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How generalist insect herbivores respond to alien plants? The case of *Aphis fabae*–*Myzus persicae*–*Rhododendron ponticum*

Arnaud Ameline,^{*}  Thomas Denoirjean, Marion Casati, Jean Dorland and Guillaume Decocq



Abstract

BACKGROUND: The enemy release hypothesis (ERH) predicts that alien plant species are unsuitable hosts for native phytophagous insects. However, the biotic resistance hypothesis (BRH) predicts that generalist herbivores may prefer an alien plant over their common host plant. In this study, we have tested these two hypotheses by comparing the potential colonization of the invasive Pontic rhododendron (*Rhododendron ponticum* L.) versus the common rearing host plants by two generalist aphid species (*Aphis fabae* and *Myzus persicae*). We assessed (i) the probing behavior using the electrical penetration graph (EPG) technique and (ii) survival and fecundity in Petri dishes.

RESULTS: The results showed the inability of *A. fabae* and *Myzus persicae* to immediately colonize *R. ponticum*. Despite their ability to feed on this invasive plant, the two aphid species hardly survived and poorly reproduced.

CONCLUSION: Our results are consistent with the ERH, since *R. ponticum* appeared as an unsuitable host for native phytophagous insects.

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

Keywords: invasive alien plant species; host plant suitability; Aphididae; EPG; demographic parameters; enemy release hypothesis

1 INTRODUCTION

One major threat to biodiversity and ecosystem functioning worldwide is the spread of invasive alien plant species.^{1,2} The native herbivore load on alien plants (abundance and species richness of herbivores) is supposed to contribute to alien plant species' invasiveness.³ In their native range, the population dynamics of alien plants is often controlled by both specialist and generalist herbivores.⁴ In contrast in the exotic range, specialist herbivores that have co-evolved with the host plant are rarely found,⁵ while resident specialist herbivores usually do not feed on newly introduced species since they miss physiological and behavioral adaptations.⁶ It is often assumed that resident generalist herbivores may newly use the alien plant, which has not evolved to resist or tolerate them,^{7,8} but several studies have suggested that native broad or single-family generalists avoid some invasive plant species.^{9,10} Moreover, alien plants may act as lethal traps for native herbivores, which often respond with low tolerance.¹¹ Consequently, alien plant species in the introduced range may experience reduced herbivore pressure with essentially polyphagous generalist herbivores, which is consistent with the enemy release hypothesis (ERH).¹² However, it has been argued that alien plants may lack the physical or chemical properties required to deter native generalists, exposing them to greater

herbivore pressure compared to native plants,¹³ so that their establishment may fail, which is consistent with the biotic resistance hypothesis (BRH).^{14,15} The exact role of generalist herbivores in fostering or impeding alien plant species invasion has been poorly tested so far, and essentially through studies of the performance of polyphagous insects but rarely using behavioral approaches.

The electrical penetration graph (EPG) technique has been developed over the past 50 years to study the host plant colonization process by aphids, which is achieved through a sequence of several behavioral steps^{16,17}: (i) probing the epidermis, (ii) stylet pathways activity in the mesophyll, (iii) sieve element puncture and salivation, (iv) phloem acceptance and sustained sap ingestion. This technique offers a vast field of investigation to study the different hypotheses and concepts related to the biology of invasion notably

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documented,¹⁸ the interaction between invasive plants and native aphids,¹⁹ or invasive aphids and native plants.^{20,21}

The Pontic rhododendron (*Rhododendron ponticum* L. subsp. *baeticum*) is a shrub native to the south of the Iberian Peninsula. It has been introduced as an ornamental plant in many parks and gardens of western Europe. It is a proven invasive species in regions with an oceanic climate, including the British Isles and the north-western part of continental Europe (France, Belgium and the Netherlands), where it mainly invades wooded areas.²² In the British isles, where the species has the longest history of invasion,²² only 31 insect species have been observed on *R. ponticum* (Orders: Coleoptera, Lepidoptera, Hemiptera), of which 25 are generalists with a high degree of polyphagy.²³ Of the generalist hemipterans, the black bean aphid *Aphis fabae* and the green peach aphid *Myzus persicae*, which have a wide host range (i.e., several hundred species) and are potentially likely to colonize this alien plant species, only *Myzus persicae* showed colonies on the underside leaves of *R. ponticum*.²³ In other studies, specialist hemipterans have been found on *R. ponticum* plants, such as *Ericolophium holsti* in the United Kingdom²⁴ and *Masonaphis lambersi* in the Netherlands.²⁵ However, according to the literature, there is no information concerning the different aphid species that would be likely to colonize *R. ponticum* in its native area.

In order to characterize the behavior of generalist insect herbivores towards *R. ponticum*, we designed laboratory experiments to investigate whether *R. ponticum* (collected in the introduced range) could be a suitable host plant for two aphid species with a very high degree of polyphagy, namely *Myzus persicae* and *A. fabae*. Our objective was to test predictions from the two previously described hypotheses: (H₀) *R. ponticum* is an unacceptable host for the two generalist aphids (ERH); (H₁) the two generalist aphid species feed on *R. ponticum* (BRH). For this purpose, we monitored the colonization of *R. ponticum* by each of these two-aphid species comparatively with that on their common host plant and more specifically (i) their probing behavior using the EPG technique and (ii) their survival and fecundity rate across time by monitoring individual fate in Petri dishes.

2 MATERIALS AND METHODS

2.1 Insects and plants

For each aphid species, we initiated colonies from a single apterous parthenogenetic female and maintained them separately in ventilated Plexiglas® cages (360 mm × 240 mm × 110 mm) in growth chambers under controlled conditions [20 ± 2 °C, 60 ± 5% relative humidity (RH), and 16 h:8 h light/dark photoperiod at 4.7 klux] to induce parthenogenesis. The individuals used to initiate the colonies correspond to the introduced area of *R. ponticum* (north of France for *Myzus persicae* and Belgium for *A. fabae*). We established the *Myzus persicae* colony from one parthenogenetic female collected in 1999 in a potato field near Loosen-Gohelle in Hauts-de-France region, France (50°28'53.8" N 2°47'22.9" E) and maintained it on pepper plant (*Capsicum annuum*). We placed plots (9 cm × 9 cm × 10 cm) containing each 2–3 pepper plants in ventilated plastic cages (24 cm × 11 cm × 36 cm). We initiated the colony of *A. fabae* from a single apterous parthenogenetic female provided in 2018 by the UCL (Université Catholique de Louvain, Belgium; 50°67'01.6" N, 4°61'25.7" E). Plantlets used in the experiments were obtained from *Vicia faba* (cv. 'Axel') seeds reared for 3 weeks in plastic pots (9 cm × 9 cm × 10 cm). All the experiments (aphid probing behavior and aphid performance) were performed using young apterous adult aphids (9 ± 2 day-

old, corresponding to their pre-reproductive period) synchronized on the leaves of their host rearing plant embedded in 1.5% agar in Petri dishes (Ø 9 cm). All tested aphids were naive, that is, they had never been submitted to *R. ponticum* volatile organic compounds (VOCs) before the experiment.

We collected 40–50 cm-long flowerless twigs of *R. ponticum* from invasive populations growing in the Hauts-de-France region (50.467456° N, 1.701729° E) in March and April 2021. Twigs were kept in a jar (diameter 15 cm, height 25 cm) containing water prior to experiments, which were carried out within 2–14 days after sampling.

2.2 Aphid probing behavior on their host plants and on *R. ponticum*

We used the direct current (DC)-EPG technique²⁶ to investigate *Myzus persicae* and *A. fabae* probing behavior on their rearing host plant used as control plants (*C. annuum* and *V. faba*, respectively) and on *R. ponticum*. We stuck a thin gold wire (20 µm in diameter and 2 cm in length) on the insect's dorsum using conductive water-based silver glue. We then connected the aphid to the DC-EPG amplifier and placed it on a plantlet leaf; we inserted a second electrode into the soil of the potted plant to complete the electrical circuit. Recordings were conducted for a period of 8 h during daytime. We placed the whole aphid–plant system inside a Faraday cage under controlled conditions (20 ± 2 °C, 60 ± 5% RH, and 16 h:8 h light/dark photoperiod at 4.7 klux). We acquired and analyzed the EPG waveforms with PROBE 3.5 software (EPG Systems, www.epgsystems.eu), and computed parameters with EPG-Calc 6.1 software.²⁷ Among the recorded parameters accounting for the probing behavior of aphids on the study plants, we analyzed the 15 following EPG parameters: waveform 'Pr' represented general probing behavior within plant tissues [parameters studied: the time to the first probe (t1.Pr), the total duration of probing (s_Pr) and the number of proings (n_Pr)]; waveform 'C' represented the intercellular apoplastic stylet pathway where the insects showed a cyclic activity of mechanical stylet penetration and secretion of saliva [parameters studied: the total duration of pathway phase (s_C) and the number of pathway phases (n_C)]; waveform 'E1' represented salivation in the sieve element [parameters studied: the pathway phase mean duration preceding the first phloem phase (tC.1E/1Pr), the total duration of phloem salivation (s_E1) and the number of salivation phases (n_E1)]; waveform 'E2' was correlated with passive phloem sap uptake from the sieve element [parameters studied: time to first phloem ingestion (t.1E2), the total duration of phloem sap ingestion (s_E2) and the number of phloem sap ingestions (n_E2)]; waveform 'G' represented active intake of xylem sap [parameters studied: the total duration of ingestion of xylem sap (s_G) and the number of ingestions of xylem sap (n_G)]; waveform 'F' represented stylet derailment mechanics which is usually attributed to mechanical stylet difficulties (parameters studied: the total duration of derailed stylets phase (s_F) and the number of derailed stylets phases (n_F)).

We recorded the probing behavior for each of the four following modalities with the following number of aphids: 20 *A. fabae* on *V. fabae*, 18 *A. fabae* on *R. ponticum*, 21 *Myzus persicae* on *C. annuum*, and 19 *Myzus persicae* on *R. ponticum*. We placed aphids on the abaxial part of two fully expanded apical leaves for the rearing plants and at the third young leaf starting from the apical part for the rhododendrons. Indeed, preliminary studies by placing rhododendron twigs in mass rearing of *A. fabae* on faba bean and *Myzus persicae* on sweet pepper allowed to identify the preferential location of settlement of both aphid species on *R. ponticum*.

2.3 Aphid performance on their host plants and on *R. ponticum*

Performance studies were performed in Petri dishes using aphids synchronized as described previously and under controlled conditions (20 ± 2 °C, $60 \pm 5\%$ RH, and a 16 h:8 h light/dark photoperiod at 4.7 klux). We placed a single 9 ± 2 -day-old aphid in each Petri dish (\emptyset 9 cm) containing a leaf (*V. faba*, or *C. annuum* or *R. ponticum*) embedded in 1.5% agar. Then, we monitored individual survival and fecundity every day for 9 days, that is a time period equivalent to that of their pre-reproductive period (*A. fabae* on *V. fabae*: $n = 36$; *A. fabae* on *R. ponticum*: $n = 34$; *Myzus persicae* on *C. annuum*: $n = 37$; *Myzus persicae* on *R. ponticum*: $n = 38$).

2.4 Statistical analysis

All analyses were performed using the R studio software version 4.1.2 (R Core Team, 2022).

Regarding aphids' probing behavior, the effect of the plant (host plant *versus R. ponticum*) on EPG data describing probing phase durations ('s_Pr', 's_C', 'tC.1E/1Pr', 's_E1', 's_E2', 's_G', 's_F') was analyzed with a generalized linear model (GLM) using a gamma (link = 'inverse') distribution as the data were not normally distributed. EPG data describing the number of probing phases ('n_Pr', 'n_C', 'n_E1', 'n_E2', 'n_G', 'n_F') were analyzed with a GLM using a Poisson (link = 'log') distribution as it is suited for count data. The hypothesis of homoscedasticity of the residuals was visually validated using the plotted residuals against predicted values. Data analysis on EPG parameters describing time before the first probe ('t.1Pr') and time to first phloem ingestion ('t.1E2') was performed using the Cox proportional hazards (CPH) regression model. Absence of an EPG reading was treated as a missing value. The number of aphids performing the different EPG phases was compared between plants (host plant *versus R. ponticum*) using a Chi-square (χ^2) test.

For aphids' performance, survival was analyzed using CPH regression models. We compared survival rates at days 4 and 9 using a χ^2 test. We analyzed daily fecundity rates using a GLM with a gamma (link = 'identity') distribution.

3 RESULTS

3.1 Aphid probing behavior on their host plants and on *R. ponticum*

Although not always significant, we observed the same trends in both aphid species (Fig. 1):

- The total duration of stylet probing activity within plant tissues (s_Pr) was significantly longer on the host plant (20% longer for *A. fabae* on *V. fabae* and 45% longer for *Myzus persicae* on *C. annuum*) than on *R. ponticum* (GLM: $\chi^2 = 7.98$, $df = 1$, $P = 0.005$ and $\chi^2 = 12.44$, $df = 1$, $P < 0.001$ for *A. fabae* and *Myzus persicae*, respectively). The total number of probing phases was around three times smaller on *R. ponticum* than on the host plant for the two aphid species (GLM: $\chi^2 = 80.99$, $df = 1$, $P < 0.001$ and $\chi^2 = 118.69$, $df = 1$, $P < 0.001$ for *A. fabae* and *Myzus persicae*, respectively) (Supporting Information Tables S1 and S2)
- The time to first probe (t1Pr) was five and seven times shorter on the host plant than on *R. ponticum* for *A. fabae* and *Myzus persicae*, respectively (CPH model: $\chi^2 = 20.35$, $df = 1$, $P < 0.001$ and $\chi^2 = 14.20$, $df = 1$, $P < 0.001$ for *A. fabae* and *Myzus persicae*, respectively).

- The total duration of the pathway phase (s_C) was 60% longer for *Myzus persicae* on the host plant than on *R. ponticum* (GLM: $\chi^2 = 6.68$, $df = 1$, $P = 0.01$) (not significant for *A. fabae*: GLM: $\chi^2 = 1.93$, $df = 1$, $P = 0.16$). The total number of pathway phases was two to three times smaller on *R. ponticum* than on the host plant for the two aphid species (GLM: $\chi^2 = 93.68$, $df = 1$, $P < 0.001$ and $\chi^2 = 137.43$, $df = 1$, $P < 0.001$ for *A. fabae* and *Myzus persicae*, respectively) (Tables S1 and S2).
- The pathway phase mean duration preceding the first phloem phase (tC.1E/1Pr) was four times shorter for *Myzus persicae* on *R. ponticum* than on the host plant (GLM: $\chi^2 = 7.06$, $df = 1$, $P = 0.008$). For *A. fabae* this parameter was not statistically different (GLM: $\chi^2 = 0.51$, $df = 1$, $P = 0.47$). The total duration of phloem salivation was not different for both aphid species (GLM: $\chi^2 = 0.01$, $df = 1$, $P = 0.91$ and $\chi^2 = 0.003$, $df = 1$, $P = 0.96$ for *A. fabae* and *Myzus persicae*, respectively). The total number of salivation phases was three to five times smaller on *R. ponticum* than on the host plant for the two aphid species (GLM: $\chi^2 = 31.92$, $df = 1$, $P < 0.001$ and $\chi^2 = 28.26$, $df = 1$, $P < 0.001$ for *A. fabae* and *Myzus persicae*, respectively) (Tables S1 and S2).
- The total duration of phloem sap ingestion (s_E2) was almost five times longer for *Myzus persicae* on the host plant than on *R. ponticum* (GLM: $\chi^2 = 5.24$, $df = 1$, $P = 0.02$), but the time to first phloem sap ingestion was not significantly different (CPH model: $\chi^2 = 3.25$, $df = 1$, $P = 0.07$). These parameters were not tested for *A. fabae*, as the number of aphids ingesting phloem sap on *R. ponticum* was too low ($n = 2$) to conduct any test on the duration of this phase. Regarding the number of aphids performing this phase and expressed as a percentage, there was a significant lower proportion of *A. fabae* individuals that succeeded in ingesting phloem sap on *V. faba* and *R. ponticum*, 65% and 11%, respectively; χ^2 test, $\chi^2 = 9.4$, $df = 1$, $P = 0.002$). For *Myzus persicae*, although the difference was not significant, this phase was observed in 71.4% of the individuals on the host plant and only 36.8% on *R. ponticum* (χ^2 test, $\chi^2 = 3.53$, $df = 1$, $P = 0.06$).
- The total duration of xylem sap ingestion (s_G) was three times shorter for *A. fabae* on the host plant than on *R. ponticum* (GLM: $\chi^2 = 10.92$, $df = 1$, $P < 0.001$). For *Myzus persicae* this parameter was not statistically different (GLM: $\chi^2 = 0.44$, $df = 1$, $P > 0.05$). For *Myzus persicae*, there was a significant lower proportion of individuals performing this phase, as 86% and 37% of individuals succeeded in ingesting xylem sap on *C. annuum* and *R. ponticum*, respectively (χ^2 test, $\chi^2 = 8.2$, $df = 1$, $P = 0.004$). Such difference was not observed for *A. fabae* for which 40% and 44% of individuals succeeded in ingesting xylem sap on *V. faba* and *R. ponticum*, respectively (χ^2 test, $\chi^2 = 0$, $df = 1$, $P > 0.05$). The total number of xylem sap ingestions (n_G) was five times shorter for *Myzus persicae* on the host plant than on *R. ponticum* (GLM: $\chi^2 = 25.89$, $df = 1$, $P < 0.001$). For *A. fabae*, this parameter was not statistically different (GLM: $\chi^2 < 0.001$, $df = 1$, $P = 0.98$) (Tables S1 and S2).
- The total duration of stylet derailment (s_F) was twice longer for *Myzus persicae* on *R. ponticum* (GLM: $\chi^2 = 8.25$, $df = 1$, $P = 0.004$). There was no significant difference of total duration of stylet derailment between *A. fabae* on *V. faba* and *R. ponticum* (GLM: $\chi^2 = 0.02$, $df = 1$, $P > 0.05$). There was a significant lower proportion of *A. fabae* showing a derailed stylet phase on *V. faba* (33%) than on *R. ponticum* (94%) (χ^2 test, $\chi^2 = 13.9$, $df = 1$, $P < 0.001$). For *Myzus persicae*, although the difference was not significant, this phase was observed on 47.6% of the individuals on the host plant and only 78.9% on

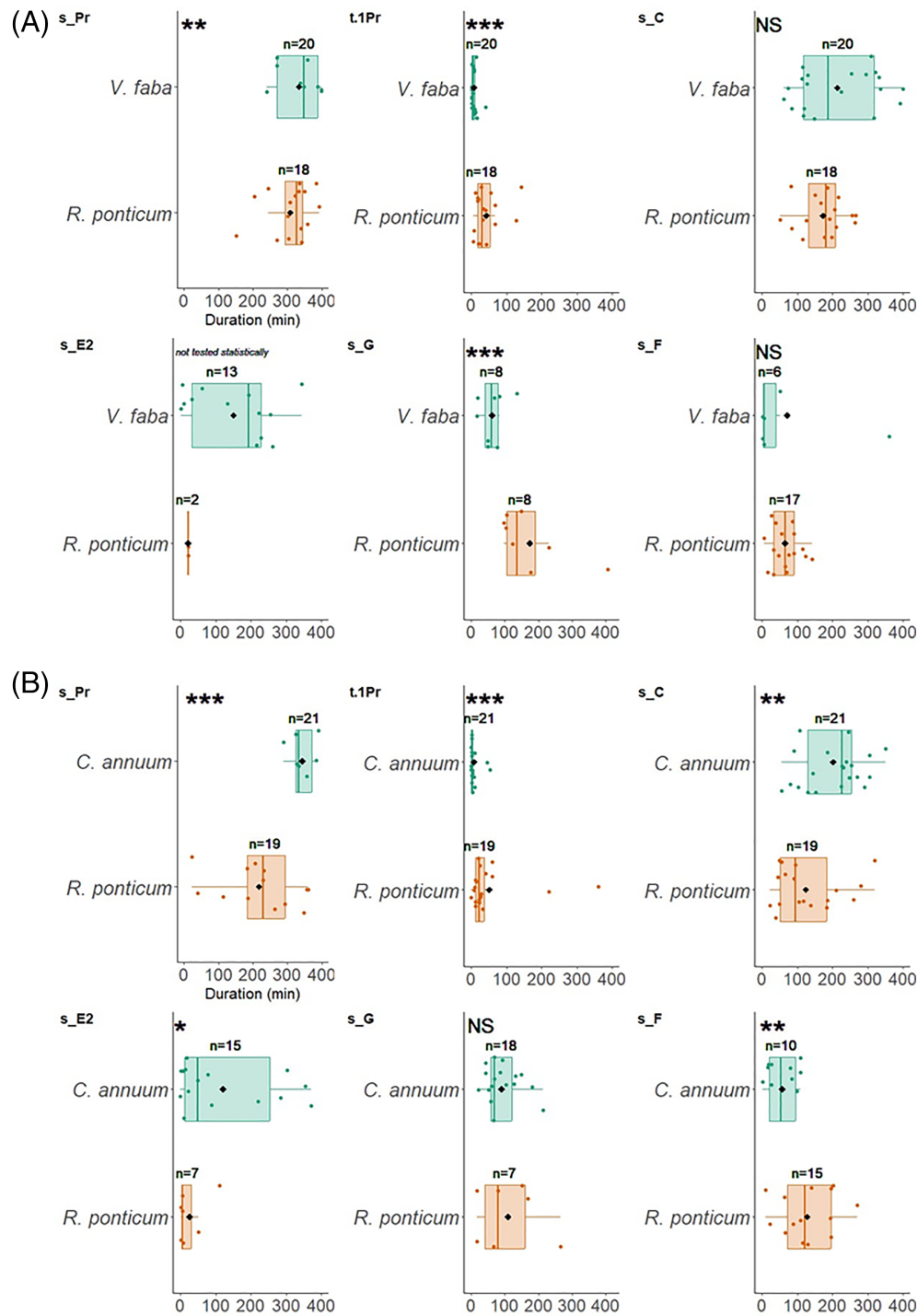


Figure 1. Probing behavior parameters (min) of (A) *Aphis fabae* aphids submitted to their host plant *Vicia fabae* (green) or to *Rhododendron ponticum* (orange) and of (B) *Myzus persicae* aphids submitted to their host plant *Capsicum annuum* (green) or to *R. ponticum* (orange). (s_Pr) total duration of probing; (t.1Pr) time to first probe; (s_C) total duration of pathway phase; (s_E2) total duration of phloem sap ingestion; (s_G) total duration of xylem sap ingestion; (s_F) total duration of derailed stylets phase. Black dots show means. The time to first probe (t.1Pr) was analyzed using a Cox proportional hazard model, while other parameters were analyzed with a generalized linear model using a gamma distribution (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant).

R. ponticum (χ^2 test, $\chi^2 = 2.95$, $df = 1$, $P = 0.09$). The total number of stylet derailment phases was twice to five times smaller on the host plant than on *R. ponticum* for the two aphid species (GLM: $\chi^2 = 4.13$, $df = 1$, $P = 0.04$ and $\chi^2 = 8.07$, $df = 1$, $P = 0.004$ for *A. fabae* and *Myzus persicae*, respectively) (Tables S1 and S2).

3.2 Aphid performance on their host plants and on *R. ponticum*

Both aphid species experienced lower survival rates on *R. ponticum* than on their host plant (CPH model, $\chi^2 = 45.6$, $df = 1$, $P < 0.001$ and $\chi^2 = 88.5$, $df = 1$, $P < 0.001$ for *A. fabae*

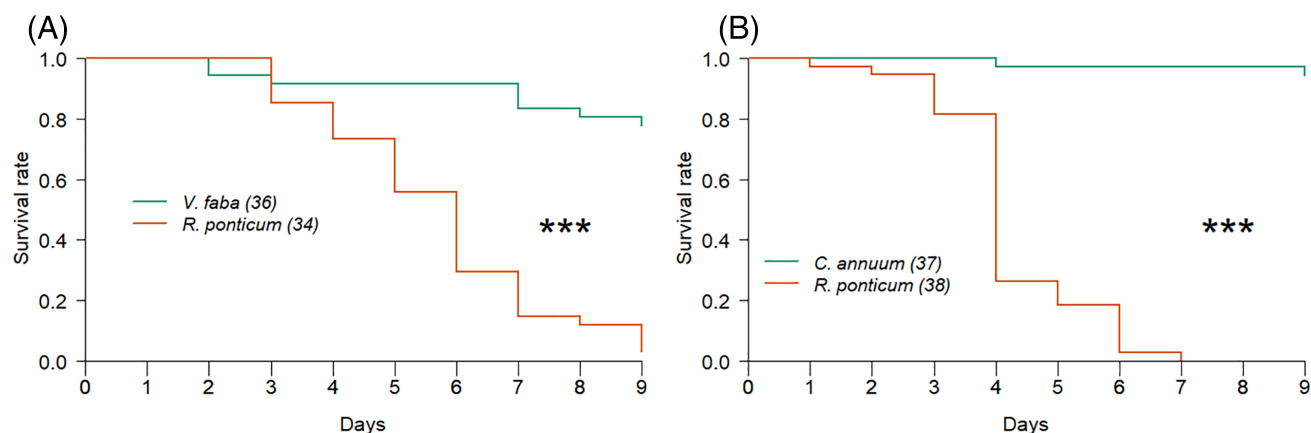


Figure 2. Survival rates of aphids on their host plant or on *Rhododendron ponticum*. (A) Survival rates of *Aphis fabae* on *Vicia faba* and *R. ponticum*. (B) Survival rates of *Myzus persicae* on *Capsicum annuum* and *R. ponticum*. Analyses were carried with a Cox proportional hazard model (***) $P < 0.001$.

and *Myzus persicae*, respectively) (Fig. 2). The survival rates were significantly different at 4 days for *Myzus persicae* but not for *A. fabae*, while at 9 days for both aphids survival rates were lower on *R. ponticum* compared to those recorded on their host plant, that is 2.9% versus 77.8% for *A. fabae* and 0% versus 94.6% for *Myzus persicae* (χ^2 test, $\chi^2 = 37.33$, $P < 0.001$ and $\chi^2 = 63.6$, $P < 0.001$ for *A. fabae* and *Myzus persicae*, respectively) (Table S3, Fig. 2).

On day 4, only *Myzus persicae* showed a significantly lower survival rate on *R. ponticum* than on *C. annuum* (26.3% versus 97.3%; $\chi^2 = 36.9$, $df = 1$, $P < 0.001$) (Table S3). On day 9, survival rates were significantly lower on *R. ponticum* than on the host plant for both species: 2.9% versus 77.8% for *A. fabae* (χ^2 test, $\chi^2 = 37.33$, $df = 1$, $P < 0.001$) and 0 versus 94.6% for *Myzus persicae* (χ^2 test, $\chi^2 = 63.6$, $df = 1$, $P < 0.001$).

The daily fecundity of both aphid species was lower on *R. ponticum* than on their host plant: 1.39 ± 0.09 versus 3.29 ± 0.14 total nymphs produced per female for *A. fabae* (GLM: $\chi^2 = 7.2$, $df = 1$, $P = 0.007$), and 0 versus 2.08 ± 0.08 total nymphs produced per female for *Myzus persicae* (GLM: $\chi^2 = 773.44$, $df = 1$, $P < 0.001$).

4 DISCUSSION

This study aimed at understanding how generalist phytophagous insects behave, survive and reproduce when facing an invasive alien plant. The ability of highly polyphagous aphids like *A. fabae* and *Myzus persicae* to immediately colonize *R. ponticum* seemed to be too limited to allow the immediate establishment of a colony (Table S1). Our results are consistent with the ERH, since *R. ponticum* appeared as an unsuitable host for generalist insects. The BRH does not apply here, since the generalist herbivores we studied preferred their common host plant to the alien plant. Hereafter we discuss these results more in detail.

EPG parameters such as frequency, duration, and sequence of the different waveforms are considered as valuable indicators for defining plant suitability or probing interference by chemical and/or physical factors in plant tissues.^{28–30} Our study reveals that both aphid species exhibited a lower total duration of phloem sap ingestion and, more generally, a shorter activity in plant tissues on *R. ponticum* compared to their common host plant. The time to first probe was delayed on the former compared to the latter, suggesting that the unsuitability of *R. ponticum* was notably due to

physical or chemical factors in epidermis tissue. Our results also showed a reduction in the duration and number of pathway phases for the two aphid species on *R. ponticum* compared to the host plant which could result from differences in the distances between the epidermis and the phloem or from chemical factors. Nevertheless, the decrease in both the duration and number of pathway phases does not enable the insects to reach the phloem vessels more rapidly. Moreover, a smaller proportion of aphids able to ingest phloem sap was observed on *R. ponticum*. The high content of phenolic compounds with toxic and deterrent properties known in *R. ponticum*³¹ may explain this decrease of palatability of the plant for the insect. The unsuitability of *R. ponticum* is further supported by stress indicators such as the high xylem sap consumption and the increased duration and number of stylet derailments for *Myzus persicae*.^{32,33} Stylet derailment is usually attributed to mechanical stylet difficulties to penetrate mesophyll tissues if they lose their proper position in the stylet sheath.²⁶

Concerning aphid performance on plants, survival and, even more, daily fecundity on *R. ponticum* also differed between the two-aphid species, which was consistent with the results obtained from the EPG study. As expected, both studied species poorly performed on *R. ponticum*, and few individuals ingested phloem sap, compared to their respective host plant. This suggests antixenosis in which the strong feeding behavior alteration on the alien plant leads to the alteration of insects' physiological features.^{34,35} Contrary to *A. fabae*, for which few neonates were produced by the few survivors on *R. ponticum*, this alteration was complete for *Myzus persicae* since the very high mortality that occurred from the fourth day revealed their incapacity to produce progeny. We do not exclude a phenomenon of intoxication for individuals who fed on phloem sap, potentially explaining the high mortality rate observed. This mortality could be due to the fact that the high phenolic compound content of *R. ponticum* leaves can also make them unpalatable and toxic to insect.³¹ Such effects of phenolic compounds have been demonstrated on a number of aphid species.^{36–38} *Rhododendrons* are indeed well-known for producing toxic diterpene secondary metabolites known as grayanotoxins (GTXs),³⁹ which are neurotoxic to a broad range of vertebrate and invertebrate organisms.⁴⁰

Finally, our results on aphids probing behavior and performance reflect the deterrence due to the physical or chemical properties of *R. ponticum* leaves. These results do not support the BRH since

A. fabae and *Myzus persicae* did not prefer the alien plant (*R. ponticum*) over their common host plant (*V. fabae* and *C. annuum*, respectively). Our results on aphids probing behavior are consistent with the behavioral constraint hypothesis, where native generalists may be able to feed on the introduced plant but lack the behavioral adaptations necessary to recognize it as a suitable food source under natural conditions.⁴¹ The fact that a small proportion of *A. fabae* and *Myzus persicae* individuals were able here to ingest phloem sap supports the work of Bernays and Chapman,⁴² in which probing behavior in generalists is often plastic. To this end, we have highlighted in several works the great plasticity of this specific population of *A. fabae* which, initially reared on faba bean, could rapidly develop on plants belonging to the Brassicaceae,⁴³ the Chenopodiaceae⁴⁴ and more recently on exotic plants belonging to the Asteraceae and the Polygonaceae (personal communication). Consequently, our specific population of *Myzus persicae* was reared and studied on taxonomically different plants species belonging to the families Brassicaceae and the Portulacaceae⁴⁵ and to the family Solanaceae.⁴⁶ It is not excluded that in the longer term, this colonization could be achieved: the phloem sap consumption highlighted in our experiment for *Myzus persicae* may serve as an initial step for explaining the presence of colonies of this species on *R. ponticum* in the wild.²³ It should be noted however that the introduction of *R. ponticum* in the mass rearing of *Myzus persicae* did not allow to initialize any colonization of the alien plant by this species (personal observation). Similarly, the ability of *A. fabae* to produce neonates and the observation of small colonies 1 month after introducing *R. ponticum* in *A. fabae* mass rearing (personal observation) suggest that this species could colonize *R. ponticum* in a few generations. Thus, there is probably a delay between the first contact of these generalist insects with the new host plant and the possibility for them to achieve full colonization. Although *Myzus persicae* and *A. fabae* exhibit a very wide host plant spectrum, the Ericaceae family, to which *R. ponticum* belongs, seems little exploited by these two aphid species.⁴⁷ Thus, herbivores must acquire new physiological or biochemical adaptations before they can exploit this plant as new host, creating a time lag before acclimation. Since *Myzus persicae* and *A. fabae* exhibit a high genetic diversity in host plant adaptation,^{48,49} in the area where it has been introduced, *R. ponticum* could well become in the long term, after a few generations, a suitable host for both aphid species. According to the behavioral constraint hypothesis defined by Lankau *et al.*,⁴¹ insects may avoid plants they do not recognize to reduce the risk of feeding on a toxic plant.⁵⁰ To explore this hypothesis further and disentangle the behavioral constraint hypothesis from the ERH, it could be interesting to assess the probing and feeding behavior of these two generalist aphid species, not with naive insects, but this time with the insects surviving from successive generations raised on *R. ponticum*.

Although our work here is consistent with the ERH (in which alien *R. ponticum* species represents an unsuitable host for the two generalist aphid species studied) but refutes the BRH (in which two generalist aphid species may prefer the alien *R. ponticum* over their common host plant), it would be worth considering the diversity of *R. ponticum* plants in terms of native versus invasive range together with the diversity of populations within each aphid species.

It could be interesting to investigate how *A. fabae* and *Myzus persicae* would behave when facing different *R. ponticum* populations originating from various native areas, in particular *R. ponticum* subsp. *baeticum* that is endemic to the Iberian

Peninsula, *R. ponticum* subsp. *ponticum* which occurs over a large part of the Black Sea region, *R. ponticum* var. *brachycarpum* which forms an isolated, relict population close to the Lebanese coast.²² Indeed, a considerable quantitative and qualitative intraspecific variation in toxic nectar (i.e., diterpene grayanotoxins) was highlighted by Egan *et al.*⁵¹ within and between the native and introduced range of *R. ponticum*. Thus, we can assume that our populations of *A. fabae* and *Myzus persicae* might not behave in the same way when subjected to native versus invasive *R. ponticum* exhibiting different chemical toxins compositions. Considering the polyphagy and the worldwide distribution of both *A. fabae* and *Myzus persicae*, these two species are naturally found in the invasive and native range of *R. ponticum*. Even if these two aphid species are not exclusively native to the north of France, it should be mentioned that *Myzus persicae* and *A. fabae* both form species complexes with a large variety of biotypes.^{52,53} Thus, validation of the ERH and the BRH could be considered by comparing populations of *A. fabae* and *Myzus persicae* originating from native versus invasive areas of *R. ponticum* and submitted to the alien plant.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

SUPPORTING INFORMATION

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

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