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Aphid performance on *Vicia faba* and two southern Italy *Phaseolus vulgaris* landraces

Anna Larocca, Paolo Fanti, Antonio Molinaro, Maria Filomena Mattia, Donatella Battaglia
Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Italy

Abstract

Plant landraces are an important reservoir of genetic variability, and their characterization can improve the selection of new cultivars with desired features. In this study we give a first contribution to the knowledge of *Aphis craccivora* Koch and *Aphis fabae* Scopoli performance on two landraces of Sarconi beans (local populations of *Phaseolus vulgaris* L. from Southern Italy), "verdolino" and "tabacchino". We also compared aphid performance on *P. vulgaris* vs. *Vicia faba* (c.v. *Aqua dulce*) because we wanted to test if the same aphid clone could utilize both plant species as a host, so being able to develop and reproduce early in the season on *V. faba* and then move to young *Phaseolus* plants. Also, we wanted to test if the different leaf trichomes density on the bean landraces could affect aphid performance. Several aphid samples of the two species were collected in the field and tested on young potted plants of both broad bean and bean landraces. Samples of *A. craccivora* were not able to complete the first generation on the two *Phaseolus* landraces tested in our study. Instead, some samples of *A. fabae* set up their colonies on *Phaseolus* plants. All *A. fabae* samples that survived and reproduced on *P. vulgaris* performed well or better on *V. faba*, whereas not all the samples surviving and reproducing on *V. faba* were able to set up on *P. vulgaris*. Life-history features (nymphal mortality and developmental time, adult longevity, and r_m) were quantified in one of the samples that it was able to colonize *Phaseolus* (fab-1). Data showed a poorer performance of *A. fabae* on *P. vulgaris* than *V. faba*. On the last host species, *A. fabae* population increased to a density that caused the host plant death. On the contrary, aphid population was stable on both bean landraces and so the infested plants were able to grow and produce seeds. Aphid performance on the two landraces differed in adult longevity, shorter on "verdolino" bean, and colony survival, longer on "verdolino".

Key words: Hooked trichomes, glandular trichomes, *Phaseolus vulgaris*, plant resistance, *Aphis fabae*, *Aphis craccivora*.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes worldwide. Local bean populations are present in Basilicata Region, Southern Italy, namely in the area of Agri river Valley (Limongelli *et al*., 1996; Marotti *et al*., 2007). These landraces have the best adaptation to the pedoclimatic conditions of this area, and have been preserved by the persistence of traditional agriculture. This local bean, identified with the name of "Sarconi bean", has obtained the PGI mark (Protected Geographical Indication) that is one of the quality recognition standards introduced by the European Community. Local names are used to identify populations of Sarconi bean different in morphological features, the most evident of which is seed colour. Local names are, for example, "verdolino", "tabacchino", "cannellino bianco", "ciuoto", and "munachedda".

Characterization of landraces is useful because they are an important reservoir of genetic variability from which one might draw when selecting new cultivars with specific features. No previous literature is available on susceptibility level of Sarconi landraces to insect pests. This study aims to give a first contribution to the knowledge of insect pest performance on Sarconi beans. In particular, we considered aphid performance on two landraces differing in seed colour.

The two most common aphid species encountered on beans are the cowpea aphid (*Aphis craccivora* Koch) and the bean aphid (*Aphis fabae* Scopoli). Although aphid control in beans is not always necessary, these species may be important pests mainly for virus transmission, but also for direct damage when plants are heavily infested (Tremblay, 1995).

A. fabae is a widespread pest in Europe. This extremely polyphagous species includes four morphological cryptic subspecies which are mostly identified by their partially distinct secondary host range species (Stroyan, 1984; Thieme, 1987; Blackman and Eastop, 1994; Thieme and Dixon, 1996): *Aphis fabae cirsiiacanthoidis* Scopoli, utilising *Cirsium* spp.; *A. f. fabae* Scopoli, utilising *Beta vulgaris* L., *Chenopodium album* L., *Papaver dubium* L. and *Vicia faba* L.; *A. f. mordwilkoi* Borner et Janisch, utilising *Arctium* spp. and *Tropaeolum majus* L.; *A. f. solanella* Theobald, utilising *Bilderdykia convolvulus* (L.) Dumort, *Cirsium* spp. and *Solanum nigrum* L.. The interclonal variation in aphid requirement for essential amino acids may be an important cause of the observed host affiliation. This is because the essential amino acids required by one or more clones of *A. fabae* (histidine, methionine, threonine, and valine) are generally variable in plant phloem sap (Wilkinson and Douglas, 2003). Processes at the level of the plant sieve elements may also contribute to host affiliation in *A. fabae*. This was demonstrated for *A. f. fabae* on *T. majus* (Tosh *et al*., 2002).

A. craccivora is well known as a very detrimental species for cowpea, *Vigna unguiculata* (L.), reducing plant growth and yields (Annan *et al*., 1995). This aphid performs better on cowpea than on broad bean, which is a better host than *Trifolium subterraneum* L., *T. repens* L. and *Lupinus angustifolius* L. (Owain, 2001; Traicevski and Ward, 2002). On the contrary, chickpea (*Cicer arietinum* L.) seems not to be a suitable host for

A. craccivora (Owain, 2001). Cowpea aphid, as *A. fabae*, shows a great amount of intraspecific variation, with significant variation in the mean relative growth rate among clones on different hosts (Owain, 2001).

Performance of different clones on *P. vulgaris* is not particularly well documented for both cowpea and black bean aphid. This crop species shows some degree of resistance towards all herbivorous insects, aphids included, because of the presence of hooked and glandular trichomes on its surface (Johnson, 1953; Pillemer and Tingey, 1978; Quiring *et al*., 1992). Johnson (1953) reported that hooked trichomes on the French bean (*P. vulgaris*) may have profoundly detrimental effects on populations of *A. craccivora*, nymphal mortality ranging from 13 to 73 percent as a function of hairs density.

Considering the level of host affiliation in the two aphid species, we preliminarily tested several aphid samples collected in the field and we pre-adapted aphid colonies before studying performance parameters. We also compared aphid performance on bean with aphid performance on broad bean. We wanted to test the possibility for the same aphid clone to feed and reproduce on both *P. vulgaris* and *V. faba* as a host, even if a lower performance on *P. vulgaris* was expected, as a consequence of the hooked trichomes that are reported to affect aphid survival (Johnson, 1953; Levin, 1973).

The internal lands of Basilicata region are characterized by the presence of small farms and traditional agriculture. Broad bean and common bean are frequently cultivated on contiguous pieces of land in the same farm. In Agry Valley broad bean is sown on March and harvested on June, while common bean is sown on May. If the same clone is able to survive and develop on both plant species then aphids could first set up colonies on *V. faba* and then move and colonize young *Phaseolus* plants.

Materials and methods

Plants and aphids

The host plants used in the experiments were the broad bean, *V. faba* c.v. *Aqua dulce*, and the common bean, *P. vulgaris* landraces "verdolino" and "tabacchino" from "Lombardi Giocoli Eduardo" farm (Marsicovetere - Potenza). Bean and broad bean plants were grown in pots (10 cm diameter) containing commercial soil (COMPO SANA® Universal Potting Soil), under glasshouse conditions, and used for the experiments when the height of 8-10 cm (broad bean) or 12-18 cm (common bean) was reached, and plants had the first true leaf completely expanded.

Several samples of both *A. fabae* and *A. craccivora* were collected in the field (table 1), and reared on the plants mentioned above. Aphids were preliminary kept starved at least for 12 hours in a Petri dish before being transferred to the new host plants. As in Italy *A. craccivora* and *A. fabae* are known as vectors of only non-persistent viruses as regards to *V. faba* and *P. vulgaris* (Crescenzi, personal communication), the abovementioned starvation period, useful to obtain a quick acceptance of the new host plant, was also suitable to clean the potential presence of viruses from the field-collected aphids.

With the term "sample" here we mean aphids collected on a single plant (herbaceous plants) or on a single sprout (woody plants), so each aphid population may not be the progeny of only one single female.

Performance of aphids on different host plants

A number of adult aphids from each sample collected in the field were moved to some plants (5-10 aphid adults for each host plant) for every species/landraces of broad bean or bean, previously grown in glasshouse, to check plant acceptance and colony formation. We collected in the field 6 samples of *A. craccivora,* for a total number of 118 adults transferred on 14 *V. faba* plants, and 198 adults transferred on 22 plants for each of the two bean landraces.

As *A. craccivora* was not able to set up successfully on bean plants, we did not use this aphid species in the subsequent set of observations. Instead, among the samples of *A. fabae* which were able to complete at least one generation on common bean (table 1) we chose fab-1 for a second set of observations, in which biological parameters were studied at two constant temperatures (21 ± 1 °C or 25 ± 1 °C), 70% RH, with LD 18:6 photoperiod in a plant growth chamber.

To study performance parameters, twenty *A. fabae* adults were moved on a new plant for each landrace/species tested (i.e., "verdolino", "tabacchino", or broad bean) and removed after 24 hours. This operation was replicated three times, for each temperature. The newborn aphids (F1) were then left undisturbed on the plants until adult stage was reached. Every day, the new adults were moved to a new plant of the same type ("verdolino", "tabacchino", broad bean) using a fine camel hair brush. During reproduction, adults were removed daily and transferred to a new plant while nymphae (F2), deposed the previous day, were left undisturbed on the same plant until they reached adulthood. The total number of host plants used for each landrace/species in the F2 generation was different as related to the adult longevity. The quantified aphid life-history components for the F2 generation were nymphal mortality, nymphal development time (birth to adulthood), adult longevity, and r_m, all calculated on data checked daily. The r_m values were calculated only for the aphids kept at the temperature of 21 °C.

Finally, in a third set of observations in order to check the aphid population dynamics on the different host plant species/landrace, we moved ten apterous adult females on a new plant and we left them and their progeny undisturbed, at the same rearing conditions described above and a temperature of 21 °C. Five repetitions were performed for each of the three host plant species/landraces tested ("verdolino", "tabacchino" and broad bean). In this set of observations the number of aphids on each plant was checked every two days.

Four strips (1 mm^2) per leaf were cut from the two sides of the main vein of *Phaseolus* leaves, cleared in a 10 percent solution of potassium hydroxide, mounted in glycerine and observed under a microscope to count hooked and glandular trichomes. The mean number of trichomes per mm^2 was calculated on a base of sixty strips for each landrace.

Table 1. Adaptation of different samples of *A. fabae* (fab) and *A. craccivora* (cr) on *V. faba*, and on two *P. vulgaris* landraces, "tabacchino" and "verdolino".

Sample	Collected		Transferred		Parturition	Completion of first generation development
	Place	Plant species	Plant species	Cultivar/landrace		
fab-1	Potenza	*P. vulgaris*	*V. faba*	*Aqua dulce*	yes	yes
fab-1	Potenza	*P. vulgaris*	*P. vulgaris*	tabacchino	yes	yes
fab-1	Potenza	*P. vulgaris*	*P. vulgaris*	verdolino	yes	yes
fab-2	Pietragalla (PZ)	*V. faba*	*V. faba*	*Aqua dulce*	yes	yes
fab-2	Pietragalla (PZ)	*V. faba*	*P. vulgaris*	tabacchino	yes	no
fab-2	Pietragalla (PZ)	*V. faba*	*P. vulgaris*	verdolino	yes	no
fab-3	Potenza	*Rumex* sp.	*V. faba*	*Aqua dulce*	yes	yes
fab-3	Potenza	*Rumex* sp.	*P. vulgaris*	tabacchino	yes	no
fab-3	Potenza	*Rumex* sp.	*P. vulgaris*	verdolino	yes	no
fab-4	Pietragalla (PZ)	*P. vulgaris*	*V. faba*	*Aqua dulce*	yes	yes
fab-4	Pietragalla (PZ)	*P. vulgaris*	*P. vulgaris*	tabacchino	yes	yes
fab-4	Pietragalla (PZ)	*P. vulgaris*	*P. vulgaris*	verdolino	yes	yes
fab-5	Calvello (PZ)	*P. vulgaris*	*V. faba*	*Aqua dulce*	yes	yes
fab-5	Calvello (PZ)	*P. vulgaris*	*P. vulgaris*	tabacchino	yes	yes
fab-5	Calvello (PZ)	*P. vulgaris*	*P. vulgaris*	verdolino	yes	yes
fab-6	Agropoli (SA)	*E. europaeus*	*V. faba*	*Aqua dulce*	no	no
fab-6	Agropoli (SA)	*E. europaeus*	*P. vulgaris*	tabacchino	no	no
fab-6	Agropoli (SA)	*E. europaeus*	*P. vulgaris*	verdolino	no	no
cr-1	Potenza	*V. faba*	*V. faba*	*Aqua dulce*	yes	no
cr-1	Potenza	*V. faba*	*P. vulgaris*	tabacchino	yes	no
cr-1	Potenza	*V. faba*	*P. vulgaris*	verdolino	yes	no
cr-2	Potenza	*V. faba*	*V. faba*	*Aqua dulce*	yes	no
cr-2	Potenza	*V. faba*	*P. vulgaris*	tabacchino	yes	no
cr-2	Potenza	*V. faba*	*P. vulgaris*	verdolino	yes	no
cr-3	Agropoli (SA)	*V. virtinica*	*V. faba*	*Aqua dulce*	yes	yes
cr-3	Agropoli (SA)	*V. virtinica*	*P. vulgaris*	tabacchino	yes	no
cr-3	Agropoli (SA)	*V. virtinica*	*P. vulgaris*	verdolino	yes	no
cr-4	Agropoli (SA)	*V. virtinica*	*V. faba*	*Aqua dulce*	yes	yes
cr-4	Agropoli (SA)	*V. virtinica*	*P. vulgaris*	tabacchino	yes	no
cr-4	Agropoli (SA)	*V. virtinica*	*P. vulgaris*	verdolino	yes	no
cr-5	Potenza	*V. faba*	*V. faba*	*Aqua dulce*	yes	yes
cr-5	Potenza	*V. faba*	*P. vulgaris*	tabacchino	yes	no
cr-5	Potenza	*V. faba*	*P. vulgaris*	verdolino	yes	no
cr-6	Potenza	*V. faba*	*V. faba*	*Aqua dulce*	yes	yes
cr-6	Potenza	*V. faba*	*P. vulgaris*	tabacchino	yes	no
cr-6	Potenza	*V. faba*	*P. vulgaris*	verdolino	yes	no

Statistical analysis

Data were analysed by one-way Analysis of Variance. Mean comparison (Tukey's test) was performed when statistical significance ($\alpha = 0.05$) occurred. All percentages were arcsine transformed before analysis (Zar, 1984) as Arcsen√ (original value/100). In the cases where it was not possible to normalize data, Kruskal-Wallis one-way analysis of variance on ranks was performed. Trichomes density was compared using Mann-Whitney test. The r_m was calculated according to Birch (1948) as: $r_m = (\log_e R_0)/T$, where R_0 is the basic reproductive rate and T is the generation length (Begon *et al.*, 1996). SYSTAT, version 9, was used for all statistical analyses (Systat, 1999).

Results

Aphids of *A. craccivora* samples died as nymphae of the first generation and were unable to set up colonies on both *Phaseolus* landraces. On broad bean, *A. craccivora* produced colonies, even though not all tested plants were accepted, and samples cr-1 and cr-2 didn't survive to complete the first generation (table 1).

All *A. fabae* samples tested here, with the only exception of that collected on *Euonymus europaeus* L., performed well on *V. faba* (table 1), producing colonies that continued to grow until plants collapsed. On the contrary, only samples collected on *Phaseolus* in the field (fab-1, fab-4 and fab-5) were able to survive on *Phaseolus* in the laboratory.

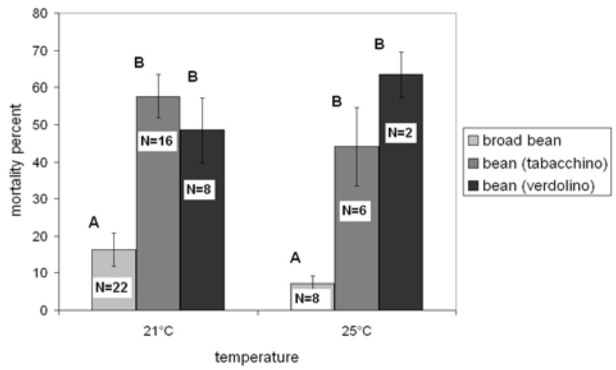

Figure 1. *A. fabae* nymphal mortality (mean ± standard error) on different host plants and under two temperature conditions. Means denoted by different letters are significantly different (Tukey's test, α = 0.05).

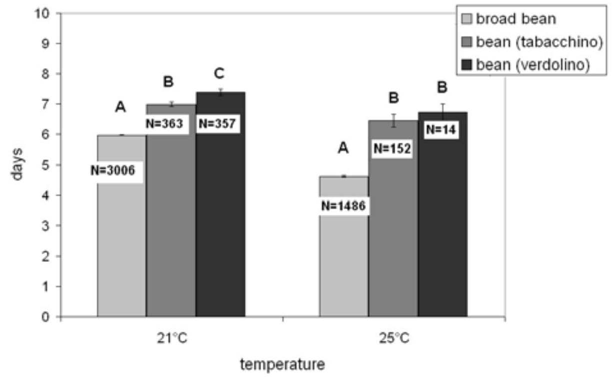

Figure 2. Pre-adult period of *A. fabae* (mean ± standard error) on different host plants and under two temperature conditions. Means denoted by different letters are significantly different (Tukey's test, α = 0.05).

Both *A. fabae* and *A. craccivora* almost always produced offspring on bean plants regardless of the ability of nymphae to survive until adulthood (table 1).

Observations on clone fab-1 performance showed that nymphal mortality (figure 1) significantly differed according to the host plant species tested (F = 23.802; d.f. 2,43; P < 0.001; F = 14.748; d.f. 2,13, P < 0.001, respectively at 21 and 25 °C). The highest mortality was observed on common bean plants, without difference between the two landraces. In this case almost half nymphal population didn't reach adult stage under both temperature conditions.

Host plant influenced also insect development time (figure 2) (F = 280.4, d.f. 2,3723; P < 0.001; F = 158.5; d.f. 2,1649; P < 0.001, respectively at 21 and 25 °C). Pre-adult period was significantly shorter on broad bean than on bean. The nymphal development at 25 °C was not statistically different between "verdolino" and "tabacchino" landraces, whereas it was significantly longer on "verdolino" landrace at 21 °C. Adult longevity was significantly different in all comparisons and under both temperature conditions (figures 3 and 4) (Kruskall-Wallis H = 104.276, d.f. 2; P < 0.001; H = 28.285; d.f. 2, P < 0.001, respectively at 21 and 25 °C). The longest adult longevity was observed on broad bean, the shortest adult longevity on "verdolino" landrace.

Aphid population dynamic was different according to the host plant (figure 5). Aphid population kept growing on broad bean plants until the heavy infestation caused the host plant death, and then the population collapsed. On the contrary, aphid population on bean was low, but stable in both landraces; also, the infestation persisted much longer on "verdolino" landrace.

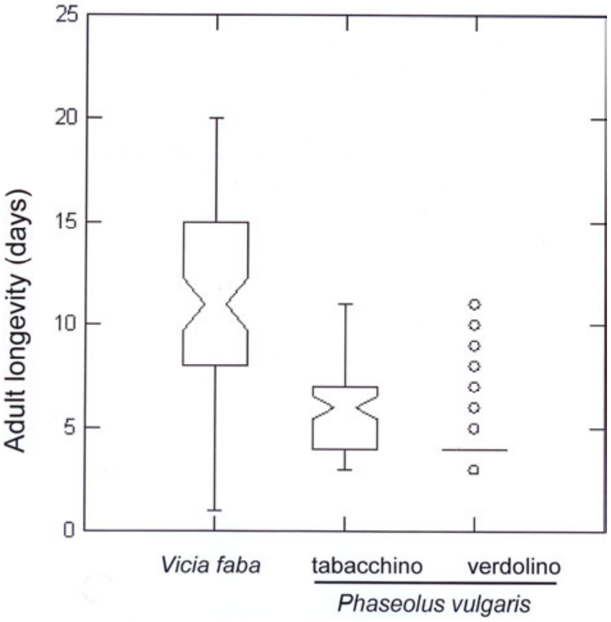

Figure 3. Notched box plot of *A. fabae* adult longevity on different host plants at 21 °C. Boxes whose notches do not overlap indicate that the medians of the two groups differ at the 5% significance level.

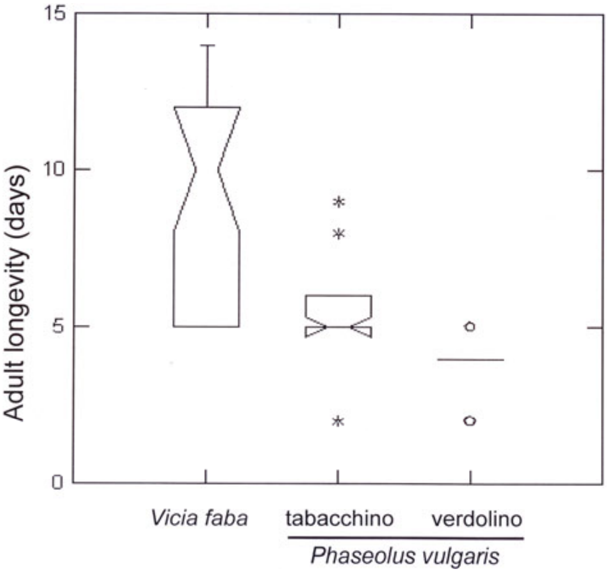

Figure 4. Notched box plot of *A. fabae* adult longevity on different host plants at 25 °C. Boxes whose notches do not overlap indicate that the medians of the two groups differ at the 5% significance level.

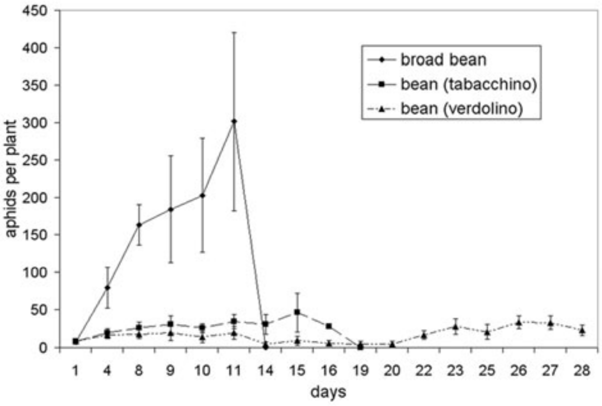

Figure 5. *A. fabae* population dynamics on different host plants.

The r_m value was significantly different in aphid samples reared on different host plants (F = 20.224; d.f. 2,6; P = 0.002), with significantly higher values on broad bean (0.67 ± 0.088, mean ± SE) than on "tabacchino" (0.25 ± 0.031) or "verdolino" (0.096 ± 0.067).

Densities of both hooked (Mann-Whitney T = 2240; d.f. 1; P = 0.02) and glandular trichomes (Mann-Whitney T = 2117; d.f. 1; P = 0.02) measured on the lower leaf surface were higher on "tabacchino" than "verdolino" landrace (figure 6).

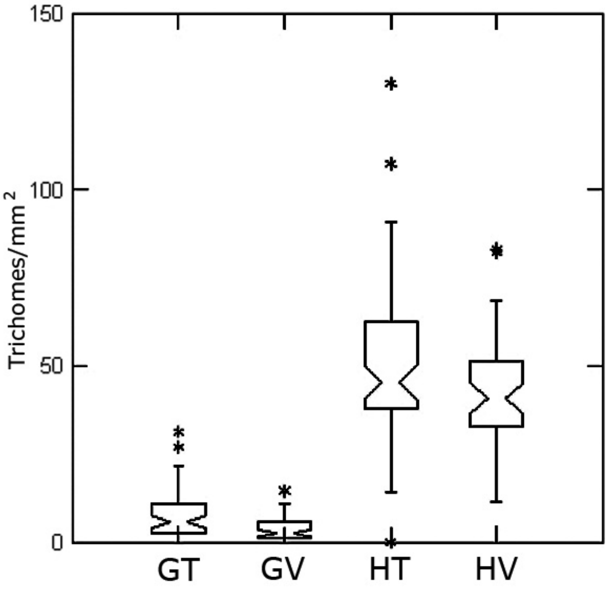

Figure 6. Notched box plot of hooked and glandular trichomes density on the lower leaf surface on "tabacchino" and "verdolino" bean landraces. Boxes whose notches do not overlap indicate that the medians of the two groups differ at the 5% significance level.
GT: glandular trichomes on "tabacchino" landrace;
GV: glandular trichomes on "verdolino" landrace;
HT: hooked trichomes on "tabacchino" landrace;
HV: hooked trichomes on "verdolino" landrace.

Discussion

Samples of *A. craccivora* tested in this work were not able to complete the first generation on the two *Phaseolus* landraces studied here. Adult females reproduced on *P. vulgaris,* but all nymphs died in one - two days. On the contrary, some samples (three out of six) of *A. fabae* were able to establish on *Phaseolus* plants, on both landraces tested. All *A. fabae* samples that survived and reproduced on *P. vulgaris* were able to do the same on *V. faba*, whereas not all the samples surviving and reproducing on *V. faba* were able to set up on *P. vulgaris*.

A. fabae includes four subspecies with partial reproductive isolation (Raymond *et al*., 2001), which perform well on subspecies-specific secondary hosts. *V. faba*, *T. majus*, *Cirsium arvense* (L.) and *S. nigrum*, are marker species, used exclusively by each of the four subspecies of *A. fabae*: *A. f. fabae*, *A. f. mordwilkoi*, *A. f. cirsiiacanthoidis*, and *A. f. solanella,* respectively (Thieme, 1987; Blackman and Eastop, 1994). Other host plant species can be shared by different subspecies (Tosh *et al*., 2004).

As our *A. fabae* samples collected on bean set up successfully on broad bean, we could argue that *Phaseolus* plants can be colonized by *A. f. fabae* subspecies. Nevertheless we observed that all our *A. fabae* samples able to infest *Phaseolus* plants in the laboratory had been collected on *Phaseolus* hosts in the field.

Our data show that *Phaseolus* can be a suitable host plant for *A. f. fabae* and for this reason contiguity between bean and broad bean fields could potentially increase the risk of infestation from one crop to the other. On the other side, bean plants have lower suitability than broad bean ones, as proved by the poor performance of our sample fab-1 and the failure of samples fab-2 and fab-3 to set up colonies on *Phaseolus* host plants. Because of the few samples we collected on bean plants, we cannot exclude the possibility that this plant species is suitable and used as an alternative host by other subspecies of *A. fabae*.

Primary hosts of *A. fabae* include *E. europaeus* and *Viburnum opulus* L.. *A. f. fabae* subspecies overwinters on *E. europaeus* together with *A. f. solanella* and *A. f. cirsiiacanthoidis* (Raymond *et al*., 2001). The presence of the other subspecies on the same primary host can explain the poor performance on both *V. faba* and *P. vulgaris* of the sample (fab-6) collected on *E. europaeus*.

Both *Phaseolus* landraces were partially resistant to *A. fabae* and youth mortality was very similar to that observed by Johnson (1953) for *A. craccivora* on plants with the same level of trichome density. The main difference between the two landraces was in the adult aphid longevity, which was shorter on "verdolino" landrace, despite the lower tricomes density. Moreover the persistence of aphid colonies was longer on "verdolino".

All parameters measured (nymphal mortality, preadult period length, adult longevity) showed a poorer performance of *A. fabae* on *P. vulgaris* than *V. faba*.

Data on both adaptation of different samples and performance of sample fab-1 prove that broad bean is a more suitable host than bean for *A. fabae*. Young broad bean plants (cultivar *Aqua dulce*) do not show effective constitutive or induced defences against the black aphid and, in the absence of natural enemies and limiting abiotic factors, *A. fabae* population increases until plants die. On the contrary, aphid population stabilizes on bean and the infested plant grows and reproduces. In view of that, it would be interesting to investigate the damage threshold and the effect of host plant trichomes on *A. fabae* natural enemies.

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Authors' addresses: Donatella BATTAGLIA (corresponding author: donatella.battaglia@unibas.it), Anna LAROCCA, Paolo FANTI, Antonio MOLINARO, Maria Filomena MATTIA, Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, viale dell'Ateneo Lucano 10, 85100 Potenza, Italy.

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