Consequences of chemical novelty: Field performance of the cardenolide-producing crucifer *Erysimum cheiranthoides* under herbivore attack and heterospecific competition.

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Abstract

1. Plants in the Brassicaceae produce glucosinolates as potent defences against generalist herbivores, but many specialists have evolved the ability to tolerate or even deactivate these compounds. As a possible co-evolutionary response to attack by specialists, plants in the genus *Erysimum* gained the ability to produce novel cardenolide defences in addition to glucosinolates.
2. We grew the dually defended *E. cheiranthoides* side-by-side with the phenologically similar *Brassica nigra* in agricultural fields surrounded by other Brassicaceae crops. Over the course of two field seasons, we monitored focal plants to characterise their associated herbivore community, and we quantified herbivore damage and measured plant growth. In the second year, we additionally attempted to manipulate herbivore density and plant competition to identify direct and indirect effects on plant fitness.
3. Both plant species were attacked by a diverse community of specialist herbivores, likely already present on the surrounding Brassicaceae crops. Most herbivores strongly favoured *B. nigra*, reaching higher densities and causing intense damage that largely suppressed growth of

these plants. A similar community of herbivores attacked *E. cheiranthoides*, but at much lower densities that caused little damage and did not impact plant growth.

4. In accordance with differential susceptibilities to herbivores, herbivore removal benefitted *B. nigra* by reducing damage and increasing plant growth, while it had no positive effect on *E. cheiranthoides*. In fact, herbivore removal had an indirect negative effect on *E. cheiranthoides* plants growing in conspecific pairs, as they suffered from increased competition by otherwise herbivore-suppressed *B. nigra*.

Synthesis: Our results agree with a successful escape of *E. cheiranthoides* from herbivory, as even though plants are still attacked when herbivores are abundant, these herbivores cause little damage and cause no reduction in fitness. However, the escape from herbivory has likely come at loss of competitive ability when herbivore pressure is low, which would explain why despite its key innovation, *E. cheiranthoides* has been unable to gain dominance in any plant community until now.

Keywords: Enemy-free space, chemical novelty, plant defence, herbivore-imposed selection

1. Introduction

In nature, plants are constantly threatened by herbivores, which consume a significant proportion of plant biomass across ecosystems and cause substantial yield losses in agriculture (Johnson, 2011). To protect their limited resources, plants have evolved multiple layers of defence, including physical barriers and chemical defences (War et al., 2012). Chemical compounds in particular play a central role in plant-herbivore interactions, as they can shape the community of herbivores that attack a plant, and in turn are shaped by herbivore-imposed selection (Agrawal, Hastings, et al., 2012; Züst et al., 2012). As a result of reciprocal adaptations between plants and their herbivores, many plants have evolved a vast diversity of toxic, repellent, or antinutritive compounds (Speed et al., 2015).

Resistance traits of plants, including chemical defences, require a significant investment of energy and resources by the plant. For example, production of defensive glucosinolate compounds by

plants in the Brassicaceae has been estimated to consume 15% of a plant's total photosynthetic energy (Bekaert et al., 2012). Defence investments therefore represent a substantial cost for plants, both by decreasing resource allocation to other plant functions (direct or allocation costs, Herms & Mattson, 1992; Züst & Agrawal, 2017), and by indirectly decreasing plant fitness through altered interactions with other species (ecological costs, Strauss et al., 2002). Importantly, both costs and benefits of defence are context dependent, and even direct costs may vary depending on the biotic and abiotic environment. For example, the growth-differentiation balance (GDH) hypothesis predicts a physiological trade-off between faster growth and higher defence levels (Herms & Mattson, 1992), yet rapid growth is primarily essential during plant establishment in highly competitive environments (Züst et al., 2011), while costs may be lower under different conditions. Similarly, high defence investments should only be beneficial in the presence of herbivores susceptible to that defence, while a lack of susceptible herbivores should favour less defended plants (Simms & Rausher, 1987).

The interplay between competition, defence, and herbivory has been well-documented in experimental studies (Hambäck & Beckerman, 2003), where the manipulation or exclusion of herbivores commonly results in substantial shifts in defence trait expression and competitive interactions among plants (Mauricio & Rausher, 1997; Züst et al., 2012). Interestingly, natural gains of novel defences may have analogous consequences to artificial herbivore exclusion: following the gain of a potent new defence, plants may at least temporarily escape herbivore-imposed selection, which likely will alter their competitive interactions and may allow plants to colonize new environments ("escape and radiate", Ehrlich & Raven, 1964).

For example, plants in the order Brassicales evolved potent glucosinolate defences approximately 80 million years ago, with subsequent biosynthetic innovations in defence triggering bouts of speciation (Edger et al., 2015). However, specialist herbivores eventually evolved strategies to disarm and overcome this defence over evolutionary time (Ratzka et al., 2002; Sporer et al., 2021; Wittstock et al., 2004). As a result, extant species of the Brassicaceae are typically attacked by a diverse community of glucosinolate-resistant herbivores (Bidart-Bouzat & Kliebenstein, 2008; Mertens et al., 2021). However, despite this apparent ineffectiveness against specialist herbivores, most Brassicaceae species nonetheless produce a diverse arsenal of glucosinolate defences (Fahey et

al., 2001), highlighting the challenges of inferring ancient evolutionary processes from current ecological interactions.

Gradual co-evolutionary adaptations might feasibly mask the processes that initially shaped new resistance traits, thus focusing on traits of recent evolutionary origin may provide a practical means to explore the costs and benefits of plant resistance before specialized antagonists could evolve. In one such example of a recent gain of a novel defence, plants of the genus *Erysimum* (Brassicaceae) evolved the ability to produce cardenolide compounds within the last 5 million years (Züst et al., 2020). Cardenolides are a potent class of toxins with distinctly different mode of action from glucosinolates (Agrawal, Petschenka, et al., 2012), and consequently at least some glucosinolate-resistant herbivores are completely repelled from *Erysimum* plants by these compounds (Sachdev-Gupta et al., 1993; Younkin et al., 2024; but see Wang & Züst, 2025). This 'escape' from herbivores may have resulted in a recent and rapid radiation in this speciose genus (Züst et al., 2020). Most *Erysimum* species co-express glucosinolate and cardenolide defences, but while variation in glucosinolates is limited among species and appears to have become fixed within at least some species (T. Züst, unpublished), variation in cardenolides is much more extensive (Züst et al., 2020), and may reflect more diverse selection pressures acting on the novel defence. To the best of our knowledge, no herbivore has evolved specialised resistance to cardenolides in the *Erysimum* system, and only one cardenolide-specialised seed bug has shifted its host range to include seeds from these plants (Petschenka et al., 2022). The *Erysimum* system thus offers a unique opportunity for studying the interplay between herbivory, defence, and fitness, before interactions are changed by coevolution.

Here we use the annual plant *Erysimum cheiranthoides* to characterise the natural community of herbivores that attack this plant in central Europe. We aim to address whether the gain of cardenolides has allowed this plant to escape from herbivory in a natural community, and to understand the selective forces that act on this species in its putative 'enemy-free space' (Jeffries & Lawton, 1984). We compared benefits of the novel defence under high herbivore pressure to potential costs of defence under high competition. To this end, we grew the dually defended *E. cheiranthoides* side-by-side with a phenologically similar Brassicaceae plant (*Brassica nigra*) over two consecutive years. Plants were grown in fields adjacent to oilseed rape (*Brassica napus*), which provided a high

density of herbivores specialized on Brassicaceae. We monitored herbivore abundance and plant damage for a full growing season in both years. In the second year we included insecticide treatments to manipulate the costs and benefits of differential investments in resistance traits, and evaluated the performance of both plant species under different levels of competition

We hypothesise that due to its novel cardenolide defence, *E. cheiranthoides* is better defended and damaged less by herbivores, and consequently achieves a higher fitness compared with *B. nigra*. Secondly, we predict that the dual investment in defence may render *E. cheiranthoides* competitively inferior to *B. nigra* when herbivore pressure is low.

2. Methods

2.1 Plant material

Erysimum cheiranthoides is an annual plant that is native to central and northern Europe and parts of Asia (Polatschek, 2010, 2013), where it grows in scattered populations in ruderal habitats. Plants commonly flower from May through September, with peak flowering in June and seed ripening in August to October (Polatschek, 2013; T. Züst, personal observations). For this study, we used a total of 38 accessions of *E. cheiranthoides*, which were originally collected from locations in western and central Europe, and which we had propagated in a common greenhouse environment for two generations. To contrast patterns of herbivory on *E. cheiranthoides* to a more typical Brassicaceae plant, we included the annual *Brassica nigra* as a control. *B. nigra* is a representative member of the Brassicaceae that is well-liked by many glucosinolate-resistant herbivores, which shares a similar phenology, and which grows at a similar rate and reaches a similar size as *E. cheiranthoides* under field conditions. Seeds of *B. nigra* used in this study were originally provided by the Botanical Garden of Krefeld, Germany. For the current study, we focus on species-level patterns between *E. cheiranthoides* and *B. nigra*, thus we treat accessions of *E. cheiranthoides* as equivalent.

Plants for field experiments were sown in late April for transplantation to the field in late May. We soaked seeds of *E. cheiranthoides* in water and kept them at 4 °C for three days to break dormancy. Three soaked seeds were placed on the soil surface of pots (7 × 7 × 8 cm) filled with a 1:1 mixture of washed sand and peat-based planting substrate (Einheitserde, Patzer Erden GmbH, Germany). Seeds of *B. nigra* were directly sown into pots without pre-treatment and lightly covered with soil. Pots were covered with transparent plastic hoods for one week to maintain high humidity, and seeds were germinated in a growth chamber (constant light, 23 °C, 60% relative humidity). Seven days after sowing, plants were moved to an open greenhouse and shaded from direct sunlight for continued growth and hardening. In the last week of May, three-week old plants were transplanted into freshly tilled fields at the Swiss federal agricultural research station (Agroscope Reckenholz, Switzerland). Plants were transplanted to the field inside their plastic pot to slow the escape of growing roots and to limit excessive biomass growth in the field. A small set of plants was kept in the

greenhouse for an additional week and used for the assessment of leaf traits (trichome density and size) and innate herbivore preference under laboratory conditions (see Supplementary Methods).

2.2 Field experiments

2.2.1 Experiment 1: 2022 herbivory

In 2022, we used a rectangular field site measuring 6 m × 80 m in a SW to NE orientation (Supplementary Figure S1a, 47.4376° N, 8.5268° E), located alongside a larger field of oilseed rape (*Brassica napus*). On either side of the experimental plot, 1 m-wide buffer strips of a commercial cover crop mixture consisting of *Raphanus sativus* and *Avena strigosa* had been sown two months earlier. We divided the experimental plot into ten blocks (5 × 7 m), and in each block we planted 38 *E. cheiranthoides* and 4 *B. nigra* plants in a randomized grid pattern, with plants spaced 1 m apart and with a 0.5 m distance to the plot edge on each side. Throughout the experiment, plants were watered as needed, and the surrounding soil was weeded regularly and kept free of other vegetation. Unfortunately, cover strips had to be mowed halfway through the experiment (week 5) to prevent weed seed dispersal.

We closely monitored plants over a period of nine weeks. In weeks 3, 6, and 9, we assessed plant damage by herbivores, and in the six remaining weeks, we recorded the herbivores that were present on each experimental plant. In weeks of herbivore community assessments, we visited each plant once and recorded the type and number of herbivores sitting or feeding on the plant. Herbivores were recorded at species level where visual identification was feasible, and as functional groups otherwise (e.g., flea beetles, weevils). Assessment of all plants was completed over 2-3 subsequent days in each week. In weeks of damage assessment, we visited each plant once to record leaf damage and plant size. We approximated total damage by counting the number of leaves on a plant with visible herbivore damage of any size, and we approximated plant size by counting the total number of leaves, as well as measuring the height of the plant stem from root base to apical meristem. A representative sample of leaves were harvested and analysed to determine nutritional quality of plants throughout the 2022 experiment. Briefly, leaves were frozen on dry ice and later freeze-dried for 48h.

Dried leaf material was then ground to a fine powder and extracted for determination of soluble sugars, digestible starch, and total digestible proteins (see Supplementary Methods).

Whole plants were sequentially harvested for biomass, with complete blocks assigned to different harvests. Block 7 was randomly selected for plants to complete development to seed production. Plants in blocks 1-3 were harvested in week 8 to capture early plant biomass, mostly before seed setting. Plants in blocks 4-6 were harvested in week 11, and plants in blocks 8-10 were harvested in week 14. A few plants had stopped vegetative growth and completely ripened their seed earlier, thus, to prevent loss of biomass we harvested these individuals ahead of schedule. For biomass harvesting, we cut the stem of each plant at soil level and placed the entire aboveground biomass in paper bags. Aboveground biomass was dried at 60°C for 48-96 hours and weighed to the nearest gram. Plants in block 7 were dug out of the soil in week 8 and transported back to a greenhouse where we let them finish their development and ripen their seeds. At the onset of seed ripening, each plant was wrapped in a paper bag to prevent seed loss. After harvesting, seeds were separated from vegetative biomass, biomass was dried and weighed, and seed mass was weighed separately for each plant in this block. Finally, we counted and weighed a subset of 100 seeds for each plant to determine average seed size.

2.2.2 Experiment 2: 2023 herbivory and herbivore exclusion

Based on the results of the 2022 field experiment, we set up a modified field experiment in 2023 with added treatments of plant competition and herbivore suppression. To test for the effect of plant-plant competition, a subset of *E. cheiranthoides* plants was grown together with a *B. nigra* plant in the same 7 x 7 x 8 cm pot. To reduce herbivore loads, half of all plants (individual and competing) were additionally treated with weekly applications of lambda-cyhalothrin (Kendo® Gold, Maag Gardens, Switzerland), a non-systemic pyrethroid insecticide.

In 2023, we used a different rectangular field site measuring 4 m × 66 m in a W to E orientation (Supplementary Figure S1b, 47.4284° N, 8.5232° E), again located alongside a larger field of oilseed rape (*Brassica napus*). On either side of the experimental plot, 1.5 m-wide buffer strips of the same cover crop mixture had been sown one week prior to transplanting, which were maintained

throughout the experiment. We divided the experimental plot into six blocks (3 × 7 m). For blocks 1, 2, 5, and 6, we planted 36 individual *E. cheiranthoides* and 4 *B. nigra* plants each in a stratified random grid pattern, with plants spaced 1 m apart and with a 0.5 m distance to the plot edge on each side. For blocks 3 and 4, we planted 40 pairs of *E. cheiranthoides* and *B. nigra* plants each, with each pair spaced apart from the next by 1 m (Supplementary Figure S1b). Starting at transplanting, we applied weekly sprays of lambda-cyhalothrin (15 mg/L water) to all plants in blocks 2, 4, and 6, completely wetting all leaf surfaces. We maintained spraying for seven consecutive weeks, increasing the total volume sprayed as plants were growing larger, from approximately 30 mL per plant in the first three weeks up to 150 mL per plant by week 7. Plants in blocks 1, 3, and 5 were sprayed with an equivalent amount of water.

Herbivore community assessments were carried out as before (weeks 1, 2, 4, 5, 7, 8), but we modified our damage assessment (weeks 3, 6, 9) to distinguishing leaves with high (leaves with more than 75% of area damaged) and low damage. With damage assessments, we also measured plant height and the total number of leaves. However, total leaf counts had high levels of observer bias for older (and therefore larger) plants in 2022, thus for weeks 6 and 9, we instead measured plant diameter and branch number. We additionally counted the total number of leaves on 20 randomly selected plants in weeks 6 and 9, and from this subset generated a predictive linear model of total leaf number as a function of plant height, diameter, branch number, assessment week and observer. We then used this model to predict the total number of leaves for all remaining plants. In the 2023 field experiment, all plants were harvested in week 10. We cut plants at soil level and placed the entire aboveground biomass in paper bags, followed by drying at 60°C for 48-96 hours and weighing to the nearest gram.

2.3 Statistical analyses

The current study is focused on differences between *E. cheiranthoides* and *B. nigra*, while variation among *E. cheiranthoides* accessions will be presented elsewhere. For the 2022 field experiment, we use herbivore community, damage, and plant size data from all 40 *B. nigra* plants and 40 neighbouring *E. cheiranthoides* plants. Neighbours were selected randomly out of the maximum of

four immediate neighbours for each *B. nigra* plant (Supplementary Figure S1a). For the smaller 2023 field experiment, we use data from all 16 individual *B. nigra* plants and 32 randomly selected neighbouring individual *E. cheiranthoides* plants. Two *E. cheiranthoides* neighbours were selected for this dataset to avoid issues with small sample sizes (Supplementary Figure S1b). We also included data from all 80 plant pairs in the competition treatment. All analyses were performed in the statistical software R v.4.3.1 (R Core Team, 2023).

2.3.1 Herbivore community composition and phenology

We summarized herbivore communities by comparing mean total herbivore abundances across entire seasons between plant species. For the 2023 herbivore data, we performed separate principal component analyses (PCAs) for *E. cheiranthoides* and *B. nigra* to evaluate similarity in herbivore communities among insecticide and competition treatments (Supplementary Figure S2). As the competition treatment had little effect on herbivore community composition, we only used individual plant data for these analyses.

We first analysed the total number of individuals observed on each plant as a function of plant species, using separate negative-binomial Generalized Linear Models (GLM, function 'glm.nb' in the *MASS* package, (Venables & Ripley, 2002)) for each year, and including insecticide treatment as an explanatory variable for 2023. To examine temporal variation in herbivore presence and abundance, we next employed a series of Generalised Additive Models (GAMs) using the 'gam' function in the *mgcv* package (Wood et al., 2016), fitting separate negative binomial GAMs to total herbivore counts in 2022 and 2023. For the 2022 data, we constructed a full model that included a parametric term of 'plant species' and a smoothing term (based on thin plate regression splines) for 'days in the field' that was allowed to vary by plant species (i.e. separate smoothers for each plant species). We determined the number of nodes (parameter *k* in GAM) by subtracting 1 from the total number of weeks insects were observed in the field. To estimate the significance of the parametric term we used the default Wald tests on deviance explained. To evaluate the support for species-specific smoothers, we constructed equivalent models without interaction terms and compared nested models based on BIC. For the 2023 data, we included an additional parametric term of 'insecticide treatment' and a

modified smoother allowing for an interaction between ‘days in the field’ and all four combinations of ‘plant species’ and ‘insecticide treatment’.

We finally fit a set of models to the individual presence and abundance data for the eight most abundant herbivores in both years. Data from the 2023 insecticide and competition treatments was excluded from this analysis to simplify results. Herbivore abundance was analysed again using negative binomial GAMs, while we also analysed herbivore presence-absence data (i.e., the likelihood of occurrence) using binomial GAMs. To avoid problems with excess zeros, we only included data from weeks in which an herbivore was observed more than once in the dataset, adjusting the number of nodes accordingly for each herbivore (Supplementary Table S2).

2.3.2 Plant damage assessments

The amount of leaf damage was analysed by fitting a Generalized Linear Mixed-effects Model (GLMM) using the glmer function in the *lme4* package (Bates et al., 2015). To account for the paired design of the competition treatment in 2023, we specified ‘neighbourhood group’ as a random effect for all models. In the competition treatment, neighbourhood group refers to individual pots with pairs of plants, whereas in the individual treatment, neighbourhood group refers to three adjacent pots each with a single plant (one *B. nigra* plant randomly paired with one or two *E. cheiranthoides* neighbours). For the 2022 field experiment, we used the numbers of damaged and undamaged leaves for each plant as a two-vector dependent variable (i.e., a binomial variable representing the proportion of leaves with insect damage). The model included fixed factors of assessment week, plant species, and their interaction, while individual plant, neighbourhood group, and observer were specified as random effects. To obtain p-values of the fixed effects, we used type III tests implemented in the ‘Anova’ function from the *car* package (Fox & Weisberg, 2019). Post hoc pairwise comparisons (Tukey’s HSD; $\alpha = 0.05$) were carried out using the *emmeans* package (Lenth, 2023).

The amount of leaf damage in the 2023 field experiment was analysed using a similar approach. We analysed the number of highly damaged leaves (leaves with more than 75% of area damaged) and the number of undamaged/low damage leaves as the two-vector dependent variable, with fixed effects of plant species, insecticide treatment, competition treatment, and their interactions.

As we did not quantify damage of plants in the competition for week 9, an interaction with assessment week could not be fit, and we instead fit three separate models for weeks 3, 6, and 9, including only random effects of neighbourhood group.

2.3.3 Correlations of herbivore observations and plant damage

Higher observed herbivore densities are expected to be correlated with higher plant damage, with the caveats that our observations only represent snapshots of the diurnal herbivore community, observed herbivores may not always have been actively feeding on the plants, and not all observed herbivores cause visible damage. To evaluate the relationship between observed herbivores and quantified damage, we combined data of the second damage quantification (week 6) with the abundances of the main herbivores in the two preceding weeks for both years. After centering and scaling of all data, we performed separate PCAs for *B. nigra* and *E. cheiranthoides* in 2022, and for *B. nigra* and *E. cheiranthoides* in ambient and insecticide-treated conditions in 2023, allowing us to compare correlations between plant damage and abundance of specific herbivore groups.

2.3.4 Herbivore impact on plant growth

Herbivore numbers dropped substantially following the early mowing of buffer strips in the 2022 field experiment. To test whether this change in herbivore pressure affected plant growth, we quantified plant growth using repeated plant height measurements from weeks 3, 6, and 9 to identify changes in herbivore impact (see Supplementary Methods). In addition to height data, we analysed biomass of plants harvested in weeks 8, 11, and 14 using a linear model (function ‘gls’ in the *nlme* package, Pinheiro, J. C. et al. 2021) of dry biomass as dependent variable and factors of plant species, harvest week, and their interaction as explanatory variables. Model fit was improved by allowing for separate variances for each harvest week (function ‘varIdent’ in the *nlme* package). Post hoc pairwise comparisons were carried out using *emmeans.*

2.3.5 Effects of herbivory and competition on plant biomass

We explicitly manipulated herbivore densities by insecticide applications in the 2023 field experiment and analysed plant biomass of plants harvested after 10 weeks using a linear mixed-effects model (function 'lme' in the *nlme* package). Dry biomass was fit as a function of plant species, insecticide treatment, competition treatment, and their interactions as fixed effects, and neighbourhood group as random effect. Model fit was improved by allowing for separate variances for each combination of plant species and insecticide treatment (function 'varIdent' in the *nlme* package). Post hoc pairwise comparisons for both models were carried out using *emmeans*.

3. Results

3.1 Herbivore community composition and phenology

We observed 14 different herbivores in 2022, with eight out of the nine most abundant herbivores occurring in substantially higher numbers on *B. nigra* than on *E. cheiranthoides* (Supplementary Table S1). Total herbivore abundance reached a peak in the fourth week, followed by a decline in the second half of the experiment (Figure 1a). This decline coincided with the removal of the buffer strips on each side of the experimental plot. We modelled herbivore abundance through time using a generalised additive model (GAM) and found a strong main effect of plant species ($X^2 = 435.30$, df = 1, $p<0.001$), reflecting the overall difference in abundance. Model comparison also revealed support for a plant-specific relationship of total herbivore abundance through time (Figure 1a, Supplementary Table S2).

Individual herbivore species differed substantially in arrival and persistence time (Figure 1b), and even though herbivores generally were co-occurring, temporal dynamics significantly differed between plant species (Supplementary Figures S3, S4). Using individual GAMs for presence and abundance data of the eight most abundant herbivores, we found significant main effects of plant species, support for plant-specific temporal relationships (i.e., plant-specific smoothers in GAMs), or both for all herbivores (Supplementary Table S3). While results differed in magnitude and significance by herbivore, we generally found a higher likelihood of herbivore occurrence, higher herbivore abundance, and longer herbivore persistence on *B. nigra* compared to *E. cheiranthoides*.

The largest exception to this pattern were the crucifer shield bugs or cabbage bugs (adult *Eurydema ornata*, *E. oleracea*, and their larvae), which occurred at similar frequencies on both plants (Supplementary Table S1), and at least during peak herbivore abundance, had a higher likelihood of occurrence on *E. cheiranthoides* (Supplementary Figure S3).

The observed herbivore community in 2023 was very similar to 2022, except for three low-abundance species that were only observed in 2022. Again, seven out of the eight most abundant herbivores were less abundant on *E. cheiranthoides* (Supplementary Table S1). As the buffer strips remained until the end of the experiment in this experiment, herbivores persisted for longer and we observed no pronounced decline in total abundance (Figure 1c). Surprisingly, the insecticide treatment in 2023 did not reduce the total observed number of herbivores (Figure 1d), but instead increased the observed number of aphids, pollen beetles, and flea beetles (Supplementary Table S1). Modelling temporal dynamics in total herbivore abundance through time, we found strong support for main effects of plant species (X^2 = 127.33, df = 1, p<0.001) and insecticide treatment (X^2 = 31.33, df = 1, p<0.001), but model comparison revealed no support for separate temporal relationships for either plant species or insecticide treatment (Supplementary Table S2).

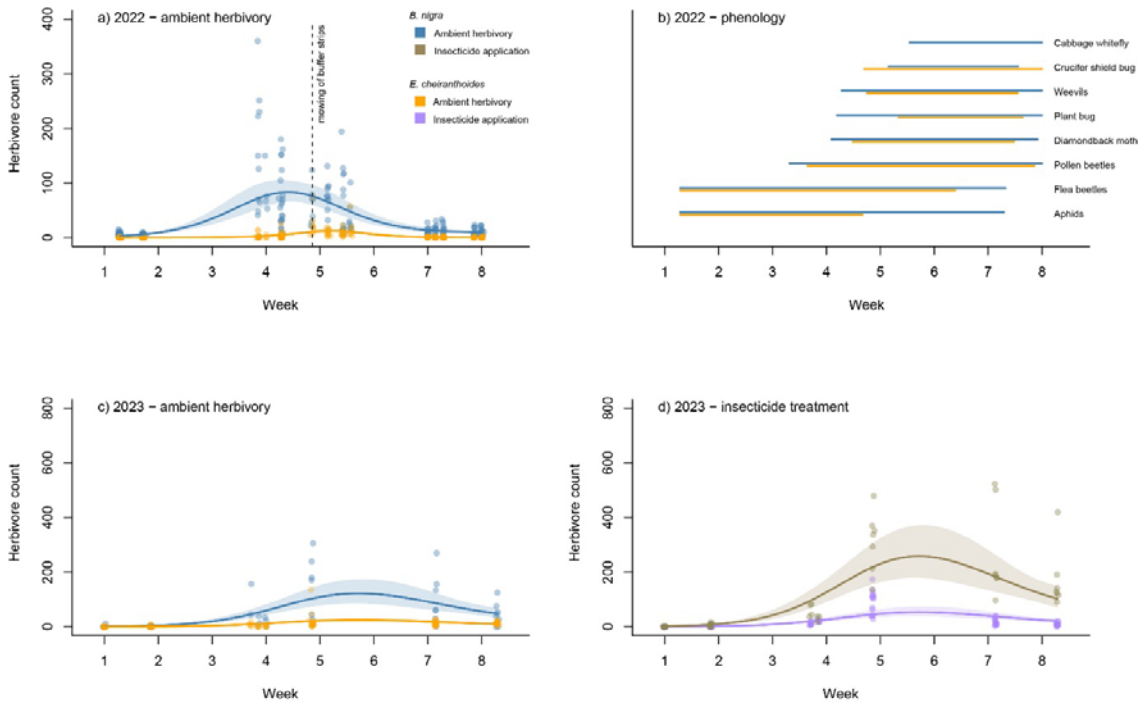

Figure 1. a) Total herbivore counts found on *B. nigra* and *E. cheiranthoides* in the 2022 field experiment. Points are observations on individual plants at a given time. Lines and shaded areas are the mean model predictions and 95% confidence intervals from a generalized additive model. The dashed line highlights the mowing of buffer strips next to the field experiment, which supported a high specialist herbivore density. b) Summarized phenology of the main herbivores observed in the 2022 field experiment. Lines connect the first and last day for which the predicted likelihood of herbivore presence (Supplementary Figure S3) was > 0.1 (more than 10% of plants occupied). c) Total herbivore counts found on untreated plants ('ambient herbivory'), and d) total herbivore counts found on insecticide-treated plants in the 2023 field experiment. All models used 'days in the field' as temporal variable, but axes show 'week' for easier comparison to other results.

Presence and abundance of individual herbivores also differed by plant species in 2023 (Supplementary Figures S5, S6), with significant main effects of plant species, support for plant-specific temporal relationships, or both for all species (Supplementary Table S3). Aphids and flea beetles persisted for much longer in 2023, likely relying on buffer strips for population maintenance. As in 2022, total abundance of crucifer shield bugs did not differ between *B. nigra* and *E. cheiranthoides* (Supplementary Table S1), and we found weak evidence for a higher abundance of this herbivore on *E. cheiranthoides* towards the end of the 2023 field experiment (Supplementary Figure S6, Table S3).

3.2 Plant damage

Mirroring the results in herbivore abundance, leaf damage on *B. nigra* was substantially higher than on *E. cheiranthoides* in the 2022 field experiment (Figure 2, Supplementary Table S4). Damage to *B. nigra* was almost complete with virtually no leaf left undamaged, while less than 20% of leaves were damaged on *E. cheiranthoides*. Damage marginally increased on *E. cheiranthoides* by week 6 (week 3 vs. 6: z-ratio = -2.11, p = 0.088), before dropping considerably in week 9 for both species (Figure 2).

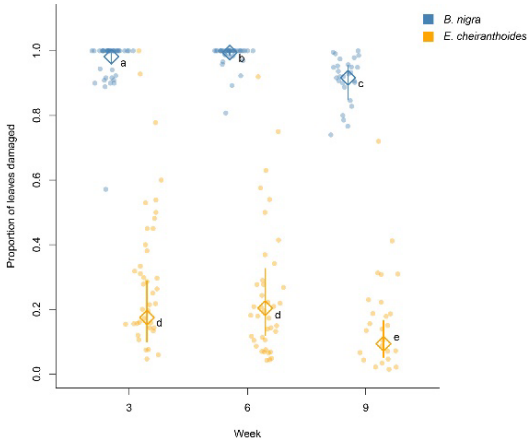

Figure 2. The proportion of damaged plants in plants of the 2022 field experiment. Points are values for individual plants, while diamonds and vertical lines are the means and 95% confidence intervals from a binomial GLMM. Letters highlight significant linear contrasts at the $p < 0.05$ level.

In the 2023 field experiment, quantified leaf damage on *B. nigra* plants exposed to ambient herbivory was again almost complete (i.e., all leaves had some damage), thus, to avoid issues with zero variance in the smaller dataset, we analysed the proportion of heavily damaged leaves (leaves with more than 75% area damaged). Quantified damage was still substantially higher on *B. nigra* compared to *E. cheiranthoides* (Figure 3, Supplementary Table S4). Insecticide treatment strongly reduced damage on *E. cheiranthoides* plants in weeks 3 (ambient vs. sprayed: z-ratio = 6.36, $p < 0.001$) and 6 (ambient vs. sprayed: z-ratio = 6.13, $p < 0.001$), while this effect disappeared by week 9 (ambient vs. sprayed: z-ratio = -0.77, $p = 0.437$). In contrast, insecticide only significantly reduced damage on *B. nigra* in week 9 (ambient vs. sprayed: z-ratio = 2.47, $p = 0.013$). Interestingly, while plant-plant competition did not significantly affect damage in week 3 (*B. nigra*: z-ratio = -1.82, $p = 0.070$; *E. cheiranthoides*: z-ratio = -1.16, $p = 0.245$), by week 6 *B. nigra* received significantly less damage in the combined competition-insecticide treatment (Figure 3).

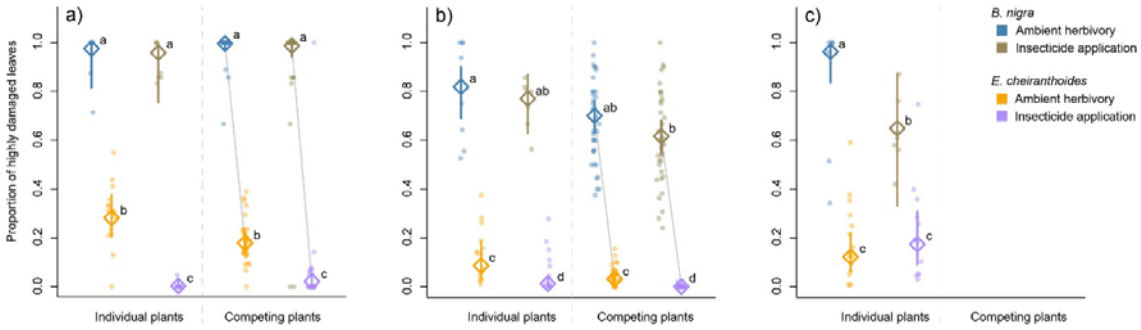

Figure 3. The proportion of highly damaged leaves (leaves with more than 75% area damaged) on plants in a) week 3, b) week 6, and c) week 9 of the 2023 field experiment. Points are values for individual plants, while diamonds and vertical lines are the means and 95% confidence intervals from week-specific binomial GLMMs. Letters highlight significant linear contrasts at the $p < 0.05$ level. Means of plants in the competition treatment are connected by grey lines to show the paired experimental design. In week 9, damage was only quantified on individual plants.

Correlation of herbivore abundances in weeks 4 and 5 with plant damage in week 6 revealed that in the 2022 field experiment, leaf damage on *B. nigra* was most closely correlated with abundances of *P. xylostella* (Supplementary Figure S7a), while damage on *E. cheiranthoides* was correlated with abundances of the crucifer shield bug (Supplementary Figure S7b). In the 2023 field experiment, patterns under ambient herbivory were similar, with abundances of *P. xylostella* and crucifer shield bugs being among the herbivores most correlated with leaf damage on *B. nigra* and *E. cheiranthoides*, respectively (Supplementary Figure S7c-d). Insecticide application changed this relationship, with abundances of most observed herbivores varying independently of damage for *B. nigra* (Supplementary Figure S7e), and aphids (causing no visual damage) being most correlated with damage for *E. cheiranthoides* (Supplementary Figure S7f). Interestingly, pollen beetles (*Brassicogethes aeneus*) were associated with leaf damage for both species in most PCAs, despite not being typical leaf-chewing herbivores.

3.3 Plant traits and innate herbivore preference

The two plant species used in this experiment differ in a wide range of other traits besides cardenolide production. Nutritional traits quantified in the 2022 field experiment showed consistent but modest

differences between the two species (see Supplementary Results, Figure S8). In contrast, trichome density and size varied substantially, with leaves of *E. cheiranthoides* being densely covered in small trichomes on both sides, while *B. nigra* primarily expressed larger trichomes along leaf veins on the underside of its leaves (see Supplementary Results, Figure S9). We further compared the role of leaf trait differences on innate herbivore preference using cut leaf disc choice assays under laboratory conditions, allowing us to exclude more complex traits such as plant size or leaf shape. Of the four tested Brassicaceae herbivores (two of which we also observed feeding in the field), each significantly preferred *B. nigra* over *E. cheiranthoides* (Supplementary Figure S10). Interestingly, this preference was least pronounced for *E. ornata*, mirroring the weaker preference for *B. nigra* of this herbivore in the field.

3.4 Plant growth and competition

To better understand herbivore impact on plants, we modelled plant growth in the 2022 field experiment using sequential measurements of stem height. We found strong differences between height growth of *B. nigra* and *E. cheiranthoides* (Figure 4a), with *E. cheiranthoides* reaching the inflection point of a logistic growth function after 29 days, while *B. nigra* reached this point only after 56 days (Supplementary Table S5). During the first growth interval (weeks 3-6, high herbivore abundance), *B. nigra* plants only grew 0.31 (0.27-0.36, 95% confidence interval) cm day^{-1}, compared to *E. cheiranthoides* growing more than 5 times faster by this point (1.74 [1.53-1.93] cm day^{-1}). Growth of *B. nigra* only increased towards the end of the experiment when herbivore numbers decreased, and by the midpoint of the second growth interval (weeks 6-9, low herbivore abundance) the order was reversed, with *B. nigra* growing 1.30 (1.11-1.51) cm day^{-1} and *E. cheiranthoides* only growing 0.86 (0.75-0.95) cm day^{-1}.

The patterns in height growth of plants in the 2022 field experiment directly translated into differences in aboveground biomass (Figure 4b). In the first subset of plants harvested after eight weeks, *B. nigra* had significantly lower biomass compared to *E. cheiranthoides* (pairwise linear contrast: $t = -4.33$, $df = 22$, $p < 0.001$). Mirroring the increased height growth of *B. nigra* in the second

half of the experiment, these differences had disappeared by week 11 (t = -0.43, df = 21, p = 0.676) and 14 (t = -0.019, df = 17, p = 0.985).

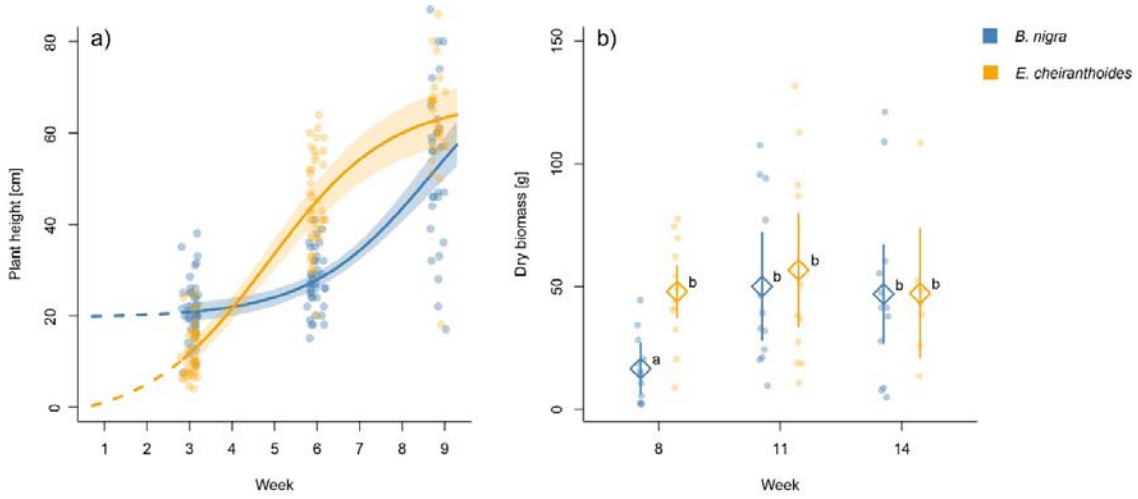

Figure 4. a) Height growth over nine weeks for *B. nigra* and *E. cheiranthoides* in the 2022 field experiment. Points are individual plant values and solid lines are model fits from a logistic regression. Shaded areas are the 95% confidence intervals (population prediction intervals). Plant height was first recorded in the third week, resulting in greater uncertainty for the initial growth period (dashed lines). b) Total aboveground biomass of subsets of plants harvested after 8, 11, and 14 weeks in the 2022 field experiment. Diamonds and lines are means and 95% confidence intervals from a linear model. Letters denote significant linear contrasts at the p<0.05 level.

Based on the strong impacts of herbivores on plant biomass we observed in 2022, we explicitly manipulated herbivore pressure and competitive environment in the 2023 field experiment. For single plants under ambient herbivore pressure, biomass of *B. nigra* plants after 10 weeks of growth was close to zero, while *E. cheiranthoides* plants could grow seemingly unimpaired (Figure 5). Even though application of insecticide did not remove herbivores (Figure 1c-d) and only partially reduced observed damage on *B. nigra* (Figure 3), insecticide-treated *B. nigra* plants grew better and reached a higher biomass (pairwise linear contrast: t = -3.37, df = 80, p = 0.006). In comparison, *E. cheiranthoides* gained no equivalent benefit from insecticide application (t = -1.05, df = 80, p = 0.723).

Growing in intense competition with *E. cheiranthoides* had little effect on *B. nigra* biomass, which again suffered heavily under ambient herbivory, but benefitted from insecticide applications (Figure 5). Biomass of *E. cheiranthoides* growing in competition with *B. nigra* under ambient herbivory was likewise largely unchanged, but application of insecticide substantially reduced *E. cheiranthoides* biomass ($t = 2.72$, $df = 80$, $p = 0.039$), likely as a direct result of increased competition from less-attacked *B. nigra* plants. A reduction in biomass has direct fitness consequences for an annual plant such as *E. cheiranthoides*, and we found a strong positive correlation between aboveground biomass and seed production among the plants we left to complete seed production in 2022 (Supplementary Figure S11).

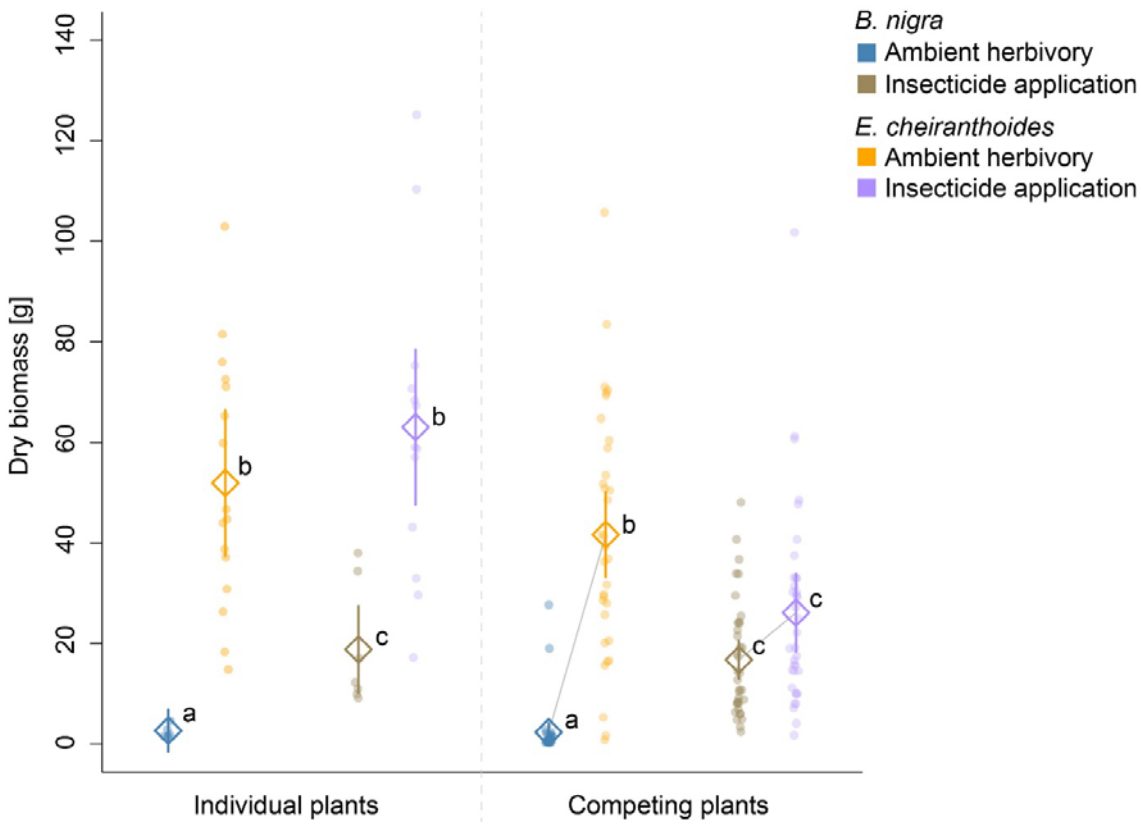

Figure 5. Biomass of individual (left) and competing (right) *B. nigra* and *E. cheiranthoides* plants harvested after 10 weeks of growth in the 2023 field experiment. Plants were growing individually or as conspecific pairs in buried pots, separated from the nearest neighbour by 0.5 m in all directions. Points are individual plant

values, while diamond symbols and lines are means and 95% confidence intervals from a linear model. Letters denote significant linear contrasts at the $p<0.05$ level.

4. Discussion

The dually defended *E. cheiranthoides* was attacked by substantially fewer herbivores than its Brassicaceae relative *B. nigra* in two years of field observations. Herbivores caused severe damage to leaves of *B. nigra*, and in consequence, growth of *B. nigra* was almost completely suppressed by herbivory when insect herbivores were abundant. In contrast, even during peak abundance, the limited herbivore damage on *E. cheiranthoides* had no apparent impact on plant growth, matching a successful escape from herbivore-imposed selection. Experimental manipulation of herbivore abundances and plant-plant competition not only revealed a lack of a negative impact of herbivore selection on *E. cheiranthoides*, but even a benefit of herbivore pressure under conditions of intense competition. If herbivore suppression of *B. nigra* was removed, increased competition from *B. nigra* reduced growth of *E. cheiranthoides* by half, suggesting that the defensive superiority of the dually defended *E. cheiranthoides* is paired with a weak competitive ability.

Herbivores attacking *B. nigra* and *E. cheiranthoides* were mostly Brassicaceae specialists with known tolerance of glucosinolate defences. These herbivores were almost certainly present in high numbers in the surrounding Brassicacaea crops and cover strips and 'spilled over' onto experimental plants (associational susceptibility, Barbosa et al., 2009). This effect was made clear by the sharp decline in herbivore numbers on experimental plants when cover strips had to be mowed prematurely in 2022. While we intentionally selected a Brassicaceae-dominated environment to ensure high specialist herbivore pressures, other field studies commonly find specialist insects to be the dominant herbivores of Brassicaceae plants as well (Bidart-Bouzat & Kliebenstein, 2008; Mertens et al., 2021), likely because the glucosinolate defence acts as potent filter against many generalist herbivores (but see Zalucki et al., 2021).

The suite of herbivores we observed is representative of the main Brassicaceae herbivores in central Europe (Mertens et al., 2021), but interestingly, we never observed larvae of the cabbage white butterfly (*Pieris rapae*) feeding on experimental plants, despite adult butterflies being present in the

environment in both field seasons (K. Wang & T. Züst, personal observations). The larvae of *P. rapae* are major herbivores of many Brassicaceae plants but they are highly susceptible to the cardenolides in *E. cheiranthoides* (Sachdev-Gupta et al., 1993). While the lack of *P. rapae* larvae on *E. cheiranthoides* is thus expected, their absence on *B. nigra* is more surprising, and it is possible that *B. nigra* plants gained associational resistance against this herbivore at the field scale (Atsatt & O'Dowd, 1976; Barbosa et al., 2009) by being surrounded by *E. cheiranthoides* neighbours. Interestingly, we also found additional evidence for associational resistance gained by *B. nigra* in the competition treatment, with insecticide-treated plants paired with *E. cheiranthoides* receiving the lowest amounts of damage overall. Competition has been shown to increase defence expression in some plant species (Lankau & Kliebenstein, 2009), thus *B. nigra* may have benefitted from growing next to *E. cheiranthoides* while suppressing its growth at the same time.

Specialist herbivores of the Brassicaceae plants commonly have low specialization among different Brassicaceae species (Mertens et al., 2021). Of the herbivores we observed, most consistently preferred *B. nigra* over *E. cheiranthoides*, regardless of taxonomic group or feeding guild (e.g., caterpillars, flea beetles, aphids). The only exception to this pattern were the crucifer shield bugs (*Eurydema ornata*, *E. oleracea*, and their larvae), which occurred on both plant species at similar abundances, and at least over select periods, were more likely to occur on *E. cheiranthoides.* Several species of *Eurydema* are known to feed on different *Erysimum* species (Gómez, 2005), and both *E. ornata* and *E. oleracea* are commonly found feeding on natural *E. cheiranthoides* plants (T. Züst, personal observations). Despite this frequent association, *Eurydema* bugs showed a slight preference for *B. nigra* under laboratory conditions, and the species remain broadly generalised herbivores of many Brassicaeae species (Piersanti et al., 2020). It thus remains currently unclear whether *Eurydema* bugs are in the process of specializing on *Erysimum*, or whether they simply favour certain traits shared by *Erysimum* and other Brassicaceae plants.

Among the specialist herbivores in our field surveys, most showed reduced total abundances on *E. cheiranthoides*, but none exhibited complete avoidance of the dually defended plant. Importantly, we did not distinguish between perching and feeding herbivores in our observations, and several of the observed herbivores are highly mobile (e.g., pollen beetles, flea beetles). It is thus

feasible that herbivores were constantly arriving on *E. cheiranthoides*, but did not settle after initial sampling of the plant. High mobility may also explain the higher numbers of observed herbivores on insecticide-treated plants in 2023. Pyrethroid insecticides are toxic upon contact but have a relatively low environmental persistence (Ripley et al., 2001), thus weekly applications likely knocked down herbivore numbers and reduced plant damage, but the resulting increase in plant quality may have subsequently attracted higher numbers of mobile (less damaging) herbivores. Correlations between herbivore abundances and plant damage supported this explanation, as under ambient herbivore conditions, plant damage on *B. nigra* and *E. cheiranthoides* was best correlated with *P. xylostella* and *E. ornata* abundance as two major damaging herbivores, respectively. In insecticide-treated plants these correlations disappeared, and damage was either not strongly correlated with any herbivore on *B. nigra* or correlated with non-damaging aphids on *E. cheiranthoides*.

Although the comparison of *B. nigra* and *E. cheiranthoides* in our study highlights differences in herbivore-imposed selection, it can only provide partial insights into the causal role of cardenolides in plant defence. Many other traits, such as foliar nutrients, trichomes and leaf architecture, also vary between these species. Our measurements revealed only modest differences in foliar nutrients between *B. nigra* and *E. cheiranthoides*, and total protein content as a key resource for herbivores did not differ between plant species for most of the experiment. Differences in trichomes were more substantial between species, and trichomes often play a role in plant defence against insects (Levin, 1973; Sato et al., 2019), yet their role and relative importance for *E. cheiranthoides* defence remain to be elucidated. Structural traits such as plant height, size, or shape could also have affected herbivore colonization (Strauss et al., 2015) but such differences are perhaps less likely to explain the observed patterns in our study, given the similarities in plant size, similar apparency of plants in a weeded bare soil environment, and consistent preference for *B. nigra* of four Brassicaceae herbivores in leaf disc choice assays. Overall, the large observed differences in herbivore preference are unlikely to be caused by the relatively modest differences in nutritional traits or plant shape. Cardenolides thus remain one of the most distinctive traits between the two species, although the additional importance of trichomes and perhaps other unmeasured traits cannot be ruled out at this point.

A direct causal role of cardenolides for defence of *E. cheiranthoides* was recently demonstrated by Younkin et al. (2024), who used a newly developed CRISPR/Cas9 knockout line of *E. cheiranthoides* with abolished cardenolide production to evaluate herbivore responses in the laboratory and field. In their study, certain specialist herbivores, including *Pieris rapae*, exhibited increased feeding on knockout plants while completely avoiding cardenolide-producing wildtype plants. Other herbivores, including flea beetles and aphids, showed no consistent differences in feeding on knockout or wildtype plants (Younkin et al., 2024), and an apparent lack of a cardenolide effect was recently reported for *P. xylostella* as well (Wang & Züst, 2025). These findings suggest a more nuanced role of cardenolides in the defence of *E. cheiranthoides*, with the novel compounds acting as highly effective qualitative defences against some herbivores, and as less effective quantitative defences – or even ineffective compounds – against others. To understand the full impact of cardenolide variation on herbivore damage, a future in-depth analysis of intraspecific variation among natural *E. cheiranthoides* genotypes may prove useful.

While *E. cheiranthoides* was mostly protected from negative impacts of herbivore feeding, the dual production of glucosinolate and cardenolide defences likely represents a major allocation cost for this species. Trade-offs between resistance traits and growth or other plant functions are expected to be ubiquitous (Züst & Agrawal, 2017), yet they may sometimes be masked by variation in other traits (Koricheva et al., 2004). The production of glucosinolates (in *Arabidopsis thaliana*) and cardenolides (in *Asclepias syriaca*) have both been shown to reduce growth under some conditions (Züst et al., 2011, 2015). The dual investment required for producing both compounds concurrently thus may simply represent too large of a cost to become masked, and such a cost is the most likely cause for the weak competitive ability of *E. cheiranthoides* demonstrated in this study. However, despite the substantial costs at lower herbivore abundances, our study clearly demonstrated a benefit of this defensive strategy in other conditions. Damage by herbivores can reduce growth of undefended plants directly by reducing photosynthetically active leaf area and depleting vital plant resources (Machado et al., 2016), or indirectly by inducing plant responses that divert resources away from growth to defence (Attaran et al., 2014; Zangerl et al., 2002), yet *E. cheiranthoides* experienced neither of these impairments under high herbivore pressure. By preferentially attacking the

competitively superior susceptible host plants, herbivores thus benefitted a competitively inferior resistant plants, which would be expected to result in an increase in its frequency over time (Crawley, 1989; Myers & Sarfraz, 2017).

Despite weak competitive ability of *E. cheiranthoides* (and likely that of its close relatives) in the absence of specialized herbivores, the genus *Erysimum* has undergone a recent radiation that coincided with the gain of cardenolides (Züst et al., 2020), suggesting that this key innovation facilitated an escape from herbivory and subsequent diversification in enemy-free space. However, *E. cheiranthoides*, and species in the genus more generally, still mostly occupy marginal habitats that are often devoid of vegetation, rarely forming large populations. *Erysimum* plants thus appear to lack the ability to become dominant species within a community, despite being better defended than most of their heterospecific neighbours. Further evolutionary change and optimization may perhaps lower costs of defence for these plants in the future (e.g., Karasov et al., 2017). However, an increase in frequency of *Erysimum* plants may also increase selection pressures for the types of specialized herbivore resistance to evolve that are common in cardenolide-producing plant systems (Dobler et al., 2012; Petschenka et al., 2017), thus continuing the never-ceasing arms race between plants and their herbivores.

In conclusion, the gain of a novel defence appears to have allowed *Erysimum* plants to escape herbivore-imposed selection, if not herbivory entirely. However, the costs required to maintain this 'enemy-free space' are so substantial that plants can only thrive under conditions where competitors are absent, or where they are suppressed by strong herbivory. As these specific conditions are rare, *Erysimum* is currently confined to survive in marginal habitats, yet changing environmental conditions might well favour this unusually well-defended plant in the future.

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Supplementary Materials

Supplementary Methods

1. Plant height growth modelling

We modelled growth of plant height for the 2022 field experiment using a 4-parameter logistic function of the form

$$H = H_0 + \frac{K - H_0}{1 + e^{\left(-r\left(t - t_{mid}\right)\right)}} \qquad \text{eqn 1,}$$

where the height H at time t is a function of the initial plant height H_0 (i.e., height at transplanting), an asymptotic height K, a growth rate r, and the time t_{mid} at which the inflection point is reached (i.e., height halfway between H_0 and K). We fitted eqn. 1 to plant height using the 'nlme' function in the *nlme* package, treating plant identity as random effects to account for repeated measures of the same individual, and fitting fixed effects of plant species on the different growth parameters. As we only recorded height three times in week 3, 6, and 9, we were limited to estimating two parameters. We therefore manually set parameters t_{mid} and r to species-specific values in an iterative process. For each species separately, we first set parameter r to 0.1 and fit models for a range of t_{mid} values (20-60 days). We compared the resulting models using AIC and selected the optimal species-specific value of t_{mid}, before repeating the procedure for a range of r values (0.05-0.5). With both parameters set to optimal species-specific values, we then analysed the full data to estimate the remaining two parameters.

To compare growth between the two species at different points in time, we estimated the absolute growth rate (AGR) using the function

$$AGR = \frac{r(K - H_0)e^{\left(-r\left(t - t_{mid}\right)\right)}}{\left(1 + e^{\left(-r\left(t - t_{mid}\right)\right)}\right)^2} \qquad \text{eqn 2.}$$

Using equation 2, we estimated AGR for the midpoints of the first (weeks 3-6) and second growth interval (weeks 6-9). For each set of AGR values, we generated 95% population prediction intervals by drawing random values from the sampling distribution of each of the two estimated parameters (Bolker, 2008).

2. Leaf traits

Leaves from subsets of plants in the field experiment were harvested in weeks 4, 7, and 10 (one week after damage assessments) to determine changes in nutritional quality of plants over time. Each plant was only sampled once, with plants from blocks 1-3 sampled in week 4, plants from blocks 4-6 sampled in week 7, and plants from block 7-10 sampled in week 10. For each sampling, we removed 5-10 leaves from *B. nigra*, or 10-20 leaves from *E. cheiranthoides*, selecting leaves from the top, middle, and bottom part of each plant. Leaves from the same plant were wrapped together in aluminium foil and immediately placed on dry ice. Frozen leaf samples were then transported back to the laboratory and stored at -80 °C, before freeze-drying under vacuum for 48 h. We homogenized

dried leaves for each plant and prepared two replicate samples weighing 10 mg each in 2 mL screw-cap plastic tubes (Avantor, USA). One replicate sample was used for determination of total protein content, and the other was used for determination of sugar and starch content.

2.1 Total protein content

The total protein content of leaves was determined using the protocol of Clissold et al. (2006). Briefly, 10 mg of ground leaf material was extracted twice using a total volume of 0.8 mL 0.1M NaOH. For extraction, samples were sonicated for 30 minutes and heated at 90 °C for 15 min, followed by centrifugation. Both supernatants were pooled and neutralized using 6M HCl. We then precipitated proteins by adding 90 µL trichloroacetic acid solution (100% w/v, Sigma-Aldrich, Switzerland), followed by centrifugation and washing of the protein pellet with cold acetone. We re-suspended dried pellets using 1 mL of 0.1M NaOH, diluted the dissolved protein 1:20 with water, and assayed 50 µL of dilute protein solution with 200 µL QuickStart™ Bradford Dye (BioRad, Switzerland), reading absorbance at 595 nm on a microplate reader. Each sample was assayed in triplicate and protein concentrations were calculated using a concentration curve of Immunoglobuline G (Sigma-Aldrich, Switzerland; 0, 1, 2, 4, 6, 8, 12 µg protein per assay).

2.2 Soluble sugars and starch content

Sugar content was determined using the protocol of Machado et al. (2013). Ground plant material was extracted with 0.5 mL 80% ethanol in water and re-extracted twice using 0.5 mL 80% ethanol in water. For each extraction, samples were vortexed and heated at 78 °C for 15 min, followed by centrifugation. All three supernatants were pooled, and 25 µL of pooled extracts were used for determination of glucose, fructose, and sucrose using an enzymatic assay as described by Velterop & Vos (2001). Briefly, glucose was converted to glucose-6-phosphate by hexokinase (Roche, Switzerland) in the presence of ATP (Sigma-Aldrich, Switzerland), and further reacted to 6-phosphogluconate by glucose-6-P-dehydrogenase (Roche, Switzerland) in the presence of $NADP^+$ (Sigma-Aldrich, Switzerland). The latter reaction releases NADPH, which was quantified photometrically at 340 nm on a microplate reader. Reactions were carried out in 50 mM HEPES buffer (pH 7, with 5 mM $MgCl_2$). After readings stabilized, we added phosphoglucoisomerase (Roche, Switzerland) to convert fructose to glucose, and finally we added invertase (Sigma-Aldrich, Switzerland) to convert sucrose into glucose and fructose. Each sample was run in triplicate, and sugars were quantified relative to a concentration curve of glucose (25 µL of 0, 0.125, 0.25, 0.5, 1, and 2 mM solutions).

We also determined starch content in the plant material remaining after sugar extraction using the protocol of Smith & Zeeman (2006). We added 1 mL of water to each plant precipitate, and diluted 100 µL of this suspension with 500 µL water. Dilute suspensions were autoclaved for 1 h and left to cool down to 37 °C. We then added 500 µL of an enzyme solution consisting of 50 mM

sodium-acetate buffer (pH 5.5), amyloglucosidase (Roche, Switzerland) and α-amylase (Sigma-Aldrich, Switzerland). Solutions were incubated at 37 °C overnight, and finally we quantified sugars in 25 µL of supernatant using the same enzymatic assay as above.

2.3 Trichome density and length

Trichomes were quantified on a separate set of plants for *B. nigra* and *E. cheiranthoides*. Plants were germinated and grown as for the field experiments but kept in the greenhouse for four weeks. We then quantified trichome densities and trichome lengths on both sides of one leaf from six plants per species. For each leaf and side, we acquired high-resolution images (Figure S10a-d) using an Emspira 3 digital microscope (Leica Microsystems, Switzerland). Trichomes were then counted within a defined area using ImageJ (Schneider et al. 2012). On three leaves per species and leaf side, we also measured the lengths of eight trichomes using ImageJ.

3. Herbivore choice assays

To contrast field observations to herbivore behaviour under more controlled conditions, we tested a set of four laboratory-reared herbivores for their innate preference between leaves of *B. nigra* and *E. cheiranthoides* in laboratory choice assays. All herbivores that were used for these assays are specialist herbivores on plants in the Brassicaceae, but feed on various species within this family due to adaptations for glucosinolate tolerance and differ in their natural association with *E. cheiranthoides*. The turnip sawfly *Athalia rosae* (Hymenoptera: Tenthredinidae) has no known association with *E. cheiranthoides* but feeds on other Brassicaceae plants as larvae and sequesters glucosinolates from its host plants (Müller & Wittstock, 2005). The cabbage white butterfly *Pieris rapae* (Lepidoptera: Pieridae) actively avoids *E. cheiranthoides* (Sachdev-Gupta et al., 1993; Younkin et al., 2024) but its caterpillars feed on other Brassicaceae plants by detoxifying activated glucosinolates enzymatically (Wittstock et al., 2004). The diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) is occasionally found feeding on *E. cheiranthoides* (Mertens et al., 2021; observations in current study) and its caterpillars enzymatically degrade glucosinolates prior to their activation (Ratzka et al., 2002), while the cardenolide coping mechanism remains unknown. Finally, the red cabbage bug *Eurydema ornata* (Hemiptera: Pentatomidae) is frequently found feeding on *E. cheiranthoides* and other *Erysimum* species both as adults and nymphs (Goméz, 2005; observations in current study). Individuals sequester glucosinolates from their host plant (Aliabadi et al., 2002; T. Züst, unpublished), but again the cardenolide coping mechanism for this species remains unknown.

Individuals of *A. rosae* were kindly provided by Prof. Caroline Müller, University of Bielefeld, and had been maintained as a laboratory rearing colony on *Sinapis arvensis*. Newly eclosed, unmated females were left to oviposit on *Brassica rapa* plants (Wisconsin Fast Plants™, Caroline Biological Supply Co., Burlington VA, USA), and the developing male larvae were used for choice assays 5 days after hatching. Individuals of *P. rapae* were kindly provided by the group of Prof.

Florian Schiestl, University of Zürich. Adults had originally been collected from multiple locations in Switzerland and were maintained in a panmictic laboratory rearing colony using *Brassica rapa* plants as oviposition substrate and leaves of savoy cabbage (*Brassica oleracea* var. *sabauda*) as caterpillar food. We placed fresh *B. rapa* plants in an adult oviposition cage for 24 hours, after which larvae were left to develop on the oviposition plant for 10 days before use in choice assays (early L2 stage). For *P. xylostella* we used a strain from a long-term rearing on a wheat germ-based artificial diet (Frontier Agricultural Sciences, Delaware, USA), originally provided by Syngenta Crop Protection AG, Stein, Switzerland. For choice assays, newly laid eggs on sheets of parafilm were placed in cups of fresh diet and left to develop for 11 days (late L2 stage). We originally collected adults of *E. ornata* from a population in Valais, Switzerland (46.312970° N, 7.675072° E, predominantly collected on *Isatis tinctoria*), and maintained a continuous laboratory rearing population on a diet of savoy cabbage leaves and young *B. rapa* plants, with yearly introductions of new individuals from the same wild population to maintain genetic diversity. For choice assays, we used mature adults (>3 days after final molt) out of the rearing population.

Choice assays were set up in petri dishes (5 cm diameter) filled with a thin layer of 3% agar, with 12 replicates per herbivore species. In each petri dish, we placed pairs of leaf discs (7.6 mm diameter), cut from the leaves of 4-week-old *B. nigra* and *E. cheiranthoides* plants grown under greenhouse conditions. We then released one larva (for *A. rosae*, *P. rapae*, *P. xylostella*) or one adult (for *E. ornata*) at the centre of each dish. To account for difference in feeding habit, larvae (leaf chewing) were left to feed for 6 hours, while *E. ornata* adults (cell-content feeding) were left to feed for 24 hours. Herbivore choice was then quantified by scanning the remaining leaf disc pairs using a CanoScan LiDE 220 scanner, followed by quantification of remaining leaf areas by ImageJ.

We subtracted remaining leaf areas from the area of an intact leaf disc (45.36 mm^2). The resulting consumed leaf areas were analysed using a linear mixed-effects model, with plant species, herbivore species, and their interaction as fixed effects. A unique identifier for each petri dish was included as a random effect to account for non-independence of leaf disc pairs from the same dish. Significance of preference for one of the plant species was evaluated using independent linear contrasts for each herbivore (function ‘emmeans’).

Supplementary Results

Species differences in plant traits

For the 2022 field experiment, we quantified several nutritional traits across the two species that might have affected herbivore preference independently of plant defence. Across three sampling points, *B. nigra* leaves contained significantly higher levels of soluble glucose and fructose (Fig. S8a-b), while *E. cheiranthoides* leaves contained higher levels of sucrose (Fig. S8c). Digestible starch content did not differ between species for most of the experiment, except for the final time point when starch content had dropped for both species, but more so for *E. cheiranthoides* (Fig. S8d). Similarly,

total hydrolysable protein content only differed at later time points, with *B. nigra* containing up to 60% more protein per unit dry mass by that point.

Trichomes differed substantially between the two plant species, with *B. nigra* expressing large linear trichomes (Figure S9a-b), while trichomes of *E. cheiranthoides* as smaller and medifixed stellate (Figure S9c-d). Trichome density was lowest on the upper (adaxial) side of *B. nigra* leaves at 0.04 ± 0.01 trichomes mm^{-2} (mean ± 1 SE). Trichome density was slightly higher on the lower (abaxial) leaf side at 0.21 ± 0.07 trichomes mm^{-2}, although trichomes were predominantly located along leaf veins (Figure S9a-b). In contrast, *E. cheiranthoides* had substantially higher trichome densities on both sides, with mean densities of 8.84 ± 0.61 trichomes mm^{-2} on the upper and 7.50 ± 0.52 trichomes mm^{-2} on the lower leaf side (Figure S9c-d). While trichome densities and shapes most likely will change with plant age and may differ between greenhouse and field conditions, these results nonetheless provide a useful measure for the relative differences between *B. nigra* and *E. cheiranthoides*.

Supplementary References

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Table S1. Mean (± 1 SE) numbers of herbivores per plant found sitting or feeding on *B. nigra* and *E. cheiranthoides*. Numbers are the cumulative per-plant counts of six observations, averaged across all plants of the same species and treatment. Flea beetles, large flea beetles, weevils, and leaf hoppers are classes of taxonomically complex species for which field determination was deemed impractical. Bold values highlight significantly different numbers between plant species in a specific year and treatment ($p < 0.05$, generalized linear model of cumulative counts using a negative binomial error structure).

				2022		2023		2023	
				ambient herbivory		ambient herbivory		insecticide treatment	
Herbivore	Scientific name	Order	Stage	*B. nigra*	*E. cheiranthoides*	*B. nigra*	*E. cheiranthoides*	*B. nigra*	*E. cheiranthoides*
				n = 40	n = 40	n = 8	n = 16	n = 8	n = 16
flea beetle		Coleoptera	adult	**25.13 ± 4.65**	**1.80 ± 0.39**	**153.5 ± 30.43**	**20.94 ± 3.12**	**225.71 ± 29.98**	**3.64 ± 0.61**
pollen beetle	*Brassicogethes aeneus*	Coleoptera	adult	**108.20 ± 14.99**	**12.10 ± 1.75**	**17.13 ± 3.79**	**9.38 ± 1.56**	**102.29 ± 21.48**	**11.79 ± 1.95**
weevil		Coleoptera	adult	**4.03 ± 0.61**	**1.08 ± 0.21**	**7.75 ± 1.43**	**1.31 ± 0.31**	**27.86 ± 5.37**	**2.50 ± 0.53**
leaf miner	*Phytomyza sp.*	Diptera	juvenile	**0.40 ± 0.16**	**0.08 ± 0.05**	0	0.12 ± 0.12	0	0
aphid		Hemiptera	adult/juvenile	**20.33 ± 4.40**	**0.83 ± 0.23**	**119.00 ± 43.71**	**19.38 ± 5.13**	**431.57 ± 73.61**	**116.29 ± 14.24**
cabbage whitefly	*Aleyrodes sp.*	Hemiptera	adult	**6.78 ± 2.03**	**0.18 ± 0.08**	0.25 ± 0.27	0.38 ± 0.27	**11.43 ± 6.79**	**0.57 ± 0.31**
leaf hopper		Hemiptera	adult	0.10 ± 0.05	0.08 ± 0.04	0	0	0	0
crucifer shield bug	*Eurydema sp.*	Heteroptera	adult/juvenile	0.55 ± 0.17	1.08 ± 0.29	1.25 ± 0.45	1.31 ± 0.33	0.29 ± 0.29	0.93 ± 0.36
plant bug	*Lygus sp.*	Heteroptera	adult/juvenile	**4.55 ± 0.71**	**0.48 ± 0.13**	**1.63 ± 0.78**	**0.06 ± 0.06**	**2.14 ± 0.55**	**0.14 ± 0.10**
plant bug	*Closterotomus sp.*	Heteroptera	adult	0.13 ± 0.06	0.03 ± 0.03	**1.00 ± 0.49**	**0.06 ± 0.06**	**1.14 ± 0.49**	**0.14 ± 0.10**
cabbage looper	*Trichoplusia ni*	Lepidoptera	juvenile	0	0.03 ± 0.03	0	0	0	0
cabbage moth	*Mamestra brassicae*	Lepidoptera	juvenile	0.03 ± 0.03	0	0	0	0	0
diamondback moth	*Plutella xylostella*	Lepidoptera	adult/juvenile	**2.35 ± 0.39**	**0.75 ± 0.17**	0.13 ± 0.13	0.31 ± 0.14	**4.00 ± 1.00**	**0.43 ± 0.18**

Table S2. Model comparison of generalized additive models (GAMs) of the temporal dynamics in total herbivore abundance in two years. For 2022, both AIC and BIC indicated better support for a model lacking a species-specific smoother. For 2023, AIC supported a model with separate smoothers for each combination of plant species and insecticide treatment ('PxT'), whereas BIC favoured a model with a single smoother, thus the simpler of the two models was retained.

Year	Model parameters	Df	AIC	BIC
2022	**~ Plant + s(Day, by=Plant)**	**11**	**2492.33**	**2536.89**
	~ Plant + s(Day)	7	2513.29	2542.03
2023	~ Plant + Treatment + s(Day, by=PxT)	18	1890.20	1953.52
	~ Plant + Treatment + s(Day, by=Plant)	11	1904.73	1944.02
	~ Plant + Treatment + s(Day, by=Treatment)	11	1908.05	1946.77
	~ Plant + Treatment + s(Day)	**8**	**1912.73**	**1940.03**
	~ Treatment + s(Day)	7	2016.12	2039.45
	~ Plant + s(Day)	7	1938.69	1962.40

Table S3. Test statistics for generalized additive models (GAMs) of the temporal dynamics in presence and abundance of the most abundant herbivores in 2022 and 2023. For each herbivore and year, a separate binomial GAM and negative-binomial GAM was fit to presence and abundance data, respectively. For each model, a parametric term of 'plant species' (main effect) and a plant-specific smoother was specified. Significance of the main effect was directly assessed by percentage deviance explained (Type III Wald chisquare tests). Support for plant-specific smoothers was assessed by comparing each full model to a nested single-smoother model using BIC. A plant-specific smoother was considered necessary to explain temporal trends in the data if the full model had a lower BIC by >2 units. The number of nodes in each smoother (parameter k in GAM) were adjusted according to the number of weeks in which herbivores were observed. For 2023, data of the insecticide and competition treatment was excluded from the analysis. Two herbivores present in 2022 had insufficient data in 2023 to fit GAMs.

	2022					2023 (ambient herbivores only)				
	Herbivore presence		Herbivore abundance			Herbivore presence		Herbivore abundance		
Herbivore	main effect plant species	plant-specific smoother	main effect plant species	plant-specific smoother	k	main effect plant species	plant-specific smoother	main effect plant species	plant-specific smoother	k
aphids	**df = 1, X^2 = 32.46, p < 0.001**	ΔBIC: 8.62	**df = 1, X^2 = 62.25, p < 0.001**	ΔBIC: 1.58	5	df = 1, X^2 = 0.21, p = 0.650	ΔBIC: 7.72	df = 1, X^2 = 1.59, p = 0.2075	**ΔBIC: -3.47**	4
cabbage whitefly	**df = 1, X^2 = 20.80, p < 0.001**	ΔBIC: 5.23	**df = 1, X^2 = 44.54, p < 0.001**	ΔBIC: 5.22	3	n/a	n/a	n/a	n/a	n/a
crucifer shield bug	**df = 1, X^2 = 6.74, p = 0.009**	ΔBIC: -0.20	df = 1, X^2 = 2.29, p = 0.130	ΔBIC: 7.87	3	df = 1, X^2 = 0.01, p = 0.917	ΔBIC: -1.08	df = 1, X^2 = 1.59, p = 0.2071	**ΔBIC: -3.06**	3
diamondback moth	**df = 1, X^2 = 8.85, p = 0.003**	ΔBIC: 9.18	**df = 1, X^2 = 18.15, p < 0.001**	ΔBIC: 10.43	3	n/a	n/a	n/a	n/a	n/a
flea beetle	**df = 1, X^2 = 7.10, p < 0.001**	ΔBIC: 0.07	**df = 1, X^2 = 11.43, p < 0.001**	**ΔBIC: -6.665**	5	**df = 1, X^2 = 7.34, p = 0.006**	ΔBIC: 0.13	**df = 1, X^2 = 73.06, p < 0.001**	ΔBIC: 13.06	5
plant bug	**df = 1, X^2 = 28.88, p < 0.001**	ΔBIC: 1.84	**df = 1, X^2 = 29.91, p < 0.001**	**ΔBIC: -2.55**	3	**df = 1, X^2 = 7.72, p = 0.005**	ΔBIC: 2.52	**df = 1, X^2 = 8.44, p = 0.004**	ΔBIC: 0.83	3
pollen beetle	**df = 1, X^2 = 50.66, p < 0.001**	ΔBIC: 5.06	**df = 1, X^2 = 17.50, p < 0.001**	**ΔBIC: -10.944**	4	df = 1, X^2 = 1.41, p = 0.234	**ΔBIC: -15.83**	df = 1, X^2 = 0.11, p = 0.736	**ΔBIC: -27.14**	3
weevil	**df = 1, X^2 = 23.35, p < 0.001**	ΔBIC: 5.61	**df = 1, X^2 = 30.25, p < 0.001**	ΔBIC: 0.96	3	**df = 1, X^2 = 5.08, p = 0.024**	ΔBIC: 6.16	**df = 1, X^2 = 26.01, p < 0.001**	ΔBIC: 8.96	3

Table S4. a) Generalized-linear mixed effects model (GLMM) for the proportion of damaged leaves in the 2022 field data using ‘Week’ and ‘Plant species’ as factorial variables. b) GLMMs for the proportion of highly damaged leaves in the 2023 field data for each week separately, using ‘Plant species’, ‘Insecticide treatment’ and ‘Competition treatment’ as factorial variables. Interactions with week could not be fit as damage was not observed for plants in the competition treatment in week 9. P-values for the fixed effects were obtained using the ‘Anova’ function from the car package.

a) Analysis of Deviance Table (Type III Wald Chisquare tests)

	Chisq	Df	(Pr > ChiSq)
(intercept)	88.242	1	< 0.001
Week	140.500	2	< 0.001
Plant species	241.374	1	< 0.001
Week:Plant species	61.521	2	< 0.001

Model: glmer(cbind(damaged,undamaged) ~ Week * Plant species + (1|Plant ID) + (1|Neighbour group) + (1|Observer ID))

b) Analysis of Deviance Table (Type III Wald Chisquare tests)

		Week 3		Week 6		Week 9	
	Df	Chisq	(Pr > ChiSq)	Chisq	(Pr > ChiSq)	Chisq	(Pr > ChiSq)
Plant species	1	14.10	**< 0.001**	57.21	**< 0.001**	38.88	**< 0.001**
Competition treatment	1	2.38	0.122	2.80	0.094	n/a	n/a
Insecticide treatment	1	0.15	0.699	0.35	0.554	6.12	**0.013**
Plant species:Competition treatment	1	3.35	0.066	0.45	0.501	n/a	n/a
Plant species:Insecticide treatment	1	4.84	**0.028**	4.82	**0.028**	8.02	**0.005**
Competition treatment:Insecticide treatment	1	0.18	0.672	0.02	0.884	n/a	n/a
Plant species:Competition treatment:Insecticide treatment	1	2.19	0.139	3.05	0.081	n/a	n/a

Model: glmer(cbind(highly damaged, low+undamaged) ~ Plant species * Competition treatment * Insecticide treatment + (1|Neighbour group))

Table S5. Parameters of the 4-parameter logistic model of plant height growth. As height was only measured three times, only two parameters could be estimated. Therefore, the parameters t_{mid} and r were set to biologically relevant values and were then further optimized by repeatedly running separate models for *B. nigra* and *E. cheiranthoides* across a range of values of t_{mid} and r. Parameters resulting in the lowest model AIC were selected for each species. With t_{mid} and r set, parameters H_0 and K were then estimated using an *nlme* model of the full data. Parameters are thus either model estimates (±1 SE) or single optimized values. Note that the negative estimate of H_0 for *E. cheiranthoides* (corresponding to negative plant height at time of transplanting) is likely due to limited data on early development.

	B. nigra	*E. cheiranthoides*
H_0 (estimated)	19.66 ± 1.08	-3.40 ± 1.61
K (estimated)	82.70 ± 4.55	66.98 ± 3.40
t_{mid} (set)	56	29
r (set)	0.1	0.1

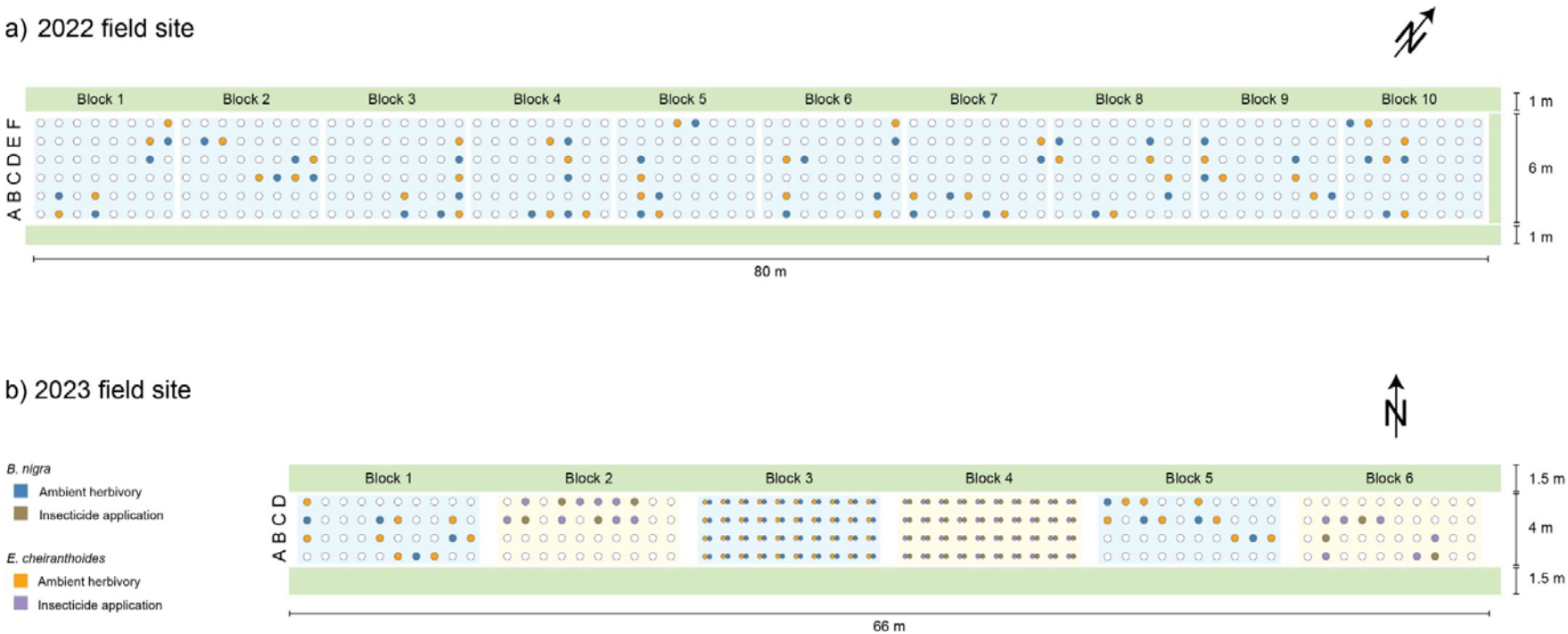

Figure S1. a) Schematic of the 2022 field site. Shown are the ten replicate blocks (light blue squares), with focal *B. nigra* plants and one matched randomly selected *E. cheiranthoides* neighbour highlighted. Empty circles are additional *E. cheiranthoides* plants not included for the analyses of this study. b) Schematic of the 2023 field site. Shown are the three blocks under ambient herbivory (light blue squares), and the three blocks receiving weekly applications of insecticide (light yellow squares). Focal *B. nigra* plants and two matched randomly selected *E. cheiranthoides* neighbour used for this study are highlighted. Paired circles in blocks 3 and 4 represent pairs of *B. nigra* and *E. cheiranthoides* plants growing closely together in the same buried pot to impose plant-plant competition. Buffer strips sown with a mixture of *Raphanus sativus* and *Avena strigosa* are shown as light green rectangles for both years. Both sites were located adjacent to oilseed rape crop fields (not shown).

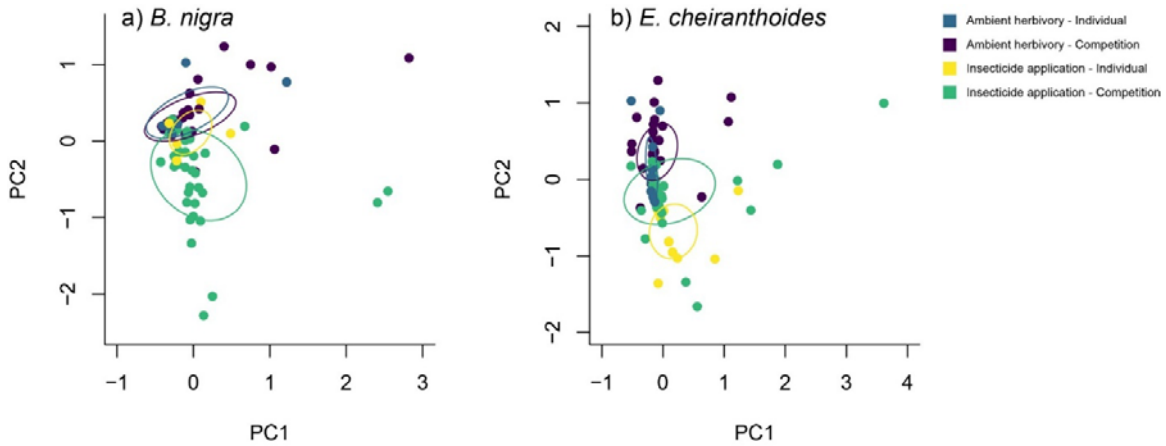

Figure S2. Principal components of herbivore communities in week 4 of the 2023 field experiment observed on a) *B. nigra* and b) *E. cheiranthoides*. Points are individual plants, coloured by insecticide and competition treatments. Ellipses encircle 95% of all datapoints belonging to a treatment.

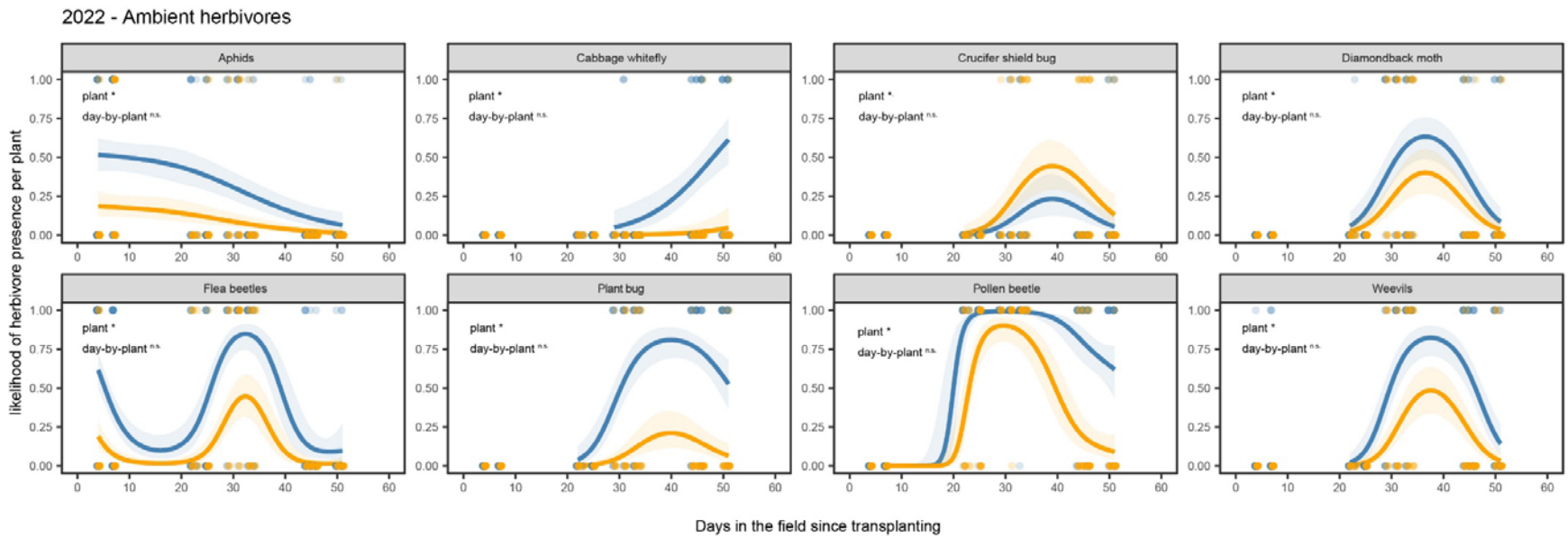

Figure S3. Likelihood of herbivore presence per plant over 9 weeks for *B. nigra* (blue) and *E. cheiranthoides* (orange) in the 2022 field experiment. The X-axis shows the days since plants were placed in the field, and the y-axis shows the likelihood of herbivore presence per plant. Points are observations on individual plants and lines are model fits from generalized additive models (GAMs). Shaded areas are the 95% confidence intervals. In each panel, 'plant' refers to statistical support of a main effect of plant species, and 'day-by-plant' for support of a species-specific smoother. See Table S3 for details.

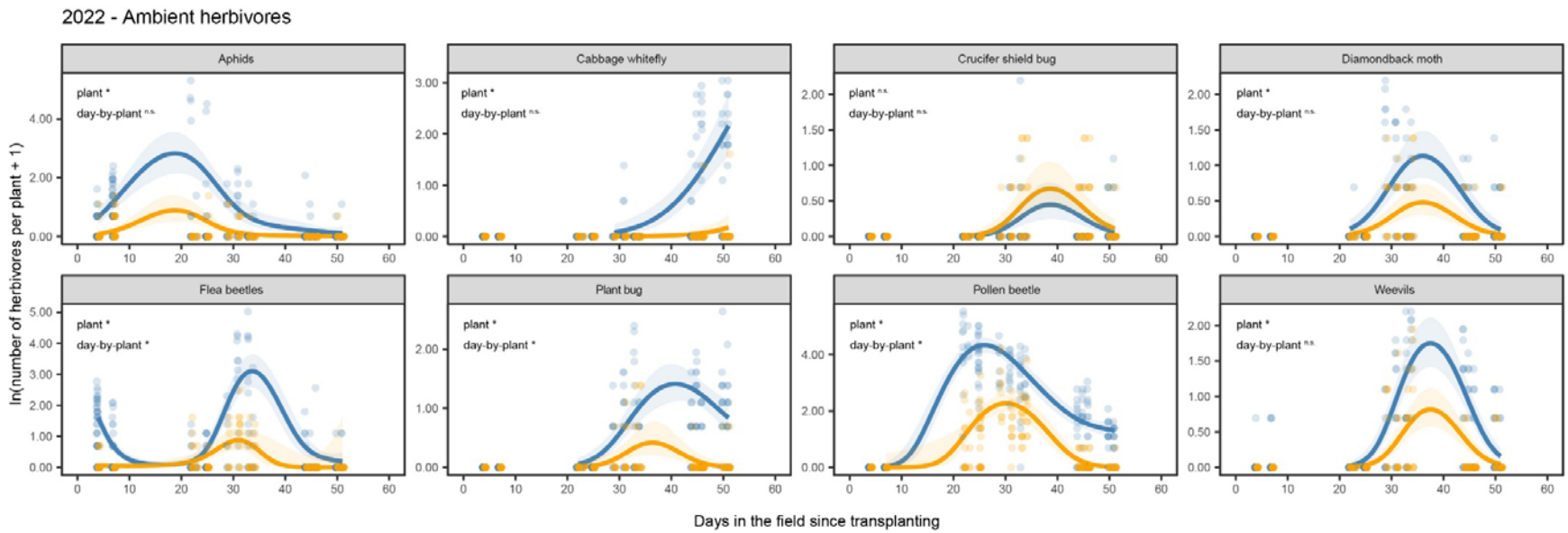

Figure S4. Number of herbivores per plant over 9 weeks for *B. nigra* (blue) and *E. cheiranthoides* (orange) in the 2022 field experiment. The X-axis shows the days since plants were placed in the field, and the y-axis shows the natural logarithm of the number of herbivores per plant. Points are observations on individual plants and lines are model fits from generalized additive models (GAMs). Shaded areas are the 95% confidence intervals. In each panel, 'plant' refers to statistical support of a main effect of plant species, and 'day-by-plant' for support of a species-specific smoother. See Table S3 for details.

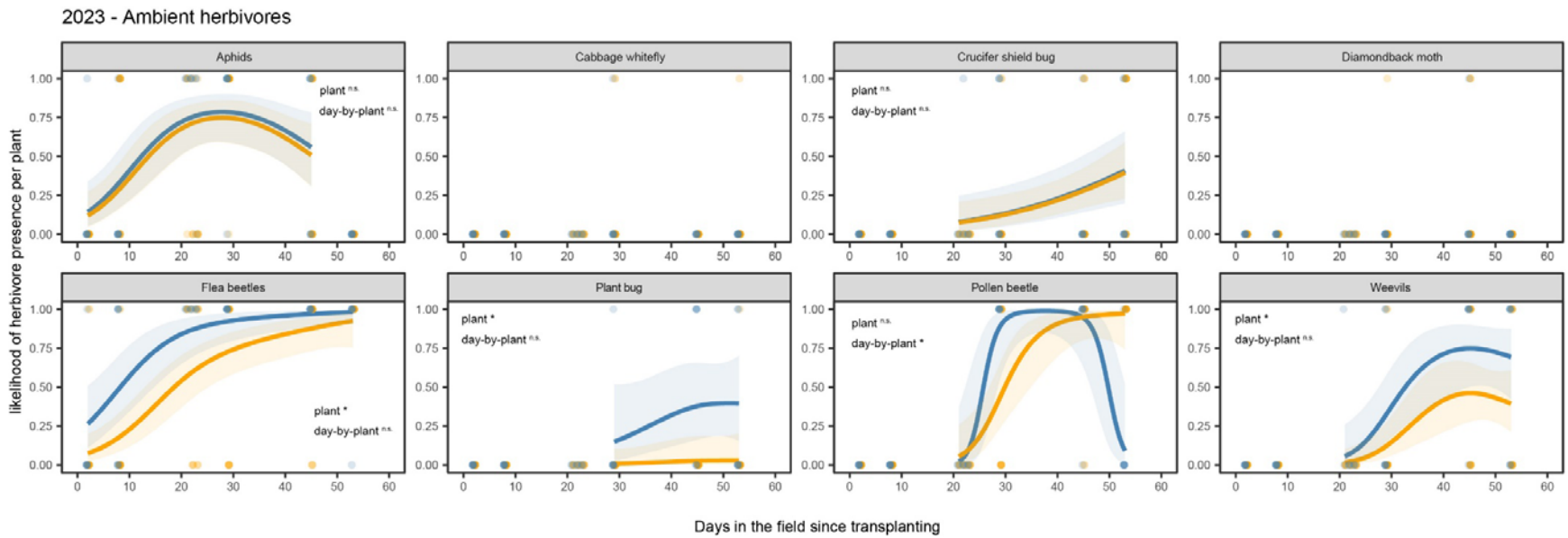

Figure S5. Likelihood of herbivore presence per plant over 9 weeks for *B. nigra* (blue) and *E. cheiranthoides* (orange) in the 2023 field experiment. The X-axis shows the days since plants were placed in the field, and the y-axis shows the likelihood of herbivore presence per plant. Points are observations on individual plants and lines are model fits from generalized additive models (GAMs). Shaded areas are the 95% confidence intervals. For the cabbage whitefly and the diamondback moth, observed numbers were too low to fit a reliable GAM. In each panel, 'plant' refers to statistical support of a main effect of plant species, and 'day-by-plant' for support of a species-specific smoother. See Table S3 for details.

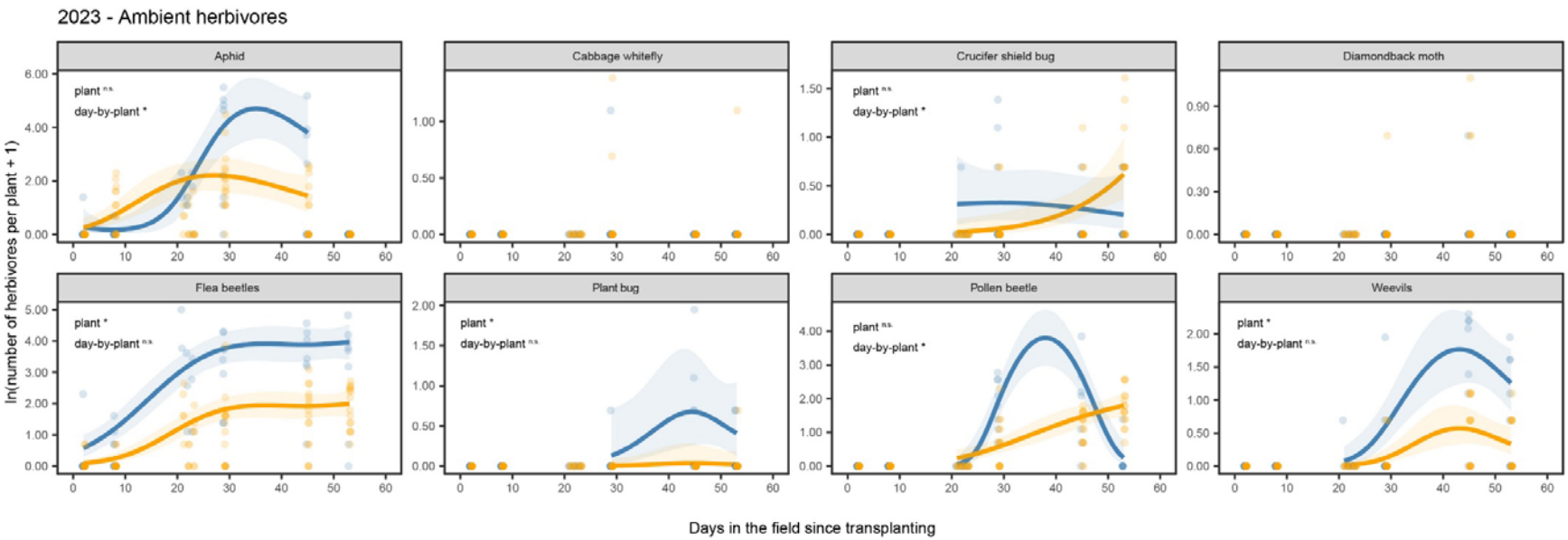

Figure S6. Number of herbivores per plant over 9 weeks for *B. nigra* (blue) and *E. cheiranthoides* (orange) in the 2023 field experiment. The X-axis shows the days since plants were placed in the field, and the y-axis shows the natural logarithm of the number of herbivores per plant. Points are observations on individual plants and lines are model fits from generalized additive models (GAMs). Shaded areas are the 95% confidence intervals. For the cabbage whitefly and the diamondback moth, observed numbers were too low to fit a reliable GAM. In each panel, 'plant' refers to statistical support of a main effect of plant species, and 'day-by-plant' for support of a species-specific smoother. See Table S3 for details.

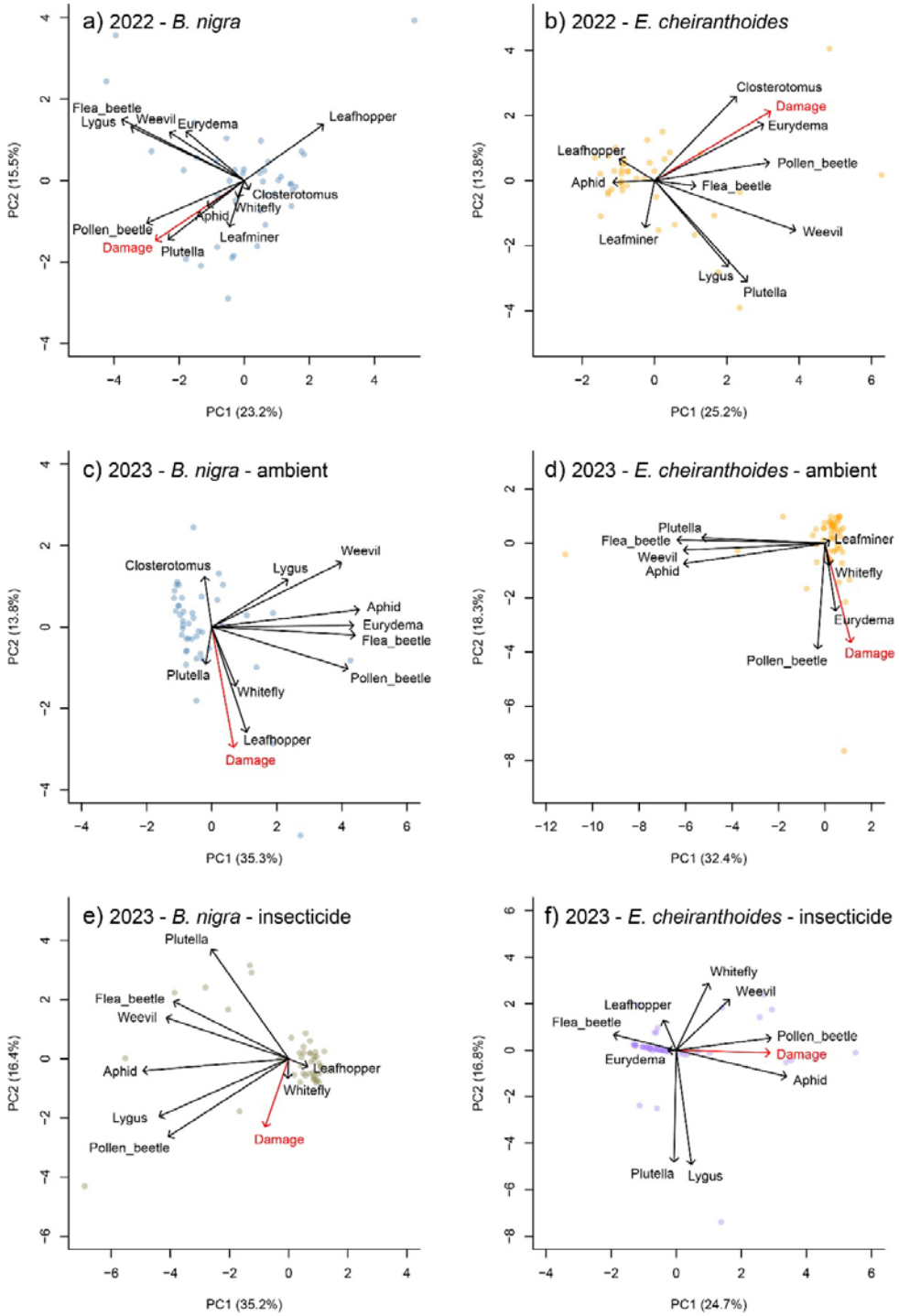

Figure S7. Principal component analyses (PCA) of the total observed herbivore numbers in weeks 4 and 5, and the quantified proportion of leaf damage in week 6. Shown are the first two principal components, with points representing values for individual plants, and arrows representing the loadings of the original variables included in the PCA. Due to substantial differences in herbivore numbers between *B. nigra* and *E. cheiranthoides*, separate PCAs were performed for each plant species and year or treatment. a-b) PCAs for the 2022 field experiment on *B. nigra* (blue) and *E. cheiranthoides* (orange). c-d) PCAs for the 2023 field experiment on *B. nigra* and *E. cheiranthoides* under ambient herbivory, and e-f) with insecticide applications.

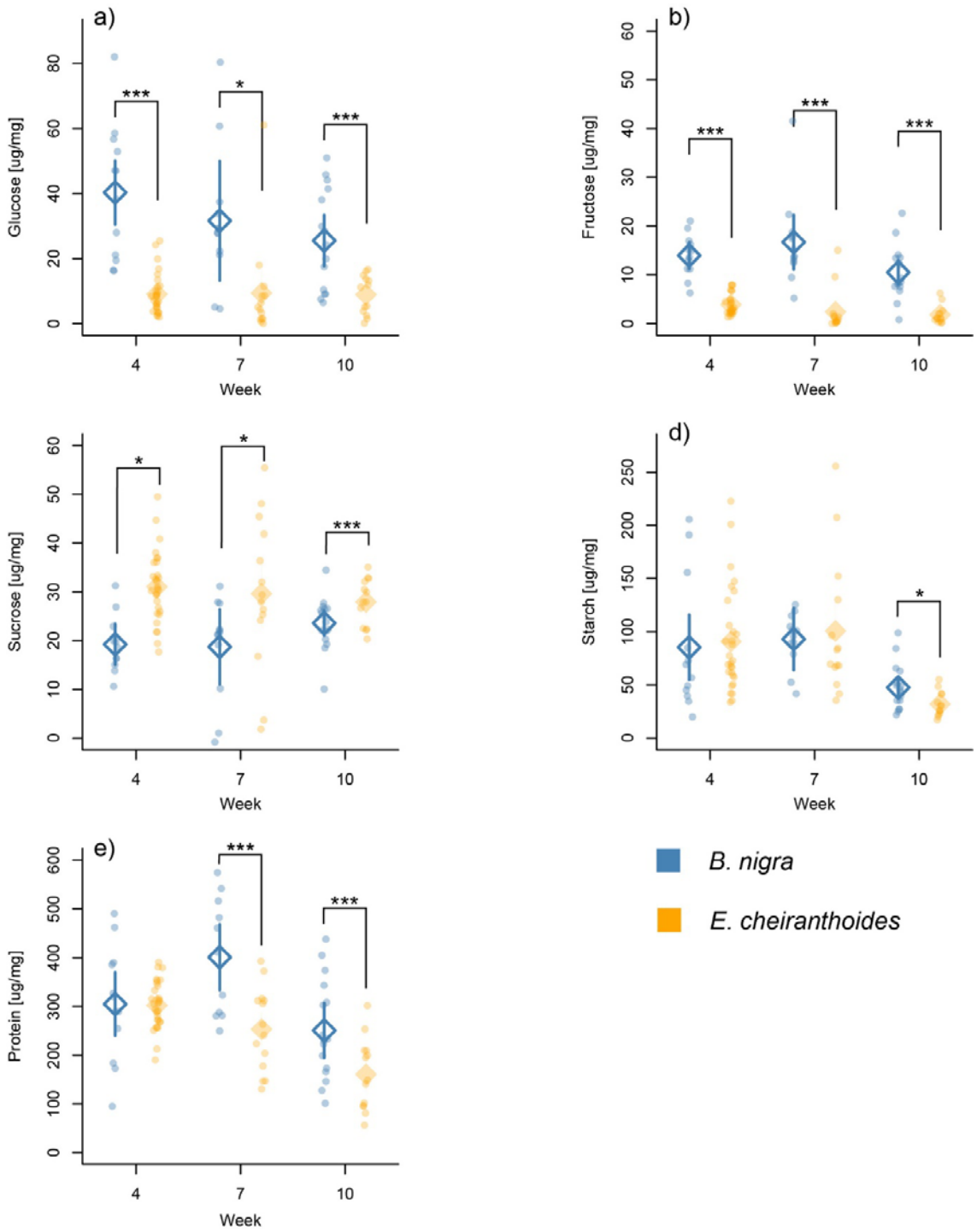

Figure S8. Concentrations of soluble sugars (a-c), starch (d), and total protein (d) in leaf tissue of *B. nigra* (blue) and *E. cheiranthoides* (orange) harvested in weeks 4, 7, and 10 of the field experiment. Points are values for individual plants, while diamond symbols and lines are means and 95% confidence intervals from a linear model. Brackets highlight significant linear contrasts at the $p < 0.05$ level.

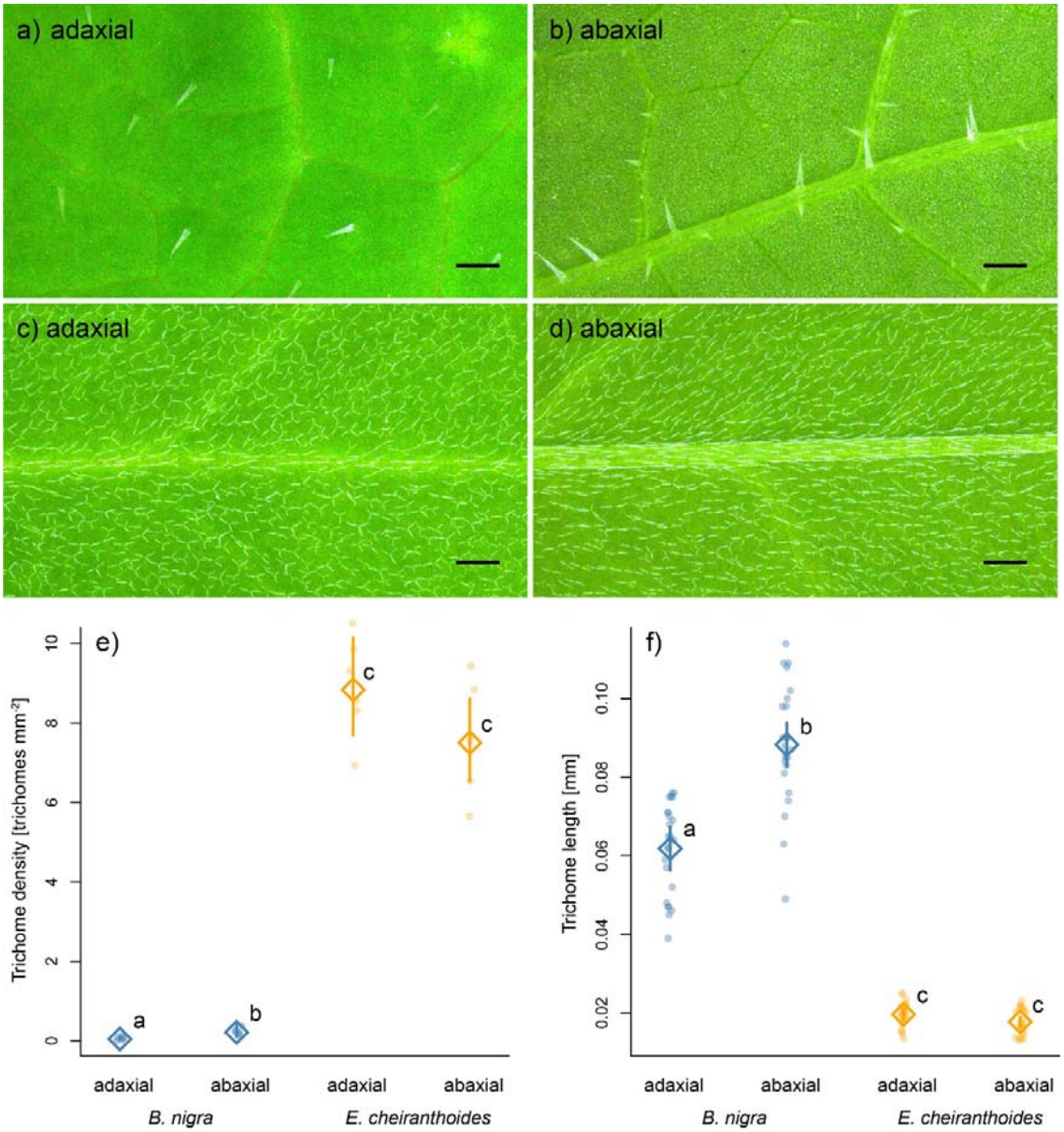

Figure S9. Photographs of the adaxial (upper) and abaxial (lower) leaf sides of 4-week-old *B. nigra* (a-b) and *E. cheiranthoides* (c-d) plants growing under greenhouse conditions. Scale bars correspond to distances of 1 mm. Six photographs per side and species were used to quantify trichome densities (e) and trichome length (f). Points are values for individual plants, while diamonds and vertical lines are the means and 95% confidence intervals from linear models. Letters highlight significant linear contrasts at the $p < 0.05$ level.

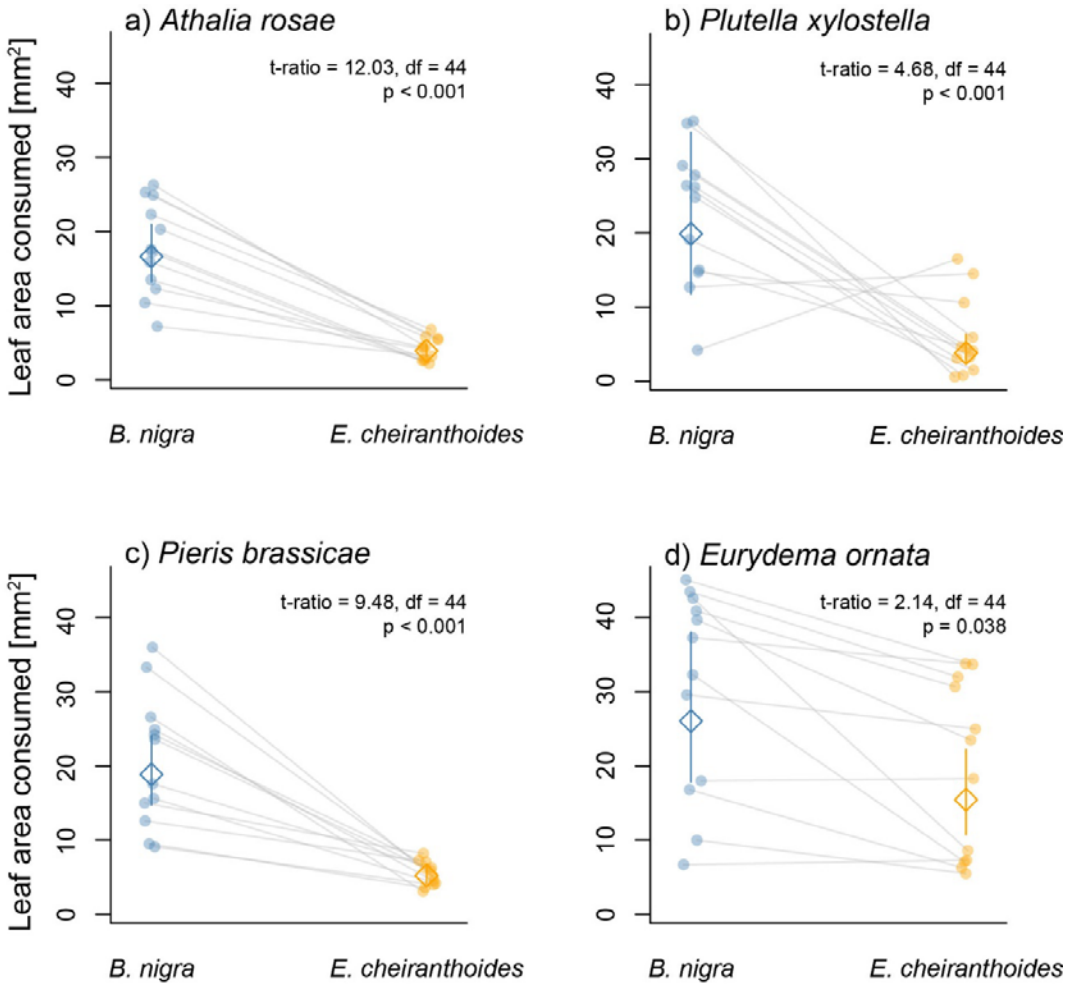

Figure S10. Relative preference of four herbivores in two-choice laboratory assays. Choice assays consisted of pairs of leaf discs (45 mm^2 area) from 4-week-old *B. nigra* and *E. cheiranthoides* plants grown under greenhouse conditions, offered to larvae (a-c) or adult (d) herbivores in petri dishes. Points are values for individual leaf discs, with grey lines connecting pairs of leaf discs from the same dish. Diamonds and vertical lines are the means and 95% confidence from a linear mixed-effects model, while statistical tests are linear contrasts comparing consumption of the two plant species for each herbivore.

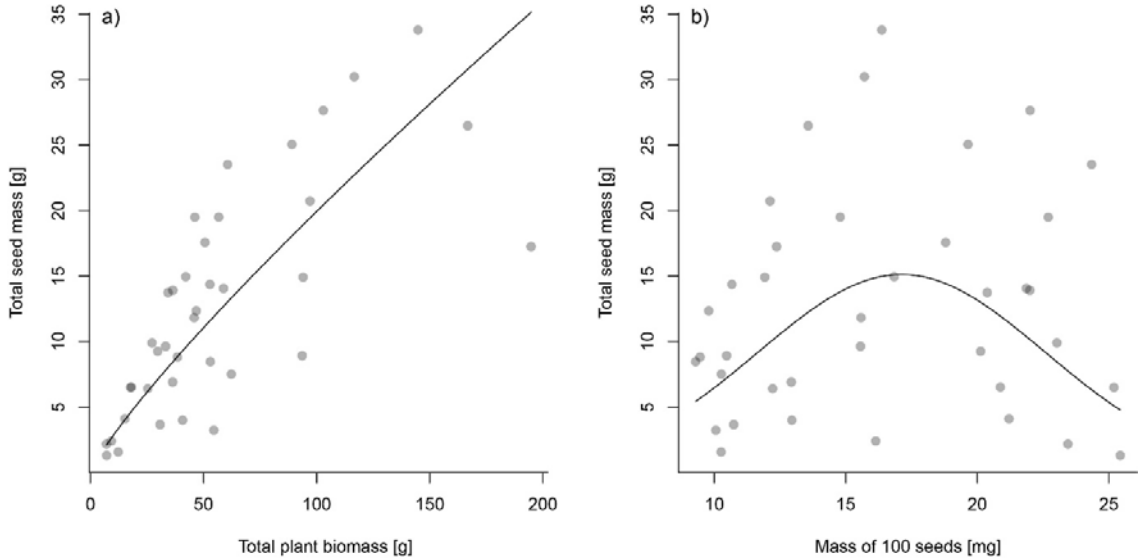

Figure S11. a) Relationship between total seed mass and aboveground plant biomass of a subset of *E. cheiranthoides* plants (2022 field experiment, block 7) that was removed from the field intact and maintained through seed ripening in a greenhouse. Points are values for individual plants, each belonging to a different accession. The solid line is a linear fit between log-transformed seed mass and log-transformed total biomass ($F_{1,36}$ = 77.48, p<0.001, R^2 = 0.67.) b) Relationship between total seed mass and mass of individual seeds, showing a non-linear relationship of total seed mass and the mass of 100 seeds, indicating a potential seed size/ seed number trade-off. The solid line is a quadratic fit between log-transformed seed mass and the combined mass of 100 seeds (quadratic term: $F_{1,35}$ = 7.15, p=0.011, R^2 = 0.12).