

Research

Spatial scale, neighbouring plants and variation in plant volatiles interactively determine the strength of host–parasitoid relationships

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Species-specific responses to the environment can moderate the strength of interactions between plants, herbivores and parasitoids. However, the ways in which characteristics of plants, such as genotypic variation in herbivore induced volatiles (HIPVs) that attract parasitoids, affect trophic interactions in different contexts of plant patch size and plant neighbourhood is not well understood. We conducted a factorial field experiment with white cabbage *Brassica oleracea* accessions that differ in the attractiveness of their HIPVs for parasitoids, in the context of different patch sizes and presence or absence of surrounding *Brassica nigra* plants. Parasitism rates of experimentally introduced *Pieris brassicae* caterpillars and the presence of naturally occurring *Pieris* spp. caterpillars in the plots were assessed throughout the growing season. The abundance of *Pieris* caterpillars was neither affected by cabbage accession nor plot size. Later in the season, when *B. nigra* plants had senesced, fewer caterpillars were found on cabbage plants in plots with a *B. nigra* border. Parasitism rates fluctuated over the season, and were not affected by plot size. However, the *B. nigra* border negatively affected parasitism rates on the accession that is less attractive to the parasitoid *Cotesia glomerata*, but not on the more attractive accession. Our results show that plant variation in HIPVs can differentially influence herbivores and parasitoids depending on characteristics of the surrounding vegetation context. These findings underscore the importance of considering the interaction between focal plant traits and neighbourhood context to reliably predict trophic cascades.

Keywords: host–parasitoid interactions, insect–plant interactions, plant neighbourhood, herbivore-induced plant volatiles



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Introduction

Organisms need to find resources in habitat patches that are embedded in a matrix of unsuitable habitat, thus effectively rendering these habitat patches small islands

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(MacArthur and Pianka 1966). In tritrophic interactions between plants, herbivores and predators, foraging behaviour of herbivores and predators may be differentially affected by characteristics of these patches, such as food-plant identity, vegetation composition and patch size. In a metapopulation composed of patches of varying quality, apparency or vegetation composition, some patches may provide a refuge for herbivores, while others may be heavily exploited by their natural enemies (Van Nouhuys and Hanski 2002, van Nouhuys 2005, Kostenko et al. 2015). Thus, variation in vegetation context may determine local strength and stability of tritrophic interactions (Abdala-Roberts et al. 2019).

Plant–host–parasitoid systems are ubiquitous and the context-dependency of the associated tritrophic interactions is relatively well studied (Cronin and Reeve 2005, Kostenko et al. 2015, Forbes et al. 2018). Host–parasitoid dynamics are expected to be tightly linked because parasitoids are strongly dependent on the abundance of their hosts at the patch scale (Hagen et al. 2012). When the host is a herbivore, tritrophic interactions can be influenced by traits of the herbivore’s food plant (Kos et al. 2011). For example, plant variation in chemical resistance can affect immune responses of herbivores to their parasitoids (Bukovinszky et al. 2009, Smilanich et al. 2009) and plant variation in the emission and chemical composition of herbivore-induced plant volatiles (HIPVs) that are used by parasitoids to locate their host can affect local parasitoid abundance (Vet and Dicke 1992, Beyaert and Hilker 2014, Turlings and Erb 2018). Because parasitoids and herbivores may respond to different cues (Steck and Snell-Rood 2018, Aartsma et al. 2019b), variation in plant traits may affect herbivores and parasitoids differentially and cause spatial variation in the strength of trophic interactions.

The attraction of parasitoids by HIPVs and the finding of food plants by herbivores can be affected by the local vegetation context. For example, the level of herbivory on a focal plant can be influenced by characteristics of neighbouring plants (Kim and Underwood 2015, Wäschke et al. 2014, Moreira et al. 2016), a phenomenon called associational resistance or susceptibility (Barbosa et al. 2009). At the same time parasitoid abundance may be affected by plant community composition (Gols et al. 2005, Bukovinszky et al. 2007, Bezemer et al. 2010, Kruidhof et al. 2015, Kostenko et al. 2015). For both herbivores and parasitoids, nearby plants may impose a physical barrier for foraging, or can limit the spread of HIPVs which are used as cues to find the food plant or host caterpillar (Kostenko et al. 2015, Aartsma et al. 2017). However, surrounding vegetation may also facilitate food location or enhance local abundance of herbivores and parasitoids, for example by increasing contrast between background and food-plant patch (Soler et al. 2007) or by providing additional resources to the insect, such as nectar (Winkler et al. 2009, Rezende et al. 2014). Moreover, food plant patch size may moderate the abundance of insects in the patch (Hambäck and Englund 2005). Large monoculture patches may hamper the ability of small parasitoids with a low dispersal capacity to reach the centre of the patches while

at the same time keeping access to floral resources in field borders (Tschardt et al. 2005). Furthermore, patch size may interact with the presence of neighbouring vegetation to govern species responses because the ratio between plants inside the patch and those surrounding the patch depends on patch size. This may, for example, affect how variation in HIPVs emitted by plant accessions influences the recruitment of parasitoids in patches. Accessions that have a weak innate potential to attract parasitoids upon herbivory may still be able to recruit a meaningful number of parasitoids when these plants are located in a relatively large patch by HIPV dose dependent effects, but not in small patches. This suggests that interactions between spatial scale, neighbourhood and emission of host plant volatiles can influence the strength and stability of parasitoid–host interactions, but this is still poorly understood.

In this study we investigated the outcome of parasitoid–host interactions for the tritrophic relationship of *Brassica oleracea*, *Pieris* caterpillars and its parasitoid *Cotesia glomerata* (Fig. 1) in spatially heterogeneous environments. We specifically tested how plant variation in the emission of HIPVs that attract parasitoids affects the strength of trophic interactions in the context of variation in plant neighbourhood and patch size. We made use of previous work on cabbage accessions that differed in attractiveness to *C. glomerata*. HIPVs of the cabbage accession ‘Christmas drumhead’ induced by *Pieris* caterpillars have been characterised in composition and found to attract more parasitoids than HIPVs of the cultivar ‘Badger shipper’ in both controlled wind tunnel and open field experiments (Poelman et al. 2009a). In the field, parasitoids were attracted to ‘Christmas drumhead’ plants over a longer distance than for ‘Badger shipper’ plants (Aartsma et al. 2019a). We therefore explored the variation in attractiveness to parasitoids, even though in addition to volatile emission the accessions differ in other traits that may contribute to strength of tri-trophic interactions. We expected that larger patch size would enhance abundance of parasitoids (Bezemer et al. 2010), but have lower density of *Pieris* caterpillars since its butterflies are known to prefer smaller plant



Figure 1. *Cotesia glomerata* parasitizing *Pieris brassicae* caterpillars. Photo by Yavanna Aartsma.

patches for oviposition (Bukovinszky et al. 2005). Presence of the wild congeneric food plant for *Pieris*, *Brassica nigra*, in the neighbourhood of the cabbage plants was expected to increase herbivore abundance as well as parasitism rates since both *Pieris* and *Cotesia* make use of the nectar in its flowers as adult food source. We present how the herbivore host and its parasitoid responded to variation in food plant accession, plant neighbourhood and patch size and discuss how these factors interact in determining the strength of tritrophic interactions. Our study advances the field of tri-trophic interactions by revealing how intricate interactions between focal plant traits and neighbourhood context affect tri-trophic interactions.

Materials and methods

Plants and insects

Seeds of white cabbage *Brassica oleracea* var. *alba* accessions ‘Badger shipper’ and ‘Christmas drumhead’ were obtained from the Centre for Genetic Resources (CGN-Wageningen, the Netherlands). For the border surrounding some of the plots, *Brassica nigra* was used. *Brassica nigra* seeds originated from a field population near Wageningen, the Netherlands, and were multiplied by open pollination for two generations. *Brassica nigra* is a wild host plant for *Pieris* caterpillars and its nectar is available to both *Pieris* butterflies and its parasitoids. All plants were grown in peat soil in a greenhouse at Unifarm, Wageningen (L16:D8, 18–26°C and 40–70% RH) and were

transplanted to the field in May 2015 (calendar week 22) as 4 week old seedlings.

Pieris brassicae caterpillars were reared on Brussels sprouts plants in a greenhouse compartment (20–22°C and 50–70% RH) and first (L1) and second (L2) instar caterpillars were used in the experiment.

Experimental design

Field plots were established according to a 2 × 2 × 2 factorial design in May 2015. Plot size was small or large (3 × 3 or 9 × 9 cabbage plants, respectively), with or without a border of *B. nigra* plants, and plots contained either the less attractive accession ‘Badger shipper’ or the more attractive ‘Christmas drumhead’. Thus, there were eight treatments, which were each replicated seven times (Fig. 2). Plants within a plot were spaced 70 cm apart, and there was at least 6 m between the plots. Two rows of white cabbage plants (variety Lennox) were planted around the full field experiment (92 × 96 m) to minimize border effects. In the days after transplanting the seedlings, the seedlings suffered from extensive damage by wood pigeons. Pigeon damage was not treatment dependent since virtually all plants were damaged. Over the next few weeks, all plots fully recovered except for one small ‘Christmas drumhead’ plot with border. This treatment thus had only six replicates.

Field observations

The abundance of caterpillars of *Pieris brassicae* and *Pieris rapae* that colonized the plots were recorded in mid-season

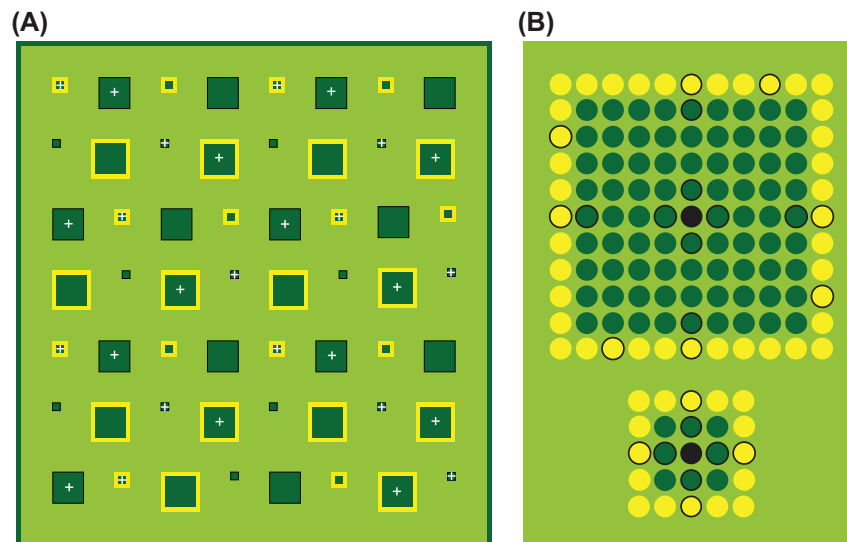


Figure 2. (A) Experimental set-up of the experiment. Large and small squares indicate plots of 9 × 9 and 3 × 3 cabbage plants, respectively. Christmas drumhead plots are indicated with “+” and plots without marker contained variety Badger shipper. Plots with *Brassica nigra* border are indicated with a yellow border, which was planted at the same time as the cabbage plants. Grass was sown between the plots and the field was surrounded by a border of white cabbage of the accession ‘Lennox’. (B) Example of a large and small plot with border. Cabbage plants and *B. nigra* plants are indicated by green and yellow circles, respectively. Plants used for herbivore monitoring are indicated with a black outline. The plant used for experimental inoculation with *Pieris brassicae* caterpillars to assess parasitism rates is indicated as a filled black circle.

(calendar weeks 29 and 30) and late season (weeks 32 and 33). *Cotesia glomerata* is a common parasitoid of *P. rapae* and *P. brassicae* in agricultural fields of the Netherlands and its responses to HIPVs induced by both caterpillars species on 'Badger shipper' and 'Christmas drumhead' are very similar (Poelman et al. 2009a). In plots with 3 × 3 cabbage plants, four *Brassica oleracea* were monitored, and in plots with 9 × 9 cabbage plants, eight cabbage plants were monitored (Fig. 2B). The central plant of the plot, which was used for assessing parasitism rates, was not used to monitor herbivore abundance. In plots with a border, four and eight *Brassica nigra* plants from the border were also monitored in small and large plots, respectively (Fig. 2B).

Parasitism was assessed on a weekly basis by placing 10 first instar (L1) *P. brassicae* caterpillars on the centre plant of each plot. Our previous work shows that using either *P. brassicae* or *P. rapae* to assess *C. glomerata* parasitism rates yield very similar results (Poelman et al. 2009a). After four days, the caterpillars were recollected and dissected under a stereomicroscope to assess the number of *C. glomerata* eggs in each caterpillar (Poelman et al. 2009a). This procedure was repeated during eight weeks from June until August 2015 (calendar weeks 24 to 32, with the exception of week 27). The experiment was terminated in week 33 because the cabbage plants were either senescent or the leaves were too tough for the 1st instar caterpillars to ingest.

Statistical analysis

Herbivores

To investigate whether the abundance of naturally occurring *P. rapae* caterpillars differed between plots with different accessions, sizes and border and at different sampling times, we used a generalized linear model with negative binomial error distribution and log link. The sum of the number of *P. rapae* caterpillars on monitored plants per plot was used as response variable. To account for the different number of plants inspected per treatment we used the number of monitored plants per plot (log-transformed) as an offset variable. Accession, border, plot size, and their two- and three-way interactions were included as explanatory variables. We used a model selection procedure using the 'dredge' function within the MuMIn package in R, which calculates all models with all fixed factor combinations and sorts them based on the Akaike information criterion corrected for small sample sizes (AICc) (Barton 2016).

Because in the late-season monitoring period *B. nigra* plants in the borders were senescing and only a single *P. rapae* caterpillar was found in the borders, we analysed mid and late season data separately. For late-season, only the count data for *P. rapae* on cabbage was analysed. For mid-season, *Pieris rapae* abundance was analysed in more detail by considering three different response variables in separate models: 1) *P. rapae* on cabbage plants only, 2) *P. rapae* on *B. nigra* border plants only, and 3) *P. rapae* on cabbage and *B. nigra* border plants combined. For all three response variables we used the same model as described above with adjusted number of monitored plants as offset variable.

Parasitism rate

Parasitism rates were calculated as the percentage of caterpillars parasitized out of the *P. brassicae* caterpillars recovered from a plot. Generalized linear mixed models with binomial error distribution were used to investigate whether parasitism rates (response variable) were affected by accession, plot size, border presence, time of the year (fixed factors) and their two-way interactions. We included an observation level random effect to account for overdispersion in the data. We used the same model selection procedure as described above for the herbivores. The interaction between factors was analysed in more detail with a Tukey post hoc test.

Superparasitism

Cotesia glomerata females rarely deposit more than 35 eggs per caterpillar in a single event of parasitism (Poelman et al. 2013). Therefore, we scored caterpillars with more than 35 eggs as being superparasitized (i.e. parasitized on two or more occasions). To investigate whether superparasitism rates were affected by accession, plot size, border presence and time of the year, we used a generalized linear mixed model with a binary response variable 'superparasitism' (i.e. presence or absence). Accession, plot size, border presence and week were included as fixed factors, and two-way interactions were included as well. Plant ID was used as a random effect in the superparasitism analysis to account for caterpillars co-occurring on the same plant at the same time. We used the 'dredge' procedure to identify the most parsimonious models. Data were analysed in R (<www.r-project.org>) using the packages lme4 (Bates et al. 2015), MuMIn (Barton 2016), MASS (Venables and Ripley 2002) and multcomp (Hothorn et al. 2008).

Results

Herbivores

Naturally occurring *Pieris brassicae* caterpillars were encountered on only 4 and 13 plants out of the 496 plants monitored during mid- and late season, respectively, and their abundance was low (0.0068 ± 0.0039 and 0.28 ± 0.21 caterpillars plant⁻¹ plot⁻¹, respectively). These numbers were too low for meaningful statistical analysis. The average abundance of *P. rapae* was 0.29 ± 0.044 and 0.38 ± 0.054 caterpillars plant⁻¹ plot⁻¹ (mean ± SE) for cabbage (Christmas drumhead and Badger shipper) and *B. nigra* plants combined in mid- and late season, respectively. In the mid-season, *P. rapae* caterpillars were present on cabbage plants within the plots and on the *B. nigra* border plants. *Pieris rapae* abundance on cabbage, *B. nigra* or combined was not significantly related to plot size, presence of a border or cabbage variety ($p > 0.05$; Table 1, Fig. 3A–C). In the late season, *P. rapae* abundance on cabbage plants was lower in plots with *B. nigra* border than plots without border ($p < 0.01$), but was not significantly different between large and small plots or between accessions Badger shipper and Christmas drumhead ($p > 0.05$; Table 1, Fig. 3D).

Table 1. Determinants of *Pieris rapae* abundance on *Brassica oleracea* and/or *Brassica nigra* plants in plots with different sizes (3 × 3 plants or 9 × 9 plants), with or without a *B. nigra* border, and with the attractive *B. oleracea* accession (Christmas drumhead) or less attractive accession (Badger shipper) in a generalized linear model with negative binomial error distribution and log link.

Factor	Level	Mid-season			Late-season
		<i>Pieris rapae</i> abundance on <i>Brassica oleracea</i>	<i>Pieris rapae</i> abundance on <i>Brassica nigra</i>	<i>Pieris rapae</i> abundance on <i>Brassica oleracea</i> and <i>Brassica nigra</i>	<i>Pieris rapae</i> abundance on <i>Brassica oleracea</i>
Size	9 × 9 plants	–	–	–	–
	3 × 3 plants	–0.105 (0.284)	0.575 (0.687)	0.053 (0.289)	–0.368 (0.256)
Border	No border	–	N/A	–	–
	Border	–0.328 (0.278)	–	–0.458 (0.286)	–0.783* (0.249)
Accession	Badger shipper	–	–	–	–
	Christmas drumhead	0.087 (0.276)	–0.351 (0.689)	0.031 (0.286)	0.446 (0.243)
Intercept		–1.036** (0.260)	–1.763** (0.575)	–1.069** (0.282)	–0.739** (0.224)
No. of plots monitored		55	27	55	55

The factor ‘border’ was not included for *P. rapae* abundance on *B. nigra* as this plant species was only present in plots including a border. In the late season, *P. rapae* was not present on *B. nigra* border plants and therefore only *P. rapae* abundance on the cabbage (*B. oleracea*) plants was analysed. Estimates with standard error (between brackets) and statistical significance (bold and with asterisks) are reported. Only main effects are shown, as interactions were not significant. Reference treatments are ‘9 × 9 plants’, ‘no border’ and ‘Badger shipper’. *p < 0.05; **p < 0.01.

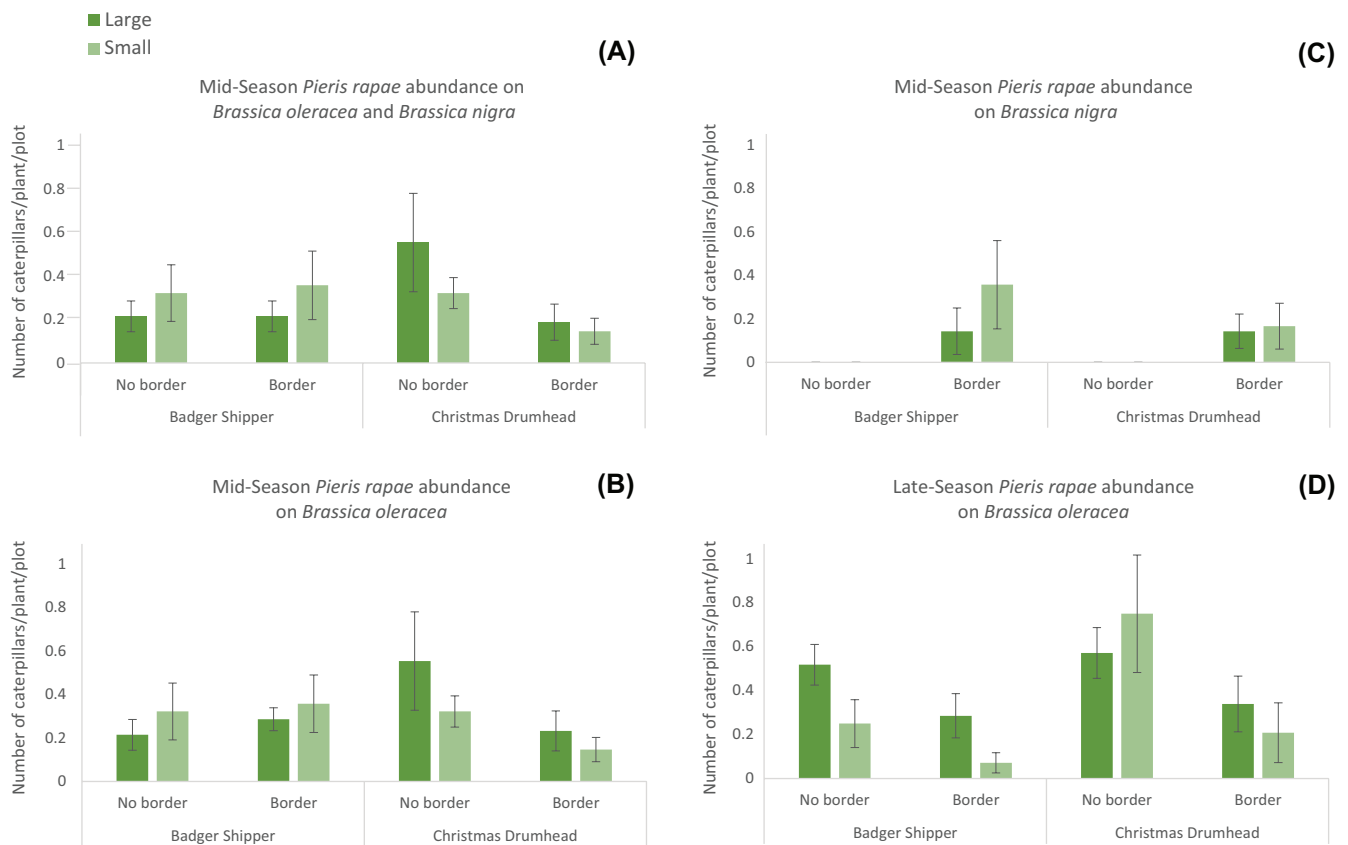


Figure 3. *Pieris rapae* abundance on *Brassica oleracea* and/or *Brassica nigra* plants in plots differing in cabbage variety (Badger shipper or Christmas drumhead), presence of a *B. nigra* border (no border, border) and plot size (large, 9 × 9 plants, or small, 3 × 3 plants). (A) shows average abundance of *P. rapae* in the mid-season on *B. oleracea* and *B. nigra* border plants combined, (B) shows on *B. oleracea* plants only and (C) on *B. nigra* border plants only. (D) shows average abundance of *P. rapae* in the late-season on *B. oleracea* only. Results of the statistics can be found in Table 1.

Parasitoids

The overall recovery rate of the experimentally introduced caterpillars on the centre plants in plots was on average 39.6%. Parasitism rates of these caterpillars varied over the season and approached 100% parasitism from week 28 onwards (Table 2, Fig. 4A–B). There was no significant effect of plot size on the parasitism of *P. brassicae* larvae on cabbage plants ($p > 0.05$; Table 2). However, there was a significant interaction between cabbage accession and border ($p < 0.05$), indicating that plots consisting of the less attractive variety Badger shipper had lower parasitism rates when a surrounding border of *B. nigra* was present, while the presence of a *B. nigra* border did not affect parasitism levels in plots of the more attractive variety Christmas drumhead (Table 2, Fig. 5).

Superparasitism incidence fluctuated throughout the season (Table 2) and mirrored the temporal fluctuations of overall parasitism rates (Fig. 4). Overall, superparasitism was more frequent on the attractive accession Christmas drumhead than on accession Badger shipper ($p < 0.001$; Table 2, Fig. 4C), but was not affected by plot size and presence of a border ($p > 0.05$; Table 2, Fig. 3D–E).

Discussion

While insect herbivores and their parasitoids are commonly confronted with heterogeneous environments, few studies have investigated how the interaction of spatial scale, neighbouring plants and variation in plant volatiles influences parasitoid–host interactions. The results of this study highlight

the differential responses of herbivores and their primary parasitoids to these environmental factors, and show the complex interaction between variation in plant volatile emission and plant neighbourhood for host finding by parasitoids, and the associated strength of tritrophic interactions. Our study has three key findings. First, herbivore infestation was lower in plots surrounded by a *B. nigra* border at the end of the season, but not in the middle of the season. Second, a *B. nigra* border was associated with lower parasitism rates, but only on the less attractive accession Badger shipper. Third, superparasitism was consistently the highest in plots with the attractive accession Christmas drumhead. We here discuss these findings for herbivores and parasitoids separately and integrate the responses in the context of spatial variation in strength of tritrophic interactions.

Herbivores

Pieris rapae abundance was not significantly different in plots with Badger shipper and Christmas drumhead at both assessments during the growing season, which aligns with results of a previous field study using the same accessions (Poelman et al. 2009b). While direct oviposition choice experiments under controlled conditions showed that *P. rapae* butterflies preferred Badger shipper plants over Christmas drumhead and that *P. rapae* larvae showed a slightly better development on Badger shipper than Christmas drumhead (Poelman et al. 2009b), we were not able to demonstrate these effects under field conditions. In the field, local abundance of *P. rapae* caterpillars may be more profoundly driven by other factors than oviposition preference for cabbage accessions.

Table 2. Determinants for parasitism as a binomial variable and superparasitism as a binary dependent variable on plants in plots with different sizes (3 × 3 plants or 9 × 9 plants), with or without a *B. nigra* border, and with the attractive *Brassica oleracea* accession (Christmas drumhead) or less attractive accession (Badger shipper) and the different weeks of the experiment in a generalized linear mixed model with binomial error distribution and log link.

Factor	Level	Parasitism (parasitized/total)	Parasitism (0) or superparasitism (1)
Border	No border	–	/
	Border	–1.621** (0.589)	
Accession	BS	–	–
	CD	0.547 (0.605)	0.946*** (0.269)
Size	9 × 9 plants	–	/
	3 × 3 plants	0.680 (0.436)	/
Border × accession	–	–	/
	CD: border	1.879* (0.873)	
Time	Week 24	–	–
	Week 25	–4.394*** (0.795)	–2.547*** (0.757)
	Week 26	–2.741*** (0.769)	0.609 (0.544)
	Week 28	2.320* (0.911)	4.757*** (0.618)
	Week 29	3.096*** (0.867)	1.711*** (0.434)
	Week 30	0.172 (0.801)	–0.718 (0.486)
	Week 31	0.607 (0.743)	–0.786 (0.466)
	Week 32	3.295** (1.178)	2.056*** (0.471)
	Intercept		2.517*** (0.670)
Observations		332 plants	1371 caterpillars

No border, 9 × 9 patch size, the less attractive accession Badger shipper and week 24 were the reference treatments for border, size, accession and week, respectively. Plant ID was used as a random effect in the superparasitism analysis to account for caterpillars co-occurring on the same plant at the same time. A slash symbol (/) indicates that this variable was not selected in this model. Estimates are shown, with standard error between brackets and statistical significance in bold and with asterisks *. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

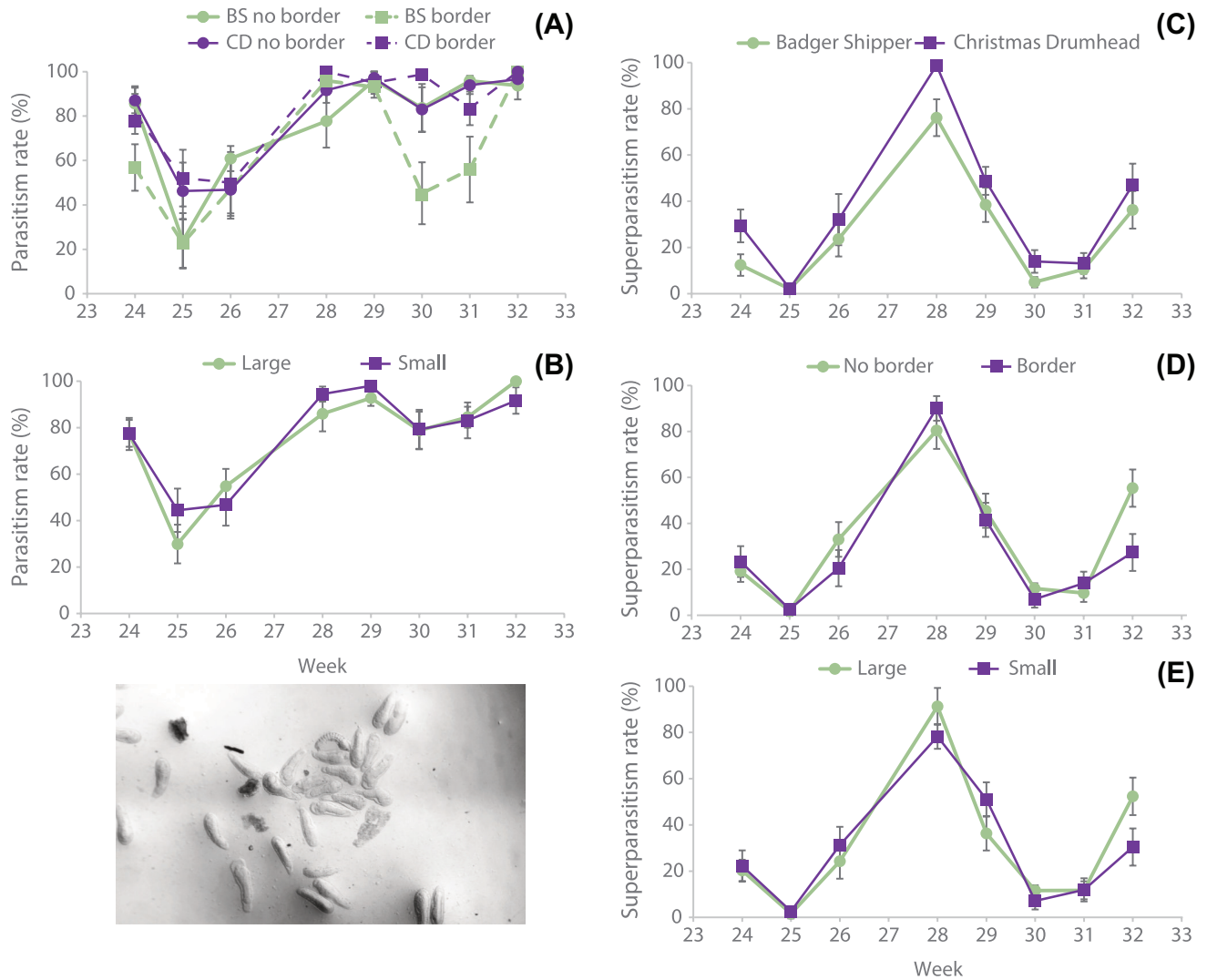


Figure 4. Parasitism rates (left) and superparasitism rates (right) of *Pieris brassicae* caterpillars that were experimentally released on the central *Brassica oleracea* plant in plots. Caterpillars with more than 35 eggs were considered to have been superparasitized, and superparasitism was calculated as the percentage of parasitized caterpillars that were superparasitized. (A) shows the interaction between accession (less parasitoid-attractive accession Badger shipper or more parasitoid-attractive accession Christmas drumhead) and border (absent or present). (B) and (E) display differences between plot sizes (9×9 plants versus 3×3 plants). (C) displays differences between Badger shipper and Christmas drumhead. (D) displays differences between border absence or presence. The photo shows eggs of *Cotesia glomerata* after dissection of the caterpillars.

For instance, Bukovinszky et al. (2005) found more oviposition by *P. rapae* in small than in large cabbage plots, regardless of the vegetation type surrounding these plots. In simulation studies, it was postulated that *P. rapae* uses visual cues to find cabbage patches and that small patches are more conspicuous in the background vegetation (Bukovinszky et al. 2005, Hambäck and England 2005). A visual search mode in combination with a high movement capacity might explain why *P. rapae* abundance was not higher in large plots of the study by Bukovinszky et al. (2005). However, our study does not confirm this hypothesis as we found no significant association between caterpillar abundance and plot size. This may be explained by the fact that the *P. rapae* abundance in our study was generally lower than in the studies by Bukovinszky et al.,

which makes it harder to demonstrate significant differences between treatments.

In the late season, there were fewer *P. rapae* caterpillars in plots with a *B. nigra* border than without border. At this moment, the *B. nigra* plants were senescent, suggesting that factors associated with development stage of the surrounding border plants may have influenced the oviposition preference of the *Pieris* butterflies. Since *P. rapae* butterflies use a visual searching mode (Bukovinszky et al. 2005), this suggests that the border obstructed the view on suitable host plants inside the plot. While visual cues such as leaf colour may reduce colonization of plants with heterospecific neighbouring plants (Finch et al. 2003), we cannot exclude the possibility that the *B. nigra* plants may have interfered with volatile cues

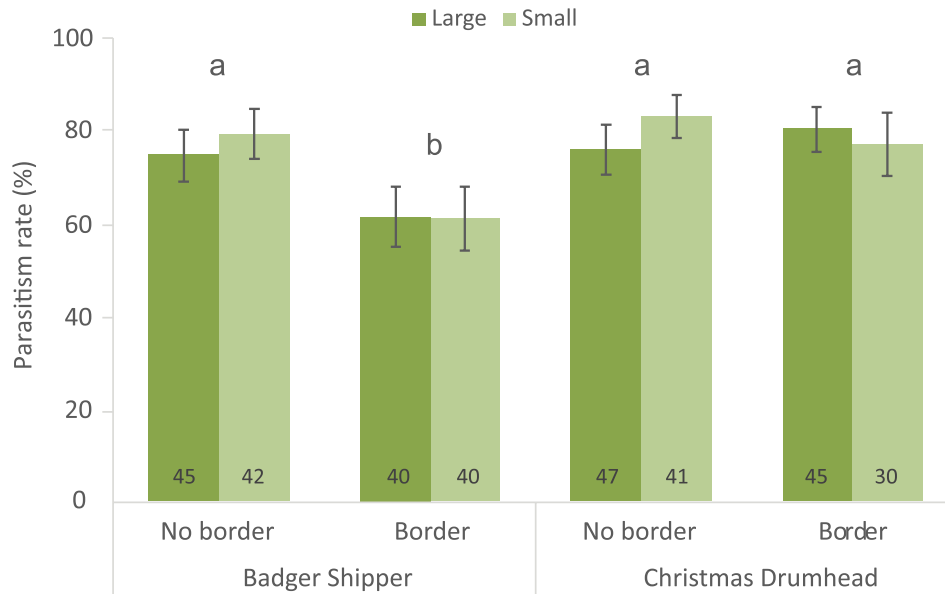


Figure 5. Average percentage of experimentally introduced L1 *Pieris brassicae* caterpillars that were parasitized on the central plant of plots differing in cabbage variety (Badger shipper or Christmas drumhead), plot size (large, 9 × 9 plants, or small, 3 × 3 plants) and presence of a border of *Brassica nigra* plants across 8 experimental weeks. Different letters indicate significant differences between combinations of border and cabbage variety (Tukey HSD). Error bars represent standard error of the mean and the number of observations is shown at the base of the bars.

from the cabbage as well. Nevertheless, the border vegetation caused associational effects by reducing caterpillar presence on *B. oleracea* and thereby reduced host availability for parasitoids in these patches (Hambäck et al. 2014).

Parasitoids

Parasitism by *Cotesia* spp. was lower on the less attractive cabbage variety Badger shipper when surrounded by a border of *B. nigra* plants compared to plots without a border. In contrast, parasitism rates on the more attractive cabbage variety Christmas drumhead were not affected by the presence of a border. While the border could provide both a physical and visual barrier for parasitoids, potentially act as a barrier for odour plumes or mask odour plumes (Kostenko et al. 2015, Aartsma et al. 2017), our data suggest that the border may hamper the perception of HIPVs from the less attractive accession, but not of the attractive accession. Thus, a more attractive variety may be more chemically apparent for parasitoids in patches with a complex vegetation structure. Previously, we found that the more attractive accession is able to attract parasitoids from a greater distance than the less attractive accession (Aartsma et al. 2019a). The relative attractiveness of *B. nigra* plants compared to Badger shipper and Christmas drumhead is not known and merits further investigation. In addition to the known differences in attractiveness of the accessions, other traits such as appearance or defence chemistry may have contributed to the observed effects on parasitism levels (Nell and Mooney 2019).

In the absence of a *B. nigra* border, parasitism rates were not significantly different in plots with attractive and less

attractive accessions. However, the absence of an accession effect may be masked by the high parasitism rates in the experiment, particularly later in the season, when parasitism rates approached 100% (Fig. 4). Interestingly, superparasitism incidence was indeed higher on the more attractive variety than on the less attractive variety, confirming higher parasitoid visitation in plots with the attractive accession (Poelman et al. 2013, Aartsma et al. 2019a). Thus, the assessment of superparasitism allowed us to demonstrate the higher visitation rates in plots with the attractive accession, despite the saturation of parasitism in plots.

Parasitism rates were not affected by plot size. Therefore, our hypothesis that larger plots might attract more parasitoids by generating a larger amount of HIPVs was not confirmed. There was also no significant interaction between plot size and the attractiveness of the plant accession. In our study, we only experimentally inoculated the central plant of the plot with caterpillars to assess parasitism. As the abundance of *Pieris* caterpillars from natural infestations was relatively low, the other plants in the plot may not have been induced to produce HIPV-blends attractive to *C. glomerata*, and therefore played a minor role in attracting *C. glomerata* to the central plants. Further studies may reveal whether the effect of plot size is more pronounced in years with higher natural infestation of *Pieris* caterpillars.

Response of herbivores vs response of parasitoids

The colonization of plots by herbivores and parasitoids were influenced by different factors. While the abundance of the herbivore *P. rapae* was negatively associated with a *B. nigra*

border late in the season, parasitism rates by *Cotesia* were affected by an interaction between cabbage accession and *B. nigra* border. The difference in responses by members of the second and third trophic level may be mediated by the type of cues they use (HIPVs versus general plant odours and visual cues) (Aartsma et al. 2019b). The spatial distribution of odour cues can be affected by structural and chemical barriers from surrounding vegetation, resulting in a spatially heterogeneous volatile mosaic (Randlkofer et al. 2010, Aartsma et al. 2017). In addition, background odours can play a role in masking of chemical cues (Vos et al. 2006, Hilker and McNeil 2008, Schröder and Hilker 2008). While a weak emitter of cues can be 'hidden' in background odours from neighbouring plants (Vos et al. 2006), a stronger emitter of these cues (i.e. a more attractive variety) might be easier to distinguish from background odours emitted by other plants. It should be noted, however, that attractiveness or apparency of host-infested plants can be influenced by both quantitative and qualitative aspects of HIPVs (Clavijo McCormick et al. 2012, Rowen and Kaplan 2016), which are difficult to disentangle under field conditions. Chemical analysis of volatile headspace composition revealed that Christmas drumhead and Badger shipper plants primarily differ in quantity and ratios of terpene emission after *Pieris* herbivory, but which compounds or ratios were causal for the difference in attraction of *Cotesia* could not be identified (Poelman et al. 2009a)

Besides the chemical composition of plant volatile cues, the dispersal capacity of insects might also affect how different trophic levels are influenced by habitat context. Herbivores tend to have a higher dispersal capacity than their parasitoids, which may be related to differences in body size (Van Nouhuys and Hanski 2002, Tscharrntke and Brandl 2004, van Nouhuys 2005). Therefore, herbivores may generally operate at larger spatial scales than their parasitoids, and may be less affected by barriers, such as the *B. nigra* border in our study. The late-season reduction in *P. rapae* abundance in bordered plots may be more a result of oviposition preference due to presence of senescing border plants than the herbivore's dispersal capacity. The contrasting responses of herbivores vs parasitoids to volatile cues and plant neighbourhood can be important for the outcome of host–parasitoid interactions. For instance, herbivores may escape parasitism by colonizing areas that are more difficult to find or reach by the less mobile parasitoids, and therefore habitat heterogeneity can stabilize host–parasitoid interactions (Gols et al. 2005, Bukovinszky et al. 2007, Karban et al. 2013). Indeed, herbivores may use less favourable plants or habitat types that are less suitable for their parasitoids to escape parasitism (Ohsaki and Sato 1994, Ohsaki and Sato 1999). In our study, we found no evidence for food plant choice by *Pieris* based on parasitism risk since *Pieris* caterpillars were less abundant in plots with a *B. nigra* border, whereas the border reduced parasitism levels in plots with Badger shipper plants. The lower abundance of *Pieris* caterpillars in plots with a border may cause a reduced local population size of the parasitoid by reduced host availability. These effects are cancelled out by

enhanced parasitoid recruitment in plots with the attractive accession Christmas drumhead. In addition to their different attractiveness to parasitoids, differences in food plant quality between the two accessions may cause indirect plant-mediated effects on parasitoid survival. Chemical defences and nutritive value of the food plant may affect vulnerability of herbivores to parasitism by affecting their development time, and hence their exposure time to parasitoids (slow growth – high mortality hypothesis) (Benrey and Denno 1997) or by affecting herbivore immune reactions to parasitoid eggs (Bukovinszky et al. 2009).

In conclusion, this study shows that plant accession variation in attractiveness to parasitoids results in variation of parasitoid recruitment in the field that is strongly dependent on the context of plant neighbourhood. In concert, these factors may also differentially affect herbivore abundance and result in local variation of the strength of tritrophic interactions. The findings of this study warrant further research on the interacting effects of variation in plant traits and plant neighbourhood on parasitoid–host interactions.

Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.2280gb5pg>> (Aartsma et al. 2020).

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