchus pisorum* (L.), although females could better discriminate volatiles from pods than males (Ceballos et al., 2015). Pod morphological traits such as surface wax, trichomes, and the toughness of the plant tissues may also play a role in the egg-laying female decision. These traits were not explored here; however, weevil resistance mechanisms and oviposition preference related to wax layers and pod thickness have been documented for *B. pisorum* (White & Eigenbrode, 2000; Mendesil et al., 2016), and for the pod borer of *Vigna unguiculata* (L.) Walp (Oghiakhe et al., 1992) although no clear relationships were found between pod structural traits and pest infestation.

## **Does egg-to-adult survival rate in *Bruchus rufimanus* differ between faba bean genotypes?**

We observed that Acequia was the genotype in which the survival of bruchids was the highest, resulting in the lowest percentage of healthy seeds, with only 16%. In contrast, 159B was the most resistant genotype with 98% healthy seeds, and although its pods received a fair amount of eggs, the larvae did not penetrate the pod and/or could not penetrate and/or survive in the seed. Genotype 159B is a consistently resistant accession, as it was also ranked as resistant to bruchid infestation in previous work on bruchid infestation in the field (Carrillo-Perdomo et al., 2019). The genotypes selected for our greenhouse experiments presented similar results in the field (Table S2). The pressure of bruchid infestation was clearly higher in the cage, especially due to optimal growth conditions and the constant release of bruchids, which resulted in high adult survival and high female oviposition.

In our greenhouse experiment, larval survival into adulthood ranged from 1 to 54%, which is not uncommon and matches field results (Seidenglanz & Huňady, 2016; Pölitz & Reike, 2019). A previous field study has shown that larval mortality in the seeds ranged from 64 to 99% and it was the most important factor in reducing bruchid populations, showing that larval development differed greatly between faba bean varieties (Seidenglanz & Huňady, 2016). Several components of seed quality may facilitate or inhibit larval performance. Seed hardness and chemistry – such as toxins, plant defense proteins, feeding deterrents, and low nutrient content – can be effective barriers to the entry and survival of bruchid larvae (Janzen, 1977; Tsialtas et al., 2019; Zhang et al., 2021). We did not test longevity of adults, but it could also be affected by chemical barriers. Further experiments testing larval development on a wider range of seeds are necessary to investigate larval and adult mortality and efficient control strategies.

### **Factors impacting bruchid infestation in the field**

The resistance of genotypes to *B. rufimanus* was evaluated in a field experiment under natural conditions. Despite the particular climatic conditions represented by unusual rain episodes and low temperatures (Figure S2), we found that phenotypic traits significantly affected bruchid infestation. Early flowering accessions provided pods first, and for that reason, the oviposition started earlier and lasted longer on the pods of these genotypes, resulting in a higher rate of bruchid infestation. This outcome has been documented in previous studies in which several faba bean varieties are grown together in the field (Seidenglanz & Huňady,

2016; Carrillo-Perdomo et al., 2019; Hamidi et al., 2021). Our results also showed that shorter plants were more attacked by bruchids, and this could be simply explained by the fact that spring-type accessions flower earlier than winter-type, being shorter.

Our field result showing that seed size (i.e., 100-seed weight) influences bruchid infestation might be discussed in light of our greenhouse results. Acequia, which also produces large seeds, was the most affected genotype and also had more holes per seed, showing that bigger seeds are an advantage for bruchids, offering more food resource for larvae and tolerating more larvae inside the seed than other genotypes.

There was a significant genotypic main effect on seed infestation in the field experiment. This could be partly explained by the influence of flowering time on bruchid damage to seeds, as this phenological trait is under genetic control (Weller & Ortega, 2015). In the faba bean plants selected for this study, there was a mix of spring and winter-type accessions, which are known to have different flowering times (Flores et al., 2012).

The influence of sowing date acted together with flowering time in affecting bruchid infestation in this study. Previous studies already pointed out the importance of timing in controlling bruchids, highlighting that delayed flowering and delayed pod-forming plants induced lower oviposition and, as a consequence, less seed damage (Szafirowska 2012; Bachmann et al., 2020; Hamidi et al., 2021). Strategies to manage bruchid infestation should take into consideration both sowing date and flowering time. Screening new lines and varieties for bruchid resistance in the field should also consider the impact of these parameters and consider dissociating the genetic component of resistance from the escape strategy.

## Conclusions

Numerous studies with bruchid species and legume crops explored key elements about insect pest resistance, such as in *Vigna* sp. (Mishra et al., 2017), in chickpea, *Cicer arietinum* L. (Shaheen et al., 2006), and in pea, *P. sativum* (Morton et al., 2000; Clement et al., 2002). However, there is still much information missing for faba bean and the biology of *B. rufimanus*, and specific studies are rare (Hamidi et al., 2021; Segers et al., 2021). Our study brings new key elements of the behavior of *B. rufimanus* and adds more questions to the field. Moreover, we showed the advantages of characterizing the mechanisms underlying bruchid resistance in faba bean under greenhouse conditions. Experiments in the field are largely influenced by weather conditions and by flowering time for some genotypes, which can both be controlled indoors. Considering our present results, the successful development of *B. rufimanus* differed substantially between the genotypes, and studies could advance into

investigating physical and chemical barriers to larval penetration and survival in the seed, especially focusing on the resistant accession 159B. Future studies could focus on exploring specific characteristics that may be selected genetically. Our methodology and results could be used to predict potential resistant genotypes, boost breeding efforts towards developing resistant varieties, and transfer knowledge to species closely-related to faba bean including pea and lentil (*Lens culinaris* Medik.), which are also highly susceptible to bruchids.

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### References

- Bachmann M, Kuhnitzsch C, Martens SD, Steinhöfel O & Zeyner A (2020) Control of bean seed beetle reproduction through cultivar selection and harvesting time. *Agriculture, Ecosystems and Environment* 300: 107005.
- Bartlet E, Blight MM, Hick AJ & Williams IH (1993) The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape (*Brassica napus*) and to some volatile isothiocyanates. *Entomologia Experimentalis et Applicata* 68: 295–302.
- Bates D, Mächler M, Bolker B & Walker S (2015) Fitting linear mixed-effects models Using lme4. *Journal of Statistical Software* 67: 1–48.
- Bruce TJ, Martin JL, Smart LE & Pickett JA (2011) Development of semiochemical attractants for monitoring bean seed beetle, *Bruchus rufimanus*. *Pest Management Science* 67: 1303–1308.
- Carrillo-Perdomo E, Raffiot B, Ollivier D, Deulvot C, Magnin-Robert JB et al. (2019) Identification of novel sources of resistance to seed weevils (*Bruchus* spp.) in a faba bean germplasm collection. *Frontiers in Plant Science* 9: 1914.
- Carrillo-Perdomo E, Vidal A, Kreplak J, Duborjal H, Leveugle M et al. (2020) Development of new genetic resources for faba bean (*Vicia faba* L.) breeding through the discovery of

gene-based SNP markers and the construction of a high-density consensus map. *Science Reports* 10: 6790.

- Ceballos R, Fernández N, Zúñiga S & Zapata N (2015) Electrophysiological and behavioral responses of pea weevil *Bruchus pisorum* L. (Coleoptera: Bruchidae) to volatiles collected from its host *Pisum sativum* L. *Chilean Journal of Agricultural Research* 75: 202–209.
- Clement SL, Hardie DC & Elberson LR (2002) Variation among accessions of *Pisum fulvum* for resistance to pea weevil. *Crop Science* 42: 2167–2173.
- Crépon K, Marget P, Peyronnet C, Carrouée B, Arese P & Duc G (2010) Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research* 115: 329–339.
- Dupont P (1990) Contribution à l'étude des Populations de la Bruche de la Fève *Bruchus rufimanus* (Boh). Analyse des Relations Spatio-Temporelles entre la Bruche et sa Plante Hôte. PhD Dissertation, Université de Tours, France.
- Flores F, Nadal S, Solis I, Winkler J, Sass O et al. (2012) Faba bean adaptation to autumn sowing under European climates. *Agronomy for Sustainable Development* 32: 727–734.
- Hamani S & Medjdoub-Bensaad F (2015) Biological cycle and populations dynamics of bean weevil *Bruchus rufimanus* (Coleoptera: Bruchinae) on two parcels: *Vicia faba* major (Seville) and *Vicia faba* minor (Field bean) in the region of Haizer (Bouira, Algeria). *International Journal of Geology, Agriculture and Environmental Science* 3: 33-37.
- Hamidi R, Taupin P & Frérot B (2021) Physiological synchrony of the broad bean weevil, *Bruchus rufimanus* Boh., to the host plant phenology, *Vicia faba* L. *Frontiers in Insect Science* 1: 707323.
- Institut du Végétal Arvalis (2014) *Feverole de Printemps et d'hiver: Guide de Culture 2014-2015*. UNIP, Paris, France.
- Janzen DH (1977) How southern cowpea weevil larvae (Bruchidae: *Callosobruchus maculatus*) die on nonhost seeds. *Ecology* 58: 921–927.
- Johansson Y (2022) *Scent Traps and Trap Crops for Broad Bean Weevil (Bruchus rufimanus) Control*. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Köpke U & Nemecek T (2010) Ecological services of faba bean. *Field Crops Research* 115: 217–233.
- Medjdoub-Bensaad F, Khelil MA & Huignard J (2007) Bioecology of broad bean bruchid *Bruchus rufimanus* Boh. (Coleoptera: Bruchidae) in a region of Kabylia in Algeria. *African Journal of Agricultural Research* 2: 412–417.
- Mendesil E, Rämert B, Marttila S, Hillbur Y & Anderson P (2016) Oviposition preference of

pea weevil, *Bruchus pisorum* L. Among host and non-host plants and its implication for pest management. *Frontiers in Plant Science* 6: 1186.

- Mishra SK, Macedo MLR, Panda SK & Panigrahi J (2017) Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties: bruchid pest management in pulses. *Annals of Applied Biology* 172: 4–19.
- Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E & Higgins TJV (2000) Bean alpha-myliase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proceedings of the National Academy of Science of the USA* 97: 3820–3825.
- Oghiakhe S, Jackai LEN & Makanjuola WA (1992) Pod wall toughness has no effect on cowpea resistance to the legume pod borer *Maruca testulalis* Geyer (Lepidoptera: Pyralidae). *International Journal of Tropical Insect Science* 13: 345–349.
- Pölitz B & Reike H-P (2019) Studies on biology and infestation dynamics of the bean seed beetle (Coleoptera, Bruchidae: *Bruchus rufimanus*) in Saxon. *Gesunde Pflanzen* 71: 79–85.
- R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- van Rossum B-J (2021) statgenGxE: Genotype by Environment (GxE) Analysis. <https://CRAN.R-project.org/package=statgenGxE>
- Roubinet E (2016) Management of the Broad Bean Weevil (*Bruchus rufimanus* Boh.) in Faba Bean (*Vicia faba* L.). Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Sabbour MM & E-Abd-El-Aziz S (2007) Efficiency of some bioinsecticides against broad bean beetle, *Bruchus rufimanus* (Coleoptera: Bruchidae). *Research Journal of Agriculture and Biological Sciences* 3: 67–72.
- Salisbury A, Cook SM, Powell W & Hardie J (2012) Odour-mediated orientation behaviour of the lily beetle *Lilioceris lili*. *Physiological Entomology* 37: 97–102.
- Segers A, Caparros Megido R, Lognay G & Francis F (2021) Overview of *Bruchus rufimanus* Boheman 1833 (Coleoptera: Chrysomelidae): biology, chemical ecology and semiochemical opportunities in integrated pest management programs. *Crop Protection* 140: 105411.
- Segers A, Dumoulin L, Megido RC, Jacquet N, Cartryse C et al. (2022) Varietal and environmental effects on the production of faba bean (*Vicia faba* L.) seeds for the food industry by confrontation of agricultural and nutritional traits with resistance against

*Bruchus* spp. (Coleoptera: Chrysomelidae, Bruchinae). Agriculture, Ecosystems and Environment 327: 107831.

- Seidenglanz M & Huňady I (2016) Effects of faba bean (*Vicia faba*) varieties on the development of *Bruchus rufimanus*. Czech Journal of Genetics and Plant Breeding 52: 22–29.
- Shaheen FA, Khaliq A & Aslam M (2006) Resistance of chickpea (*Cicer arietinum* L.) cultivars against pulse beetle. Pakistan Journal of Botany 38: 1237–1244.
- Szafirowska A (2012) The role of cultivars and sowing date in control of broad bean weevil (*Bruchus rufimanus* Boh.) in organic cultivation. Vegetable Crops Research Bulletin 77: 29–36.
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologia Experimentalis et Applicata 47: 3–14.
- Titouhi F, Amri M & Mediouni Ben Jemâa J (2015) Status of coleopteran insects infesting faba bean in Tunisia with emphasis on population dynamics and damage of *Bruchus rufimanus* (Chrysomylidae). Basic Research Journal of Agricultural Science and Review 4: 225–233.
- Titouhi F, Amri M, Messaoud C, Haouel S, Youssfi S et al. (2017) Protective effects of three *Artemisia* essential oils against *Callosobruchus maculatus* and *Bruchus rufimanus* (Coleoptera: Chrysomelidae) and the extended side-effects on their natural enemies. Journal of Stored Products Research 72: 11–20.
- Torres AM, Roman B, Avila CM, Satovic Z, Rubiales D et al. (2006) Faba bean breeding for resistance against biotic stresses: towards application of marker technology. Euphytica 147: 67–80.
- Tran B, Darquenne J & Huignard J (1993) Changes in responsiveness to factors inducing diapause termination in *Bruchus rufimanus* (Boh.) (Coleoptera: Bruchidae). Journal of Insect Physiology 39: 769–774.
- Tran B & Huignard J (1992) Interactions between photoperiod and food affect the termination of reproductive diapause in *Bruchus rufimanus*. Journal of Insect Physiology 38: 633–642.
- Tsialtas JT, Irakli M & Lazaridou A (2019) Exit of seed weevil and its parasitoid changed testa color but not phenolic and tannin contents in faba beans. Journal of Stored Products Research 82: 27–30.
- Ward RL (2018) The Biology and Ecology of *Bruchus rufimanus* (Bean Seed Beetle). PhD

Dissertation, Newcastle University, Newcastle, UK.

Weller JL & Ortega R (2015) Genetic control of flowering time in legumes. *Frontiers in Plant Science* 6: 207.

White C & Eigenbrode SD (2000) Effects of surface wax variation in *Pisum sativum* on herbivorous and entomophagous insects in the field. *Environmental Entomology* 29: 773–780.

Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer, New York, NY, USA.

Yus-Ramos R, Ventura D, Bensusan K, Coello-García P, György Z & Stojanova A (2014) Alien seed beetles (Coleoptera: Chrysomelidae: Bruchinae) in Europe. *Zootaxa* 3826: 401-448.

Zhang Q, Yan Q, Yuan X, Lin Y, Chen J et al. (2021) Two polygalacturonase-inhibiting proteins (VrPGIP) of *Vigna radiata* confer resistance to bruchids (*Callosobruchus* spp.). *Journal of Plant Physiology* 258–259: 153376.

### Figure captions

**Figure 1** Proportion of (A,C) females and (B,D) males of *Bruchus rufimanus* choosing faba bean flowers in a (A,B) one-flower and (C,D) two-flower choice experiment using a Y-tube glass olfactometer. Grey bars are choices towards the no-flower side of the tube; coloured bars are choices towards the genotypes. Pairs of columns represent separate experiments.

Results from naïve and sexually mature bruchids are combined as no effect of state was seen. Asterisks indicate a significant preference (Pearson's  $\chi^2$ : \*P<0.05).

**Figure 2** (A) Density of faba bean pods that received new *Bruchus rufimanus* eggs in relation to pod age in days since pollination. Curves represent the density and white bars represent the frequency of each genotype. (B) Mean ( $\pm$  SD) total number of eggs laid in each genotype during the 4 weeks of the trial. Means within a week capped with different letters are significantly different (Tukey's test: P<0.05).

**Figure 3** (A) The number of damaged seeds per faba bean pod for each genotype was related to the number of eggs per pod. (B) The number of emergence holes per seed was related to single-seed weight (g) for each genotype.

**Figure 4** Relationship between the date of faba bean flowering and the percentage of healthy seeds. Flowering time corresponds to the number of days after sowing. Early flowering plants had less healthy seeds (%) in both sowing dates (flowering time:  $\chi^2 = 31.5$ ; replicate:  $\chi^2 = 32.8$ , both d.f. = 1,  $P < 0.001$ ).

### Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Table S1** Number of females and males of *Bruchus rufimanus* used in each olfactometer test, states tested and total number of individuals evaluated at the end of the experiments.

**Table S2** Mean percentage of healthy seeds on the selected *Vicia faba* accessions under field conditions in previous studies (PROFABA, not published) and in the present study.

**Table S3** Field data for the two replicates: flowering time (days since sowing), plant height at maturity (cm), 100-seed weight (g), and healthy seeds (%).

**Figure S1** (A) Y-tube glass olfactometer used in the flower preference experiment. Size of the glass tube: 10 cm upper arms, 12 cm base part, and 32 mm outer diameter. At the end of each arm: mid chamber for bruchids and external chamber for flowers. A white mesh divided the two chambers, preventing bruchids to reach the flowers. Chambers were made with 50-ml Falcon tubes. (B) Funnel at the end of the glass arm which let the individuals go in, but prevent them to go back.

**Figure S2** Climatic data recorded from the sowing date (24 February) to the harvest date (2 August) in 2021 at the field in Bréteniere, France. Blue bars: precipitation (mm). Red line: average temperature (°C). Dashed line: 15 °C and 15 mm rainfall. Orange rectangle: flowering period (10 May–29 June). Black rectangle: Period in which bruchids have been seen in the field (3–25 June).

**Table 1** Oviposition preference and larval survival into adulthood of *Bruchus rufimanus*

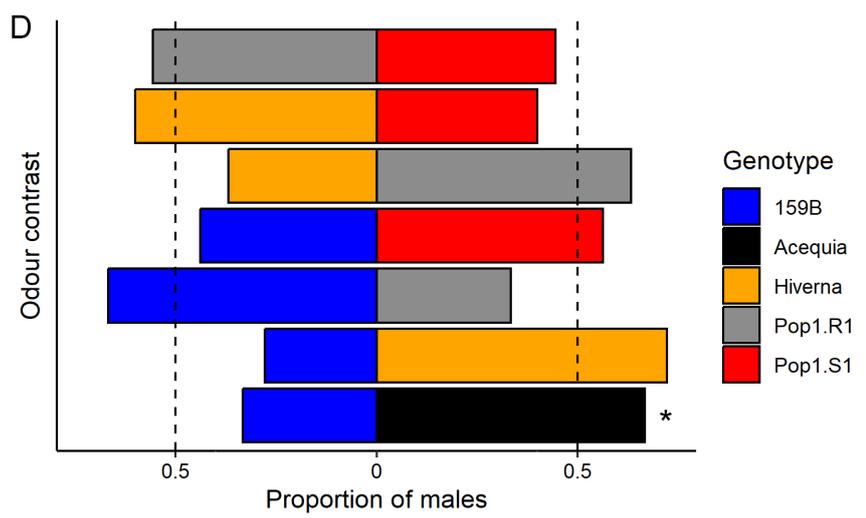
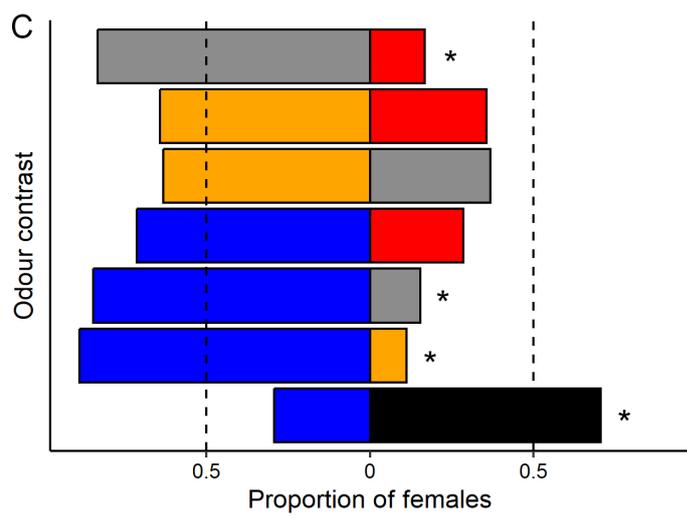
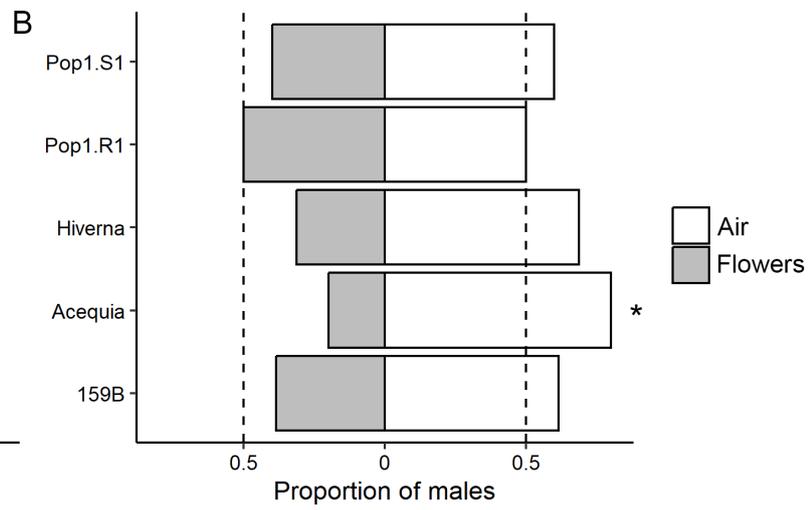
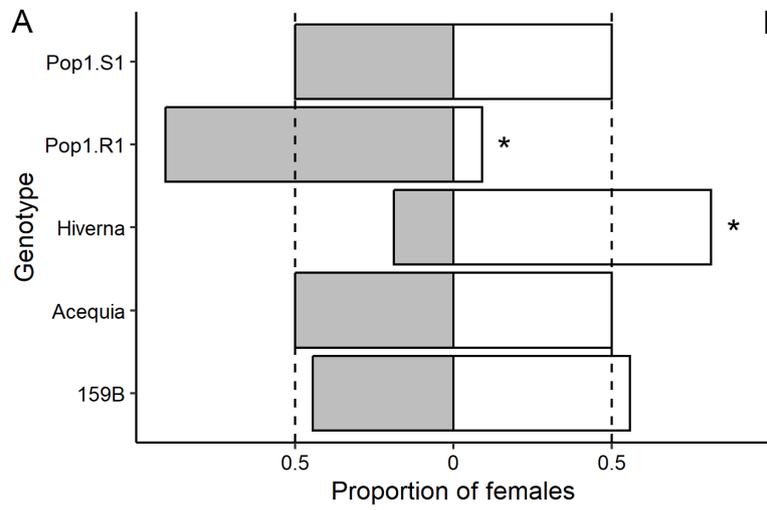
Genotype	Total no.					Mean ( $\pm$ SD)	Egg-to-adult success (%)	Healthy seeds (%)	Mean ( $\pm$ SD)
	eggs	Pods	seeds	damaged seeds	emergence holes	no. eggs per pod			
159B	209	41	83	2	2	5.1 $\pm$ 2.7	1.0	98	0.33 $\pm$ 0.05
ACEQUIA	264	47	107	91	143	5.6 $\pm$ 3.9	54.2	16.1	0.84 $\pm$ 0.21
HIVERNA	132	23	46	33	43	5.7 $\pm$ 2.4	32.6	33.3	0.43 $\pm$ 0.17
POP1.R1	164	37	63	26	27	4.4 $\pm$ 2.8	16.5	60.8	0.46 $\pm$ 0.11
POP1.S1	485	77	218	89	98	6.3 $\pm$ 3.9	20.2	61.3	0.49 $\pm$ 0.07
Total	1254	225	517	241	313	-	-	-	-
Mean	250.8	45	103.4	48.2	62.6	5.4 $\pm$ 3.5	24.9	53.9	0.52 $\pm$ 0.21

**Table 2** ANOVA table for the linear fitted model applied to healthy seeds' percentage of genotypes in the field and its correlation to phenotypic factors

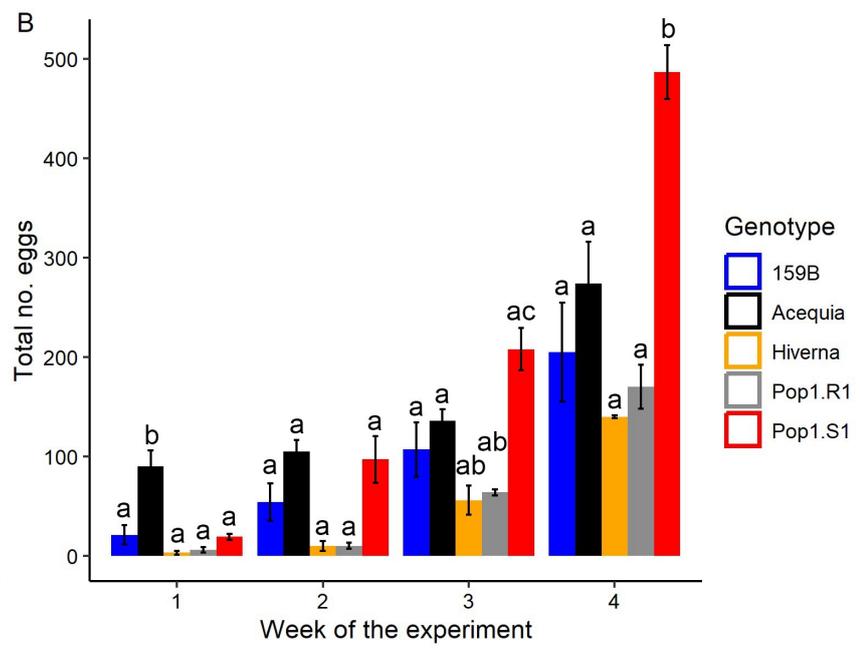
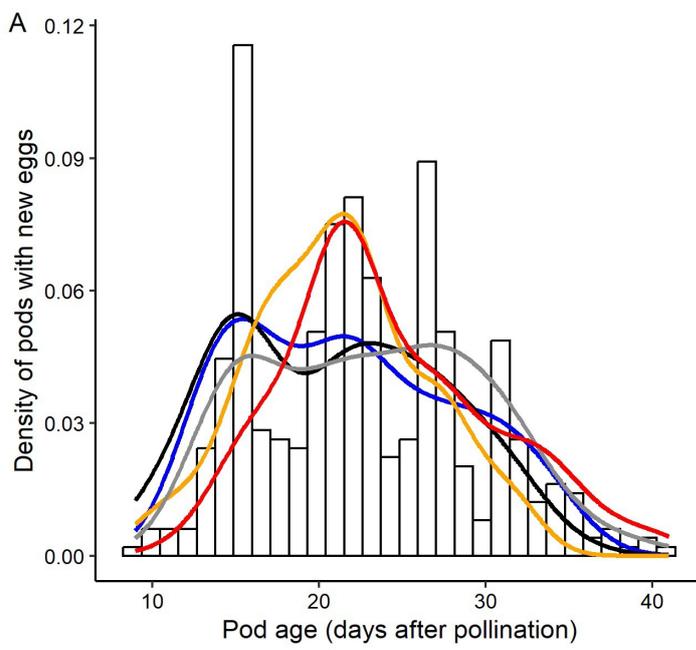
	$\chi^2$ (d.f. = 1)	P	Pearson's correlation
Flowering time	5.15	0.023	0.22
Plant height at maturity	18.01	<0.001	0.51
100-seed weight	14.69	<0.001	-0.60

**Table 3** ANOVA table for the fitted model. Effects of the environment (sowing) and genotypes on the healthy seeds' percentage in the field trials

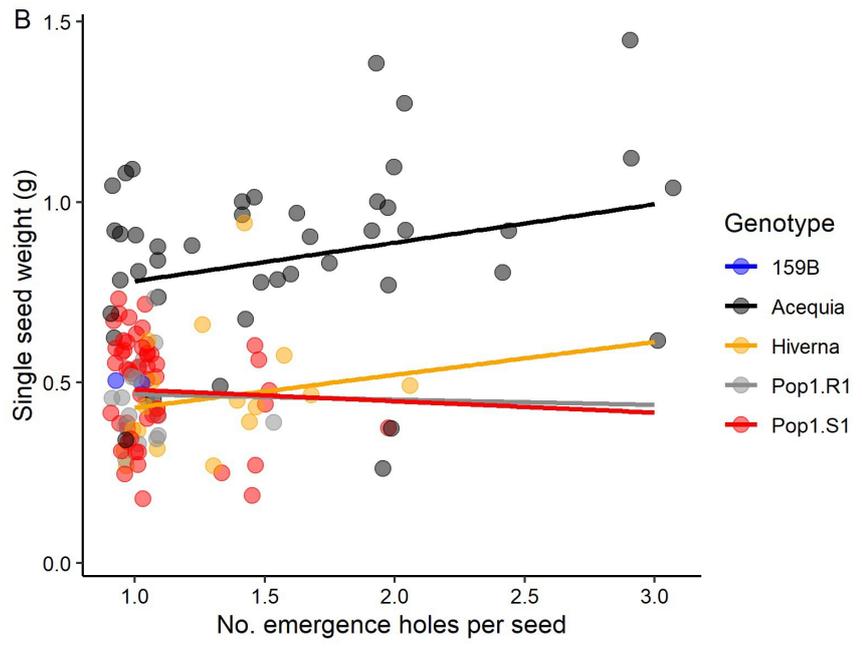
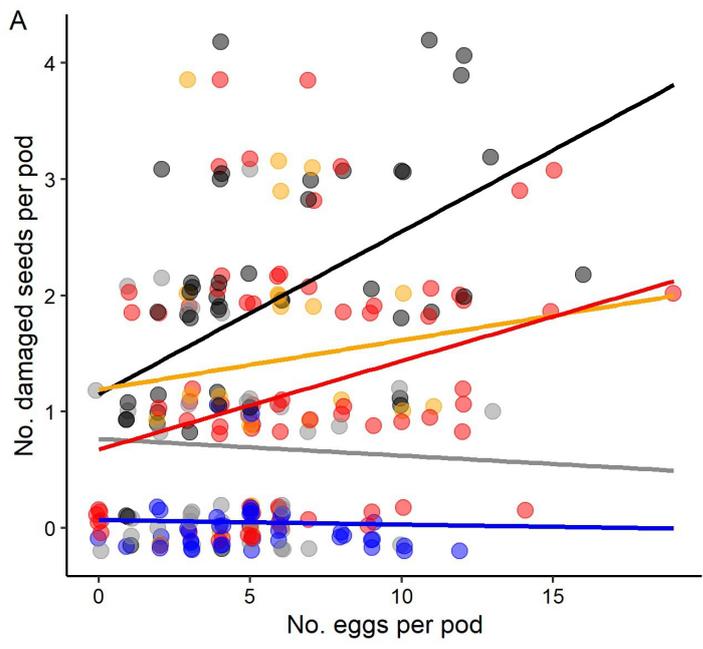
	d.f.	SS	MS	% variance explained	F	P
Sowing	1	2999.1	2999.05	13.94	15.8	0.0004
Genotype	31	28698.2	925.75	58.16	4.9	<0.001
Residuals	27	5095.7	188.73	27.90		
Total	59	36793				



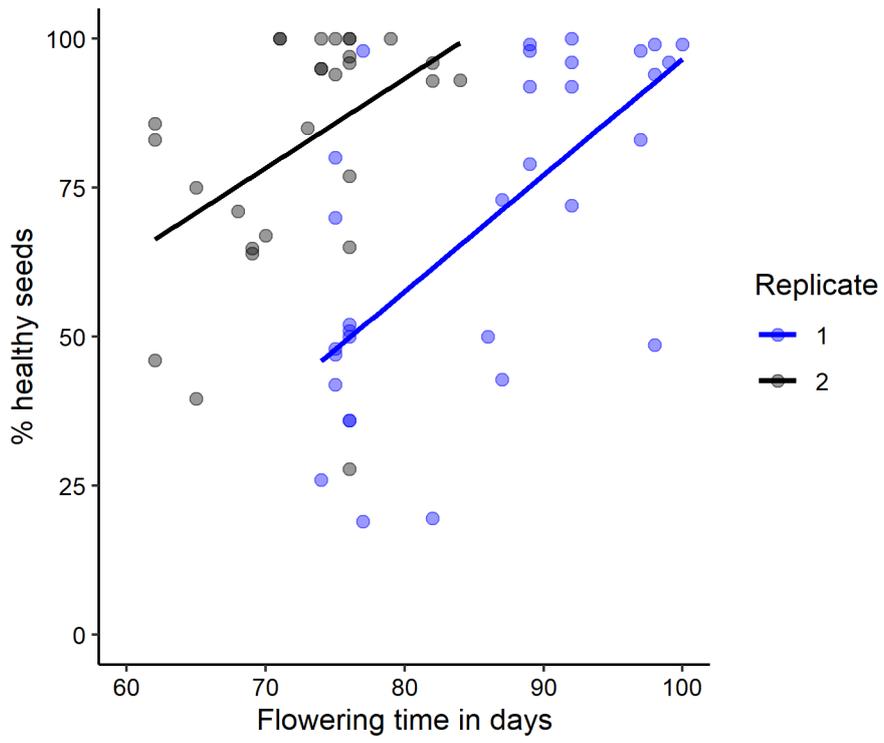
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