

Testing optimal defense theory: Root resistance selection in chicory (*Cichorium intybus*) reduces foliar defense and alters aphid performance and feeding behavior

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Abstract

BACKGROUND: Plants are constantly exposed to a wide range of herbivores at both aboveground (AG) and belowground (BG) levels, involving multiple constitutive defenses in leaves and roots. However, the Optimal Defense Theory predicts that the energy cost of defenses prevents plants from defending all organs equally. In an agroecological context, selecting genotypes for their root-specific resistance may therefore create trade-offs in the allocation of constitutive defenses, potentially leaving leaves more vulnerable to aerial herbivores. To test this hypothesis, we assessed the resistance to the root-feeding aphid *Pemphigus bursarius* (BG) and the foliar aphids *Myzus persicae* and *Nasonovia ribisnigri* (AG) in two chicory (*Cichorium intybus* var *foliosum*) genotypes: AX, considered susceptible, and B18, considered resistant to *P. bursarius*. Aphids' survival and fecundity were measured, and their feeding behavior was evaluated using the electrical penetration graph (EPG) technique.

RESULTS: Only the root-feeding aphid *P. bursarius* exhibited reduced survival, fecundity and feeding activity on the B18 genotype, compared with the susceptible AX genotype, confirming the root-level resistance for which this line was originally selected. In contrast, the foliar aphid *N. ribisnigri* showed enhanced feeding activity and fecundity on B18, whereas *M. persicae* displayed only increased feeding activity, suggesting increased foliar susceptibility relative to AX.

CONCLUSION: Selection for root resistance may promote a shift in resource allocation toward root defenses, potentially reducing constitutive defenses in leaves. These results highlight potential trade-offs in plant defense allocation and underline the importance of considering multi-organ herbivory when breeding for resistance traits.

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Keywords: constitutive defenses; electrical penetration graph; *Myzus persicae*; *Nasonovia ribisnigri*; *Pemphigus bursarius*

1 INTRODUCTION

Plants in agroecosystems are simultaneously challenged by a wide range of herbivores that attack both their aboveground (AG) and belowground (BG) organs. These dual pressures are of particular importance because they act concurrently on different plant tissues and functions. Most studies have traditionally considered AG and BG herbivory in isolation rather than as interconnected processes.¹ These two compartments host different communities of herbivores, including aphids, caterpillars, and beetles on the aboveground, and root-feeding nematodes, insect larvae, or root aphids on the belowground, causing distinct but often concurrent selective pressures on plant physiology.²

To cope with such a wide range of herbivores, plants have evolved both induced and constitutive defense mechanisms that occur in AG and BG organs, and that directly or indirectly affect the herbivore.³ Induced defenses trigger after herbivore contact or damage and rely on the mobilization of secondary metabolite

compounds, such as the emission of volatile organic compounds (VOCs).⁴ Constitutive defenses rely on physical barriers such as leaf thickness and toughness, trichomes and cell walls,^{5–7} or the accumulation of secondary metabolites such as phenolic

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compounds, flavonoids and proteins.⁸ Those defenses structure the resistance of the plant against herbivores, either by modulating their feeding behavior (antixenosis) with physical barriers and chemical repellents that limit the access to the resource (i.e., xylem and phloem), or by deterring the herbivore's physiology (antibiosis) once feeding takes place, through reduced survival, growth, or fecundity.⁹ Those aspects of the defense are particularly evident in insects that rely on sustained ingestion, such as aphids, and can partially be assessed with the electrical penetration graph technique (EPG), which allows the monitoring of the probing activity in the plant's tissues.¹⁰ The host acceptance and the performance of the aphid then rely partially on the nature and the localization of the constitutive defenses in the plant. However, regarding the family or even the plant species, secondary metabolites and their accumulation may vary from AG to BG organs.¹¹ Moreover, the storage of secondary metabolites does not necessarily occur in the tissues where they are produced. Instead, these compounds can be transported between plant compartments, reflecting systemic defense mechanisms. For instance, in Brassicaceae, glucosinolates are synthesized in the roots and subsequently transported to the leaves.^{12,13} As a result, both the level and the expression of constitutive defenses may differ between AG and BG compartments, underlying their dual role in the defense of the plant.^{14–16} Nevertheless, maintaining uniform constitutive defense levels throughout the entire plant is theoretically unlikely, given the substantial energetic costs associated with the production and transport of defensive compounds.¹⁷

Initially proposed by McKey,¹⁸ the Optimal Defense Theory (ODT) posits that plants would allocate their defensive resources to organs with high fitness value and greater probability of attack, such as young leaves and flowers. Most studies have supported this hypothesis,¹⁹ but mainly from AG-centered perspectives focusing on flowers and leaves, while belowground systems have received less attention. Recent work, however, indicates that ODT also shapes the distribution of chemical defenses within the root system.²⁰ For instance, Tsunoda *et al.*²¹ showed that glucosinolate levels vary among roots according to their age and function. The highest concentrations were observed in older roots, such as the taproot (i.e., the plant's storage organ) and lateral roots, rather than in the younger distal roots. Remarkably, the larvae of the specialist root herbivore fly (*Delia radicum*) preferred feeding on tap roots rather than distal (i.e., less defended) ones. These results highlight that ODT can also depend on plant–herbivore interactions, which are themselves shaped by the herbivore's degree of specialization, since generalists are more constrained by a wide range of constitutive defenses while specialists evolve adaptations to bypass or exploit them, thereby exerting contrasting selective pressures on defense allocation.^{22–24}

The ODT becomes especially relevant in an agronomical context, where varietal selection, aiming to optimize yield and/or resistance, often leads to trade-offs between growth and defense.²⁵ Domesticated plants may thus exhibit reduced constitutive defenses or a biased allocation favoring specific plant parts over others. For instance, selecting for resistance to certain pests may unintentionally compromise resistance to another,²⁶ thus complicating the development of resilient crop varieties in multi-herbivore environments.

A representative example of varietal selection focused on foliar traits, with little consideration for BG compartments, is the witloof chicory (*Cichorium intybus* L. var *foliosum*). This herbaceous species from the Asteraceae family is widely cultivated across Europe

for its compact foliage, through a growth in the field followed by leaf development in a forcing chamber. Recent breeding efforts have focused on developing uniform hybrids using molecular markers (SSR, SNP) to modulate traits such as head compactness and reduced bitterness.²⁷ Yet, the search for resistance to its broad array of herbivores is overlooked in current breeding processes. Aboveground pests such as aphids (*Myzus persicae*, *Aphis fabae*, *Nasonovia ribisnigri*) (Hemiptera: Aphididae) or leaf mining flies (*Napomiza cichorri*, *Liriomyza strigata*, *Ophiomyia pinguis*) (Diptera: Agromyzidae) can directly damage the plant or act as virus vectors,^{28–31} affecting the crop quality and yield. Meanwhile, belowground pests such as the aphid *Pemphigus bursarius* or nematodes (*Xiphinema spp.*) affect the integrity of roots and, consequently, the growth of the plant. Among studies addressing resistance to insect pests, only a few have focused on identifying genotypes resistant to belowground herbivores.³² The dual pressure from AG and BG herbivores raises the question of optimal defense allocation across chicory compartments. Indeed, selective breeding for AG resistance, such as the reduced bitterness or improved tolerance to foliar pests, may have unintentionally compromised root defenses.³³ Thus, chicory offers a pertinent biological and agronomical model for studying how domestication and varietal selection may shape the allocation and balance of constitutive defense mechanisms.

In this study, we investigated the interactions between two chicory genotypes and three aphid species, considering both aboveground and belowground herbivory. The two genotypes were selected based on their level of resistance to the lettuce root aphid, *P. bursarius*,³² a species known to colonize the underground organs of lettuces and chicories as secondary hosts.³⁴ Aboveground interactions were explored using the green peach aphid *M. persicae*, a generalist species, and the lettuce-currant aphid *N. ribisnigri*, which primarily targets lettuces and chicories.³⁵ Aphid performance and feeding behavior are widely used as functional proxies to infer plant suitability and to indirectly assess plant resistance. Here, we examined whether a chicory genotype selected for reduced susceptibility to root aphids would also show altered suitability to aphids at the leaf level, and whether aphid responses would be consistent with patterns that are typically associated with antibiosis or antixenosis. In a second step, we aimed to infer potential constitutive defenses involved in the suitability of each genotype, and to determine whether these responses varied between aboveground and belowground organs. We therefore formulated two alternative hypotheses: (i) the plant suitability is similar across organs, leading to comparable aphid responses on roots and leaves, or (ii) the suitability differs between organs, resulting in contrasting aphid performance and feeding patterns.

To test these hypotheses, we exposed the three aphid species to each chicory genotype to assess their survival rate and fecundity on leaves or roots embedded in agar within Petri dishes, and to monitor their feeding behavior using the electrical penetration graph (EPG) technique.

2 MATERIALS AND METHODS

2.1 Plants and insects

The experiments were conducted on witloof chicory (*Cichorium intybus* var *foliosum*). Two genotypes were used in all experiments: the inbred line AX, considered susceptible to *P. bursarius* and sown on May 9, 2024, and the B18 hybrid (Barbucine®), considered resistant to *P. bursarius* and obtained from a cross

between the Barba di Capuccino (BC) and witloof groups, sown on June 7, 2024. The susceptibility and resistance features are based on the work of Benigni *et al.*,³² which showed that the AX genotype was 8 to 16 times more severely infested (number of apterous aphids on the root) by *P. bursarius*, after a 3-month exposure, than the BC × Witloof group to which B18 belongs. Both chicory genotypes were provided by the Association des Producteurs d'Endive de France (APEF, Arras, France), harvested in December 2024, and subsequently planted in 1.2 × 1 × 1 m containers filled with Loire River sand before being stored in a greenhouse under controlled temperature and humidity conditions. The plants were then planted in 23 × 17 cm pots containing potting soil (Universal potting soil, Botanic®, France; product ref. 227 032) 7 days before the beginning of the experiments under controlled conditions (21 ± 1 °C, 60 ± 5% relative humidity (RH), and 16 h:8 h LD photoperiod at 2.5 klux). Across all experiments, plants exhibited an average weight of 116 ± 18 g, a well-developed taproot measuring 15–22 cm, and leaves shorter than 20 cm.

The *M. persicae* colony originated from a parthenogenetic female collected in 1974 in Colmar (Haut-Rhin, France) on sugar beet (*Beta vulgaris vulgaris*), and was maintained on rapeseed (*Brassica napus* L.) placed in 50 × 50 × 50 cm ventilated plastic cages. The *N. ribisnigri* colony came from a parthenogenetic female collected in 1989 in Colmar on lettuce (*Lactuca sativa*), and was maintained on escarole chicory (*Cichorium endivia* var. *latifolium*) placed in a 30 × 30 cm pop-up cage. These two colonies were provided by Santé de la Vigne & Qualité du Vin, INRAE Grand Est-Colmar (France). Experiments for both AG species were performed using 9 ± 2-day-old (corresponding to the pre-reproductive period)^{36,37} individuals synchronized on detached host leaves placed in 25 mL of 1.5% agar in 90 mm Petri dishes. The root aphid *P. bursarius*, provided by APEF, originated from apterous adults collected in June 2024 in galls of its primary host, *Populus nigra* var. *italica* Münchh, near Arras (Pas-de-Calais, France, 50°16'40.5" N 2°48'11.4" E), and was maintained on roots of the chicory hybrid 'Darling' (Hoquet Endives, Raillencourt-Sainte-olle). Experiments used 12 ± 3-day-old apterous adults, synchronized on 0.5 cm tap-root sections placed in 25 mL of 1.5% agar in 90 mm Petri dishes. The duration of the synchronization period was determined by an estimated pre-reproductive period based on the results of unpublished preliminary studies.

All three aphid species were reared under identical controlled conditions (21 ± 1 °C, 60 ± 5% RH, 16L:8D photoperiod, and 2.5 klux light intensity).

2.2 Aphid performance

The daily fecundity and the survival rate of aphids were assessed using chicory leaves (for *M. persicae* and *N. ribisnigri*) or 0.5 cm tap-root (3–4 cm diameter) circular sections (for *P. bursarius*) embedded in 25 mL of 1.5% agar in 90 mm Petri dishes.^{36,38} All experiments involving *M. persicae* and *N. ribisnigri* were conducted on 10–15 cm intermediate leaves located between the inner heart and the external circle,³⁹ due to a marked difference in cuticle thickness between the oldest and youngest leaves.

For all experiments, a single aphid was laid on the chicory organ. Then, its survival and the number of nymphs produced were recorded every day for a maximum period of 10 days. To avoid overlapping generations, ensure individual monitoring, and limit the expression of induced defenses caused by numerous aphids (in alignment with the feeding behavior study using EPG), nymphs were removed from the Petri dish after counting.

Because daily fecundity data for *M. persicae* were highly heterogeneous and zero-inflated, conventional day-by-day analyses were not appropriate. Fecundity was therefore summarized as mean daily fecundity (total offspring divided by lifespan), providing a robust measure of individual reproductive output that integrated both survival and reproduction. Aphids dead within 24 h were considered to be affected by handling and were excluded from the analyses. A total of 32 and 34 *M. persicae* individuals were used on 12 AX and 14 B18 plants, respectively. For *N. ribisnigri*, 26 aphids were tested on 5 AX and 7 B18 plants, respectively. For *P. bursarius*, 45 and 30 aphids were used on 13 AX and 5 B18 plants, respectively. Each plant was assigned a unique identification number (ID). The chicory organ sections in the Petri dishes originated from the same plants used for the EPG recording. Leaves or root sections were not replaced during the 10-day monitoring period to avoid stressing or injuring the aphids.

2.3 Aphid probing behavior

The probing behavior of the three aphid species was measured using the electrical penetration graph (EPG) technique^{10,40} on the AX and B18 genotypes of chicory. Aphids were attached to a 2 cm gold wire (Ø 20 µm) with a water-based silver glue (EPG systems, Wageningen, the Netherlands) on the abdomen. The end of the gold wire was connected to a 5 cm long copper wire (2 mm in diameter), which was soldered with tin to a brass nail. The assembly was then connected to the input of the EPG head amplifier with an input resistance of 10⁹ Ω. The main electrode was then inserted into the potting soil. The probing behavior was recorded by the Giga-8 DC-EPG amplifier inside a Faraday cage in a room under controlled conditions (20 ± 1 °C, 60 ± 5% RH, and a 16L:8D photoperiod). For *M. persicae* and *N. ribisnigri*, the feeding behavior was recorded over an 8-h period on whole plants by placing the aphid on the lower surface of the leaf. This recording duration is sufficient to assess the response of the aphids to constitutive defenses and the plant suitability,¹⁰ and is standard for detecting varietal effects. The set-up to study the probing behavior of the root aphid *P. bursarius* was adapted from Cole *et al.*,⁴¹ by laying the aphid on the surface of the tap root of a whole plant, positioned at a 45° angle in a 10 × 8 × 7 cm glass container filled with 1 cm of water. The probing behavior of *P. bursarius* was recorded in darkness to mimic underground conditions. Preliminary experiments showed that aphids might require up to 6 h before initiating the first probe. To ensure a representative probing time, the recordings were thus set to a total of 14 h. To optimize the signal transmission, the wax was gently removed from the abdomen of *P. bursarius* aphids with a brush.

Eight aphids were connected simultaneously, each individually attached to a recording electrode. Acquisition and analysis of the EPG waveforms were performed using the EPG Styler + software (v01.30; EPG Systems, <http://www.epgsystems.eu>). EPG parameter calculation from the recorded waveforms was performed using the Workbook for Automatic Parameter Calculation of EPG Data (©) version 5.0.⁴² The parameters characterizing the global probing behavior in the plant tissues (Pr) were the Total duration of probing (s_Pr), the time to first probe (t_1stPr) and the number of probes (n_Pr). The pathway phase (C) corresponding to the stylet pathways in intracellular apoplastic plant tissues was studied using the Total duration of pathway phase (s_C) and the Number of pathway phases (n_C). Then, the phloem phase (E) comprising the salivation phase in the phloem vessels (E1) and the ingestion phase of elaborated sap (E2), was studied using the Time to first phloem phase (t_1stE), the Total

duration of phloem phase (s_E), the number of phloem phases (n_E), the Total duration of salivation phase (s_{E1}), the Number of salivation phases (n_{E1}), the Total duration of ingestion phase (s_{E2}) and the Number of ingestion phases (n_{E2}). To compare probing behavior between species despite different recording durations, the proportion of time spent probing (P_{s_Pr}) and the proportion of time spent in the phloem phase (P_{s_E}) were calculated. A total of 28 and 27 *M. persicae* individuals were used on 7 AX and 7 B18 plants, respectively. For *N. ribisnigri*, 27 aphids were tested on 13 AX and 11 B18 plants. For *P. bursarius*, 23 and 15 aphids were used on 16 AX and 10 B18 plants, respectively.

For each feeding behavior experiment, aphids were placed on plant organs of the same phenological characteristics as those used in Petri dish. The aerial aphids *M. persicae* and *N. ribisnigri* were placed on leaves that had not been exposed to feeding before, to limit the localized defensive responses of the plant, which could have potentially affected the next aphid.⁴³ Similarly, roots used for EPG recordings were replaced between recordings whenever possible, depending on the availability of plant material.

2.4 Statistical analysis

All analyses were performed in R (v. 4.5.0)⁴⁴ using RStudio(v. 2024.12.1).⁴⁵

Data analysis of count data (i.e., number of probing events) was conducted using Generalized Linear Models (GLM), fitted with a Poisson distribution (link = 'log') or a Negative Binomial distribution (link = 'log') (MASS package v.7.3), depending on the outcome of the overdispersion test performed using the `check_overdispersion()` function from the `performance` package (v.0.13.0).

For continuous and positive-valued data (i.e., duration of probing phases and mean daily fecundity), GLMs fitted with a Gamma distribution (link = 'log') were used. In cases of excess zeros, Zero-Adjusted Gamma (ZAGA) models were applied. To analyze the proportion of probing time (P_{s_Pr}), a Beta regression model was used, adapted to these kinds of values. Due to the high number of zero values in the Proportion of time spent in the phloem phase (P_{s_E}), a Zero-Inflated Beta model was used. All ZAGA, BEZI and Beta regression models were fitted using the `gamlss()` function from the `gamlss` package (v.5.4). Zero values were retained in all analyses, except GLMs fitted with a gamma distribution, which require strictly positive values. Survival data were modelled using Cox proportional hazards models (`coxph()` function, `survival` package v.2.2.22), with plant genotype as a factor. Aphids that did not experience mortality during the 10-day observation period were treated as censored. The proportional hazards assumption was tested with Schoenfeld residuals using the `cox.zph()` function. Finally, the median survival time (t_{50}) was estimated for each group.

3 RESULTS

3.1 Aphid performance

The mean daily fecundity (Fig. 1) of *M. persicae* did not differ significantly between the two chicory genotypes (ZAGA model, $P = 0.323$), with 0.56 ± 0.11 and 0.36 ± 0.10 nymphs per day on AX and B18, respectively. The mean daily fecundity of *N. ribisnigri* was around 40% greater on B18 compared to AX (1.77 ± 0.16 vs. 1.27 ± 0.15 nymphs per day, GLM, $P = 0.03$). Conversely, the mean daily fecundity of *P. bursarius* was approximately halved

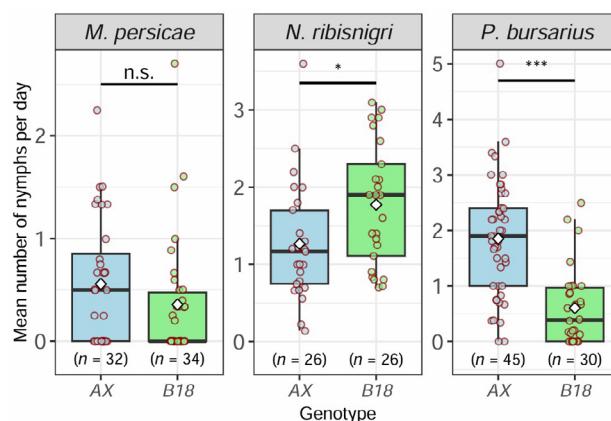


Figure 1. Mean daily fecundity (mean number of nymphs per day) of *Myzus persicae*, *Nasonovia ribisnigri* and *Pemphigus bursarius* on AX and B18 genotypes. The fecundity of *M. persicae* was analyzed using a ZAGA model, whereas those of *N. ribisnigri* and *P. bursarius* were analyzed using a GLM with a gamma distribution. ns: non-significant, *: $P < 0.05$, ***: $P < 0.001$. White diamonds indicate the mean value.

on B18 compared to AX (0.86 ± 0.15 and 1.94 ± 0.15 nymphs per day on B18 and AX, respectively; $P < 0.001$).

The survival rate (Fig. 2) did not differ between chicory genotypes for *M. persicae* (AX: 0.03, B18: 0.00, CPH model, $P = 0.589$) and *N. ribisnigri* (AX: 0.48, B18: 0.62, CPH, $P = 0.385$). However, the survival rate of *P. bursarius* on B18 was significantly smaller (AX: 0.27, B18: 0.03, CPH model, $P = 0.015$).

3.2 Aphid probing behavior

The data for each feeding behavior parameter of the different aphid species on the two tested chicory genotypes are summarized in Table 1. For all aphid species, no significant differences in general probing (Pr) parameters were observed between individuals feeding on AX and B18 genotypes.

For *P. bursarius*, the number of pathway events (n_C) was 34% smaller on B18 than on AX (10.53 ± 2.22 vs. 16.00 ± 1.70 , $P = 0.027$). In contrast, the time to first phloem phase (t_{1stE}) was about 70% longer on B18 than on AX (530.61 ± 79.27 vs. 313.22 ± 48.33 min, $P = 0.026$). Conversely, when compared to those on AX, aphids recorded on B18 displayed lower values ($P < 0.001$) of total duration of the phloem phase (s_E) (5.68 ± 1.28 vs. 58.24 ± 21.90 min), number of phloem phases (n_E) (1.67 ± 0.61 vs. 5.91 ± 1.05), total duration (s_{E1}) (5.48 ± 1.66 vs. 38.70 ± 13.30 min) and number of salivation phases (n_{E1}) (1.67 ± 0.61 vs. 5.91 ± 1.05), and the total duration of ingestion phases (s_{E2}) (1.37 vs. 155.66 ± 31.25 min). For *M. persicae*, the total duration of the phloem phase (s_E) was twice as long on the B18 plant genotype as on AX (37.23 ± 6.66 vs. 16.48 ± 2.70 min, $P = 0.002$), as were the total durations of the salivation phase (s_{E1}) (20.10 ± 3.50 vs. 10.90 ± 1.51 min, $P = 0.008$) and the ingestion phase (s_{E2}) (26.86 ± 8.31 vs. 11.18 ± 3.03 min, $P = 0.043$). The number of ingestion phases (n_{E2}) was twice as great on B18 as on AX (1.59 ± 0.29 vs. 0.86 ± 0.19 , $P = 0.015$). For *N. ribisnigri*, the time to first phloem phase (t_{1stE}) was approximately 40% shorter on B18 than on AX (107.25 ± 22.55 vs. 180.40 ± 27.00 min, $P = 0.049$).

The proportion of probing time (P_{s_Pr}) (Fig. 3) significantly differed among aphid species on AX and B18 genotypes. On AX, *N. ribisnigri* displayed values that were approximately 20% greater than those of *M. persicae* (0.88 ± 0.02 vs. 0.76 ± 0.02 , BE model,

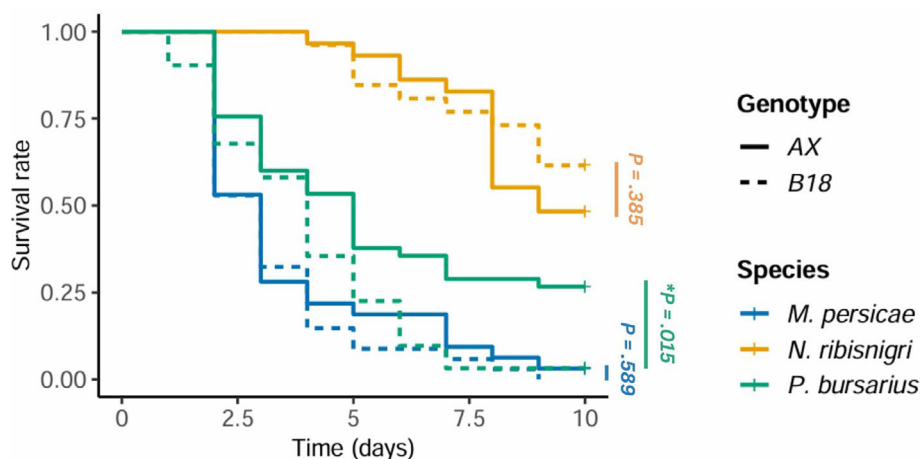


Figure 2. Survival rate of *Myzus persicae*, *Nasonovia ribisnigri* and *Pemphigus bursarius* on both genotypes AX and B18. The statistical analysis was performed using a Cox Proportional Hazard (CPH) model. *: $P < 0.05$.

$P < 0.001$) and twice those of *P. bursarius* (0.44 ± 0.04 , BE model, $P < 0.001$), while *M. persicae* exhibited a proportion of probing time 70% greater than *P. bursarius* (BE model, $P < 0.001$). A similar trend was observed on B18, where *N. ribisnigri* showed values almost 20% greater than those of *M. persicae* (0.92 ± 0.01 vs. 0.77 ± 0.02 , BE model, $P < 0.001$) and nearly twice those of *P. bursarius* (0.40 ± 0.06 , BE model, $P < 0.001$), whereas *M. persicae* values were around 90% greater than those of *P. bursarius* (BE model, $P < 0.001$).

Regarding the proportion of probing time spent in the phloem phase (P_sE) (Fig. 4) on AX, no significant difference was observed between *P. bursarius* and *M. persicae* (0.05 ± 0.01 vs. 0.16 ± 0.05 , BEZI model, $P = 0.457$). In contrast, *N. ribisnigri* exhibited values that were approximately twice as high as those of *P. bursarius* (0.35 ± 0.05 , BEZI model, $P = 0.006$) and seven times greater than those of *M. persicae* (BEZI model, $P < 0.001$). Considering B18, the proportion of probing time spent in the phloem phase (P_sE) significantly differed among the three aphid species. The highest values were displayed by *N. ribisnigri*, with a mean of 0.47 ± 0.06 , compared to *P. bursarius* (0.011 ± 0.01 , BEZI model, $P < 0.001$) and *M. persicae* (0.10 ± 0.01 , BEZI model, $P < 0.001$), which also significantly differed from each other (BEZI model, $P = 0.003$).

4 DISCUSSION

In this study, we assessed the response of *P. bursarius* at the root level by measuring aphid performance and feeding behavior using EPG recordings on two chicory genotypes to infer the plant resistance and the potential defense mechanisms. We compared the responses of *P. bursarius* on the B18 genotype, previously selected for root resistance, with those on the AX genotype. We then tested whether the two aerial aphids, *M. persicae* and *N. ribisnigri*, would display similar physiological and behavioral responses to *P. bursarius* at the AG level to determine whether selection for root-targeted resistance could also influence aphid responses in aerial tissues. Our results suggest that chicory–aphid interactions involved both antibiosis and antixenosis, expressed differently between roots and leaves. Compared with AX, the B18 genotype strongly restricted *P. bursarius* feeding, and subsequently survival, but simultaneously favored foliar aphids, particularly the specialist *N. ribisnigri*, which exhibited higher fecundity.

These contrasting changes in probing behavior could suggest differential allocation of constitutive defensive resources between above- and belowground compartments, consistent with the predictions of the ODT, which proposes that plants allocate defenses unequally among organs according to their fitness value or exposure to herbivory.

The defense of the plant can act through two main mechanisms against herbivorous insects: antixenosis (non-preference) that modulates plant attractiveness or acceptability, leading to changes in insect behavior before or during feeding,⁴⁶ and antibiosis, which causes detrimental effects on insect survival or reproduction following ingestion of plant tissues or sap.⁴⁷ These two mechanisms may operate independently or simultaneously. The frequency and duration of EPG waveforms provide essential information about the plant palatability, reflecting chemical and mechanical properties of plant tissues, and can thus explain aphid performance.⁴⁸

As described by Cole *et al.*,⁴¹ the EPG technique provided an effective monitoring of the aphid's feeding behavior on the taproot. Similar patterns to those commonly observed on leaves were recorded¹⁰ although differences in waveform duration and frequency were noted. At the BG level, the strong reduction in survival and fecundity of *P. bursarius* on B18 compared to the AX genotype confirmed its effective resistance to root-feeding aphids. Such effects are typically associated with antibiosis, where ingestion of plant sap alters aphid physiology and reproduction.⁴⁹ However, EPG recordings provided more elements for the interpretation. The prolonged pathway phases, delayed access to sieve elements, increased salivation, and reduced phloem ingestion observed on B18 suggest that aphids encountered pre-ingestive barriers, either structural (e.g., thicker cell walls, cell lignification)⁶ or chemical (secondary metabolites in prephloemic tissues),⁵⁰ limiting access to phloem rather than directly impairing post-ingestive metabolism. Thus, what initially reflected antibiosis may in fact have arisen from antixenotic mechanisms, which appears consistent with the continuum between these two resistance types.

The responses of *M. persicae* and *N. ribisnigri* observed at the AG level on B18 differed from *P. bursarius*'s performance and behavior at the BG level. Both aerial aphids *M. persicae* and *N. ribisnigri* exhibited greater feeding capacity on this genotype. For *M. persicae*, the unchanged survival and fecundity indicated the

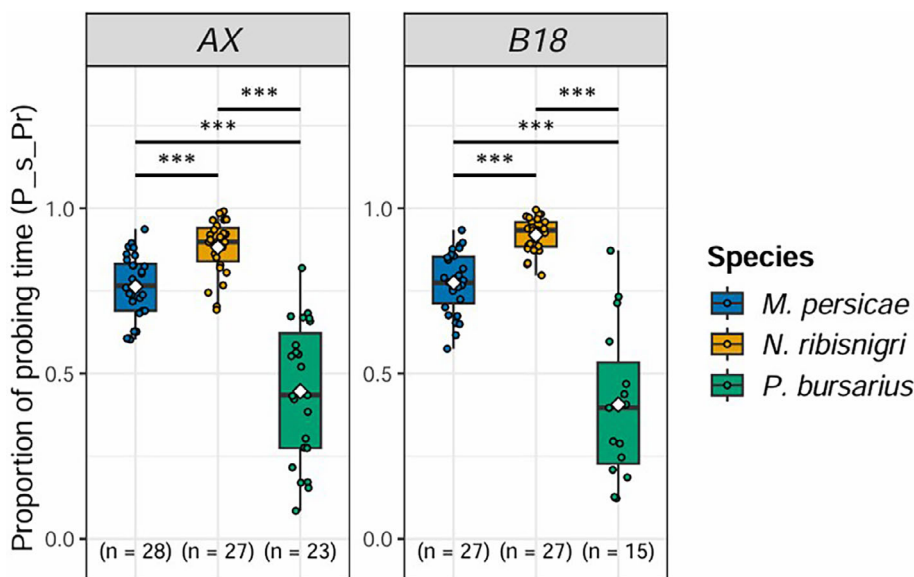


Figure 3. Proportion of time spent in the probing phase (P_sPr) of *Pemphigus bursarius*, *Myzus persicae* and *Nasonovia ribisnigri* on both plant genotypes. Statistical analyses were conducted using a Beta regression (BE) model. ***: $P < 0.001$. White diamonds indicate the mean value.

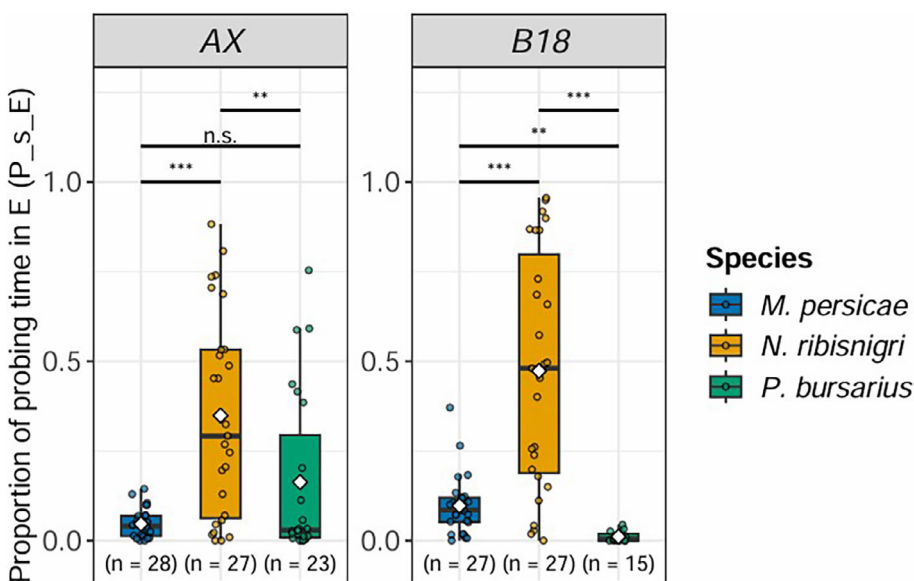


Figure 4. Proportion of time spent in the phloem phase (P_sE) by *Myzus persicae*, *Nasonovia ribisnigri* and *Pemphigus bursarius* on both plant genotypes. Statistical analyses were conducted using a Beta Zero Inflated (BEZI) regression model. ns: non-significant, **: $P < 0.01$, ***: $P < 0.001$. White diamonds indicate the mean value.

absence of post-ingestive toxicity, suggesting that no antibiosis mechanism was involved. However, the longer salivation, the greater frequency and longer duration of ingestion phases showed a prolonged feeding within the sieve elements, suggesting potential lower concentrations of deterrent metabolites or phloem-based barriers.⁵¹ Since the parameters reflecting a potential delay in reaching the phloem remained similar for aphids on the two genotypes, the hypothesis of a physical resistance in the form of a barrier can be ruled out. These patterns suggest that the aphid may have encountered fewer chemical defenses inside the phloem of the aerial tissues of B18, allowing more efficient

feeding activity.⁵² The absence of a positive effect on *M. persicae* performances when feeding on B18 despite a facilitated ingestion may suggest that the quality of the phloem sap was lower,^{53,54} which could be partly due to cut organs. The aerial aphid *N. ribisnigri* displayed greater fecundity and a shorter time to reach the phloem on B18. The EPG data indicated that this greater fecundity could have resulted from altered feeding behavior rather than direct physiological impairment. It therefore seemed to have arisen from an enhanced ability to reach and feed on the phloem, as previously observed in lettuce lines.⁵⁵ Thus, while the B18 genotype exhibited root-level antixenosis, its aerial parts

appeared more susceptible to foliar aphids, conversely to *AX*, which expressed stronger foliar antixenosis. It should also be noted that plant's production of secondary metabolites may vary over the monitoring period, potentially influencing the aphid responses.

The present results appear to be consistent with the ODT, which predicts that plants allocate their defensive resources to organs with the highest fitness value or greatest risk of attack, instead of a uniform distribution. Without any direct quantification of physical barriers or secondary metabolites potentially involved in the two genotypes studied, we cannot fully assert a shift in defense allocation between organs. However, numerous studies describe the metabolites present in chicory varieties and their distribution in the plant. The most likely secondary metabolites involved here could be the sesquiterpene lactones (STLs) and phenolic compounds, as they are known to be present in both leaves and roots.^{9,56} STLs such as lactucin, lactucopicrin, and 8-deoxylactucin are synthesized in leaves and roots, then transported via the phloem and stored in laticifers within both organs.⁵⁷ Their biosynthesis depends on the Germacrene A synthase (*CiGAS*) gene, mostly expressed in the epidermis of leaves and external tissues of the taproot.⁵⁸ These compounds can act as feeding deterrents and disrupt insect metabolism by inducing oxidative stress,⁵⁹ which appears consistent with the lower palatability observed here on *B18* roots and *AX* leaves. Likewise, chlorogenic acids, especially 3- and 5-caffeoylquinic acids, and isochlorogenic acids such as 3,4-dicaffeoylquinic acid occur in all organs of chicory in various concentrations⁶⁰ and exhibit feeding deterrent, anti-nutritive or toxic properties toward aphids and other sap-sucking insects.^{61–63} A higher accumulation of these metabolites in *B18* roots may explain the strong antixenosis observed against *P. bursarius*, as shown by Cole *et al.*,⁶⁴ who measured higher concentrations of isochlorogenic acids in resistant cultivars of *L. sativa*. In contrast, we suppose that higher concentrations in *AX* leaves may explain the reduced feeding activity observed in both *M. persicae* and *N. ribisnigri*, as well as the lower fecundity of the latter.⁵⁵ Such variations of chemical defenses between cultivars have been documented in other Asteraceae species. Beharav *et al.*⁶⁵ reported marked differences in the contents of STLs between *L. sativa* cultivars, showing that specific STL contents could be lower in the leaf of a given cultivar (British Hilde) compared to another (Iceberg), but greater in the roots. Although the model is relatively different from ours, a similar situation was observed on maize (*Zea mays mays*) and its wild ancestor teosinte (*Zea mays parviglumis*) by Gaillard *et al.*²⁶ They showed that the concentrations of chemical defenses against herbivores, the benzoxazinoids, were globally higher in teosinte's leaves whereas they were higher in maize's roots. Our results suggest that the selection may influence the allocation of chemical constitutive defenses of the plant in chicory. Although comparable chemical analyses have not yet been performed between organs across chicory genotypes, the opposite response of aerial and edaphic aphids observed here between *B18* and *AX* should rely on such an allocation pattern, caused by the selection for root resistance of *B18*. However, our experimental design tested each aphid species independently to prevent any induction of defenses across the plant and, therefore, ensure that the measured responses of aphids primarily reflect constitutive defenses,³ in contrast to a field approach that would consider a multiherbivory system.

However, aphids response to the reallocation of constitutive defenses depends on their degree of specialization.²⁶ At the aerial

level, the generalist *M. persicae* engaged in probing and in the phloem phase for a smaller proportion of time than the specialist *N. ribisnigri*, regardless of the chicory genotype. This outcome first highlights that *N. ribisnigri* appeared better adapted to the *Cichorium* genus, having been reared on lettuce (*L. sativa*) for years and then on *C. endivia* for several weeks.³⁵ Moreover, it is consistent with the expectation that generalists are more strongly affected by plant constitutive defenses⁶⁶ such as STLs,⁶⁷ which specialists can tolerate or adapt to. Moreover, the unchanged performance of *M. persicae* across host plant genotypes observed here may reflect not only its physiological plasticity,⁶⁸ but also its behavioral flexibility, despite the intraspecific variations of the host plant.^{69,70} Such results suggest that mixing chicory genotypes with complementary aerial and root resistance may reduce aphid pressure by disrupting the colonization of specialist herbivores that benefit from homogeneous hosts, as currently practiced in wheat and rice through the use of cultivar mixtures.⁷¹

5 CONCLUSION

Our study revealed contrasting aphid performance and feeding behaviors between genotypes and organs, suggesting that the *B18* genotype selected for root resistance may be more susceptible to aerial aphids than *AX*. However, resistance was inferred indirectly from aphid traits rather than direct measurements of defensive compounds, and plants were exposed to single herbivores under controlled conditions, providing only partial support for the ODT. Despite these limitations, our findings highlight important implications for breeding, as selecting for root protection may increase vulnerability of aboveground tissues and affect the commercial value of chicory, cultivated for both leaves and roots. Increasing inter- and intra-row genetic diversity by combining genotypes with complementary root and leaf resistance could help limit specialist herbivore colonization and mitigate pest risks, as already observed in wheat and rice cultivar mixtures. Future studies should quantify defensive metabolites and assess simultaneous infestations under field conditions to validate these patterns.

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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

The authors declare no conflict of interest.

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