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The effect of direct and indirect defenses in two wild brassicaceous plant species on a specialist herbivore and its gregarious endoparasitoid

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Abstract

Most studies on plant defenses against insect herbivores investigate direct and indirect plant defenses independently. However, these defenses are not necessarily mutually exclusive. Plant metabolites can be transmitted through the food chain and can also affect the herbivore's natural enemies. A conflict may arise when a natural enemy is attracted to a plant that is suboptimal in terms of its own fitness. In addition, plant defenses are often studied in cultivated plant species in which artificial selection may have resulted in reduced resistance against insect herbivores. In this study, we investigated both direct and indirect plant defenses in two closely related wild brassicaceous plant species, *Brassica nigra* L. and *Sinapis arvensis* L. The herbivore *Pieris brassicae* L. (Lepidoptera: Pieridae), which is specialized on brassicaceous plant species, developed faster and attained higher pupal mass when reared on *B. nigra* than on *S. arvensis*. In contrast, *Cotesia glomerata* L. (Hymenoptera: Braconidae), which is a gregarious endoparasitoid of *P. brassicae* caterpillars, developed equally well on *P. brassicae* irrespective of the food plant on which its host had been reared. The feeding strategy of the parasitoid larvae, that is, selectively feeding on hemolymph and fat body, is likely to allow for a much wider host-size range without affecting the size or development time of the emerging parasitoids. In flight chamber experiments, *C. glomerata*, which had an oviposition experience in a host that fed on Brussels sprout, exhibited significant preference for host-damaged *B. nigra* over host-damaged *S. arvensis* plants. Headspace analysis revealed quantitative and qualitative differences in volatile emissions between the two plant species. This parasitoid species may use a range of cues associated with the host and the host's food plant in order to recognize the different plant species on which the host can feed. These results show that there is no conflict between direct and indirect plant defenses for this plant–host–parasitoid complex.

Introduction

Plants have evolved several defense traits to prevent or reduce feeding by insect herbivores. These traits can be broadly divided into direct and indirect defenses. Direct plant defenses refer to plant traits that negatively affect development of the herbivore, whereas indirect defenses refer to traits that promote the effectiveness of the herbivore's natural enemies. Examples of direct defenses are morphological characteristics, such as spines and wax layers, which may hamper colonization and movement on the plant (Schoonhoven et al., 2005). Furthermore, the production of chemicals, such as toxins and digestibility reducers, may interfere with the physiology of the herbivore and reduce growth and survival (Schoonhoven et al., 2005). Indirect plant defenses include the provisioning of

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alternative food sources and shelter for the herbivore's natural enemies, and the production of volatile attractants. It has been demonstrated for many plant species that herbivore damage induces the release of volatile chemicals that attract parasitoids and predators. These herbivore-induced plant volatiles can be used by the natural enemies of herbivores to locate plants infested with their prey or hosts (Dicke, 1999; Paré et al., 1999; Sabelis et al., 1999; Vet, 1999; Turlings et al., 2002).

A conflict between direct and indirect plant defenses may arise when plants have high levels of direct defense compounds and at the same time are also highly attractive to the insect's natural enemies (Havill & Raffa, 2000). It has been demonstrated that plant secondary chemicals, such as toxins, not only affect development of insect herbivores but also that of higher trophic levels (reviewed by Harvey, 2005; Ode, 2006). As direct and indirect plant defenses are usually studied independently, the occurrence of conflicts between different defensive traits is rarely addressed. Furthermore, direct and indirect plant responses do not act independently of each other. For instance, the plant hormone jasmonic acid plays an important role in the induction of direct as well as indirect plant defenses (reviewed by Dicke & van Poecke, 2002). In order to better understand the evolution of plant defenses against arthropod herbivores, plant defenses should also be studied in wild plant species.

Research exploring evolutionary aspects of interactions between plants, herbivores, and their natural enemies in a multi-trophic framework has often employed crop plants in stead of their wild relatives. Plant breeding programs may have disrupted the original plant defense strategies that were present in wild progenitors of cultivated plants that evolved under natural selection (Evans, 1993; Rosenthal & Dirzo, 1997). Artificial selection of crop plants has in some cases reduced the level of secondary plant compounds and may have altered the strength of indirect plant responses as well. Therefore, it is even more important to compare the chemistry of crop plants with their wild relatives and to determine how this affects the behavior and development of associated insect consumers (van der Meijden & Klinkhamer, 2000).

In this study, we investigated both direct and indirect plant defenses in two closely related wild plant species, *Brassica nigra* L. and *Sinapis arvensis* L. (Brassicaceae). Both species are native to much of Eurasia, including The Netherlands, and belong to the large family Brassicaceae, which is characterized by the production of glucosinolates (GS). Hydrolyzed metabolites of GS, which are produced in response to tissue damage, play an important role in defense of Brassicaceae against insect herbivory (Chew, 1988; Rask et al., 2000). Some insect species have evolved mechanisms to detoxify GS (Ratzka et al., 2002; Wittstock et al., 2004). Insects that are specialist feeders on plants containing GS may even use these chemicals as feeding stimulants (van Loon et al., 1992; Bartlet et al., 1994; Renwick, 2002) or sequester them and employ them for their own defense (Müller et al., 2001; Aliabadi et al., 2002). However, high levels of GS breakdown products can also have a negative effect on the development of specialist herbivores (Agrawal & Kurashige, 2003). Furthermore, different brassicaceous plant species not only vary in food plant quality for hosts specialized on plants in this family, but also for their endoparasitoids (Ohsaki & Sato, 1994; Harvey et al., 2003; Sznajder & Harvey, 2003; Harvey & Wagenaar, 2006).

Pieris brassicae L. (Lepidoptera: Pieridae) is a specialist herbivore that feeds exclusively on plants producing GS. Larvae are often considered to be pests of cultivated crucifer species (e.g., *Brassica oleracea*), but also readily feed on related wild species in the Brassicaceae, such as *B. nigra* and *S. arvensis* (Feltwell, 1982). *Cotesia glomerata* L. (Hymenoptera: Braconidae) is a fairly specialized gregarious endoparasitoid that primarily attacks first to third instars of *P. brassicae* and a related species, *Pieris rapae*, in Eurasia (where all three species are native). Larvae of *C. glomerata* feed primarily on their host's hemolymph and fat body before emerging through the side of the host to pupate.

The aims of this study were (i) to investigate whether *B. nigra* and *S. arvensis* differ in plant quality for *P. brassicae* as well as for its larval endoparasitoid, *C. glomerata*, and (ii) to determine whether female *C. glomerata* wasps are differentially attracted to the two plant species when damaged by *P. brassicae* larvae. In addition, we measured GS concentrations in leaf tissue and described the chemical composition of the herbivore-induced volatile blend emitted by the two plant species. Differences and similarities in herbivore and parasitoid performance, respectively, are discussed in relation to GS content and parasitoid feeding biology. Furthermore, we discuss the dynamic response of *C. glomerata* to different host plant species that emit quantitatively and qualitatively different volatiles blends.

Materials and methods

Plants

Seeds for *B. nigra* and *S. arvensis* were obtained from large, single populations growing within 2–10 km of Wageningen, The Netherlands (coordinates *B. nigra* 51°58′N, 5°38′E and *S. arvensis* 51°57′N, 5°48′E). Plants of both species were grown in pots (20 × 25 cm) containing a soil mixture consisting of approximately 30% sand, 5% clay, and 65% peat. Plants were reared in a climate-controlled greenhouse

at 25 ± 2 °C, 50% r.h., and a photoperiod of at least 16 h. If the light dropped below 500 µmol photons/m^2/s during the 16-h photoperiod, supplementary illumination was applied by high-pressure mercury lamps. Plants were watered daily. In the experiments, we used non-flowering plants that were approximately 4 weeks old.

Insects

The herbivore, *P. brassicae*, and its larval endoparasitoid, *C. glomerata*, were originally collected from Brussels sprout (*B. oleracea* variety *gemmifera*) fields in the vicinity of Wageningen. Cultures of *P. brassicae* and *C. glomerata* were reared on Brussels sprout (cultivar Cyrus) in climate-controlled rooms at 22 ± 2 °C, 50% r.h., and an L16:D8 photoperiod. The parasitoid, *C. glomerata*, was reared from *P. brassicae.* A leaf infested with first (L1) or second (L2) instars was placed in the rearing cage with adult wasps for 10–30 min, depending on the density of the wasps. The leaf with parasitized larvae was then transferred to a cage containing cabbage plants. After approximately 2 weeks, when the host caterpillars were late L5 stage, the parasitoid larvae egressed in preparation for pupation. Parasitoid cocoons were collected and transferred to a new cage until emergence of the adult wasps. The wasps were supplied with 10% (wt/vol) sucrose dissolved in tap water. Wasps were used either for rearing or experimental purposes.

Plant quality for development of the herbivore and its parasitoid

Neonate larvae of *P. brassicae* were placed in groups on leaves of the two crucifer species in cages (40 × 70 × 50 cm) and kept in climate room facilities at 25° ± 2 °C, 50% r.h., and an L16:D8 photoperiod. Approximately 30 caterpillars were kept in each cage to prevent overcrowding and to standardize rearing conditions. First instars were allowed to feed and develop on their respective food plants until the second day. At this stage, for each food plant, one cohort of 54 larvae was parasitized by *C. glomerata*, whereas a second cohort of 32 larvae served as unparasitized control. Caterpillars of *P. brassicae* were individually presented to parasitoid females in plastic vials. Wasps were allowed to parasitize the host once. The completion of the oviposition of a single brood by *C. glomerata* normally takes more than 10 s; hosts from which females removed their ovipositor within 5 s were assumed to be rejected. None of the hosts offered to the wasps was rejected. More than 20 female wasps were used to parasitize 108 larvae.

Following parasitism, hosts were returned to their respective food plants in rearing cages with a maximum of four plants in a single cage. Unparasitized larvae were kept in separate cages on their respective food plant. Plants were added every 3 days or earlier, if insufficient leaf material remained for feeding. Approximately 24 h prior to larval parasitoid egression and pupation, host caterpillars entered the wandering phase and left the food plants by climbing to the top of the cage. From here they were removed from the cages and maintained in labeled 9.5-cm Petri dishes until parasitoid egression and pupation. The number of parasitoid larvae that egressed from a single host (= secondary brood size) was recorded. The still-living host was separated from the cocoons and weighed on a microbalance (1 µg accuracy) (Sartorius AG, Göttingen, Germany).

The remaining parasitoid cocoons were checked several times daily for adult parasitoid emergence. Unparasitized larvae also leave the plant when larval development is completed and pupate on the cage walls. For each pupa, the day of pupation and their fresh weight was recorded. Upon eclosion, adult *C. glomerata* wasps were killed by freezing. They were then segregated by sex and brood (i.e., wasps emerged from a single caterpillar) and weighed. The mean body masses of male and female wasps per brood were obtained by dividing the total mass of emerged wasps by the number of wasps in the brood. In order to ensure that fresh mass is an efficient index of parasitoid size, a subsample of male and female wasps from five broods originating from each plant (= 10 in total) were dried in an oven at 60 °C for 48 h, and then weighed on the microbalance. Development time of male and female parasitoids was recorded in days. Most parasitoids from a single brood emerged between 08:00 and 15:00 hours on the same day; however, wasps that emerged after 21:00 hours were assumed to emerge on the following day. Development time was determined as the number of days between parasitism and adult emergence. Offspring sex ratios were determined only for *C. glomerata* that produced mixed broods (n = 47 in *B. nigra* and n = 43 in *S. arvensis*).

Glucosinolate analyses

Samples for GS analyses were taken during the host plant-quality experiment from both *S. arvensis* (n = 9) and *B. nigra* plants (n = 8). After the plants had been damaged by *P. brassicae* for 3 days, all remaining leaf tissue was collected. Immediately after harvesting, samples were frozen at –20 °C. Fifty mg aliquots of freeze-dried and pulverized material were used for GS analysis. GS were extracted with boiling 70% methanol, desulfated and separated by means of high performance liquid chromatography (for a detailed description of the method see van Dam et al., 2004).

Flight response of *Cotesia glomerata* to *Brassica nigra* and *Sinapsis arvensis* plants infested with *Pieris brassicae*

The flight response of *C. glomerata* was tested in a greenhouse compartment at 22 ± 2 °C and 60% r.h. in a tent-like

structure (330 × 175 × 280 cm), which was made of white nylon sheets. The greenhouse compartment was continuously ventilated, which created a turbulent air current inside the tent. In the bioassay, we placed two plants, each of a different treatment (see below), on a table 60 cm apart. *Cotesia glomerata* females were released in vials at one end of the table 100 cm away from the plants. Female wasps were collected from the mass-rearing before using them in the bioassay by putting a leaf of a Brussels sprout plant with L1 or L2 *P. brassicae* larvae in the rearing cage for less than a minute. Females were allowed to oviposit and were collected from this leaf with a fine paintbrush and transferred to 5-ml vials (one wasp per vial). Oviposition experience enhances the motivation of the wasps to forage for hosts. As wasps do not leave the vials instantaneously, about 10 vials were placed on the table simultaneously. Wasps were allowed to fly freely within the tent. As soon as a wasp landed on one of the two plant species, it was collected and the species on which it had landed was recorded. If some of the wasps that were initially released had not alighted on one of the plants within 30 min, additional wasps were released. The position of the two plants was exchanged after five wasps had landed.

Experiment 1: undamaged vs. damaged plants. In this experiment, we tested the response of female wasps to non-infested clean plants vs. plants damaged by 20 L1 *P. brassicae* larvae for 96 h for both plant species separately. For each plant species, we conducted three replicates on different experimental days with 10–15 wasps in each replicate. Wasps were only used once.

Experiment 2: damaged Brassica nigra *vs. damaged* Sinapsis arvensis. In this experiment, we tested the two plant species against each other. Plants had been infested with 20 L1 larvae of *P. brassicae* and were tested in the flight chamber 1, 2, 3, and 4 days after the caterpillars had been placed on the plants. Plants had been labelled and the two plants were tested again on three successive days in the same combination. Wasps were not allowed to search for hosts and oviposit on *B. nigra* and *S. arvensis*. Each wasp was only used once on an experimental day, after which it was returned to the culture. A total of 10 wasps were recorded per set of plants per day. In total, we used 18 sets of plants, which were tested in two blocks of four experimental days with approximately 400 different females.

Headspace collection and analyses

Plants were infested with 20 L1 larvae of *P. brassicae*, as in the flight chamber bioassay. Larvae were allowed to feed for 4 days before the plants (with the caterpillars) were transferred to 35-l glass collection containers. Pressurized air was filtered over silica gel, molecular sieves 4Å (Sigma, St Louis, MO, USA) and activated charcoal before entering the sampling container. Prior to sampling, the container was purged for 1 h. Subsequently, the headspace volatiles were trapped on Tenax TA (90 mg, 20/35 mesh; Chrompack, Middelburg, The Netherlands) for 4 h at 100 ml min^{-1}. Headspace collection was conducted in five replicated experiments for both plant species in a climate-controlled room 22° ± 1 °C with mercury lamps (40 ± 10 µmol photons/m^2/s) hanging above the pots.

The collected volatiles were released from the Tenax by heating in a Thermodesorption Cold Trap Unit (Chrompack) at 250 °C for 10 min and flushing with a helium flow of 10 ml min^{-1}. The released compounds were cryofocused in a cold trap (0.52-mm inside diameter deactivated fused silica) at a temperature of –85 °C. The volatiles were transferred to the gas chromatograph (GC)-column (Supelcowax 10 fused sillica capillary column; 60 m × 0.25 mm i.d., 0.25 µm film thickness) by ballistic heating of the cold trap to 220 °C. The GC-column was connected to a Finnigan MAT 95 mass spectrometer (Bremen, Germany) and programmed from 40 °C (3 min hold) to 270 °C (4 min hold) at 4 °C min^{-1} and the initial helium flow rate was 30 cm s^{-1}. The mass spectrometer was operated in the 70 eV EI ionization mode and scanning was done from mass 24 to 400 at 0.7 s per decade. Compounds were identified by comparison of the mass spectra with those in the Wiley library and in the Wageningen Mass Spectral Database of Natural Products and by checking the retention index.

Statistical analysis

Two-sample t-tests were used to compare pupal mass and development time of *P. brassicae* on the two plant species. We also used t-tests to reveal difference in brood size and sex ratio (number of males divided by the total number of wasps) of the parasitoid on the two plants species. Parasitoid development time and body mass were analyzed by analysis of variance (ANOVA) with food plant (*B. nigra* and *S. arvensis*), offspring sex (male and female), and their interaction as explanatory variables. Post-hoc multiple comparisons were made using Tukey–Kramer tests. We used plant species as treatment and brood size as a covariate to determine the relationship between fresh mass of the host carcass after emergence of the parasitoid larvae and brood size on the two plant species. The relationship between fresh and dry mass of the adult parasitoids was determined using linear regression analyses.

For the volatile data, we used two-sample t-tests for each of the compounds to reveal differences in emissions by the two plant species. Peak areas were log transformed to meet assumptions of normality. To test whether the duration

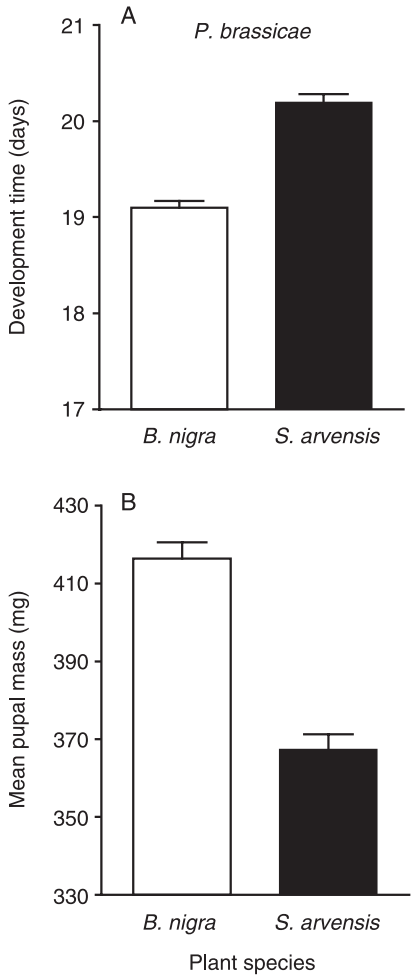

Figure 1 Development of *Pieris brassicae* reared on *Brassica nigra* (white bars) and *Sinapis arvensis* (black bars) plants; (A) Larva-to-pupa development time (mean + SEM, days) and (B) pupal fresh mass (mean + SEM, mg).

of feeding by *P. brassicae* had an effect on the landing preference of *C. glomerata*, we used a generalized linear model with binomial distribution for errors and a logit link function. The response variable was the fraction out of 10 wasps that chose *B. nigra* over *S. arvensis*. Duration of feeding (1, 2, 3, and 4 days) and trial (18 sets of plants) were entered as explanatory variables in the regression model. To determine whether *C. glomerata* landing responses were significantly different from a no-preference situation, we conducted two tailed Z-tests on arcsine-transformed ratios (H_0: μ = arcsine 0.5). These Z-tests were performed separately for each of the time points (i.e., after 1, 2, 3, and 4 days of feeding by *P. brassicae*). Analysis was carried out using SAS 8.2. (SAS Institute Inc., Cary, NC, USA).

Results

Plant quality for development of the herbivore and its parasitoid

The success of *P. brassicae* development, based on survival from newly hatched L1 larvae to adulthood, did not differ significantly between the two food-plant species. Not a single caterpillar failed to pupate, and only one pupa (out of 32) reared on *S. arvensis* died prior to adult emergence. In contrast, there was a significant effect of food-plant species on the duration of development between larval hatching and pupation ($t = 12.8$, d.f. = 59, and $P<0.001$; Figure 1A). *Pieris brassicae* took just over 24 h longer to pupate when reared on *S. arvensis* compared to *B. nigra*. Furthermore, pupal weight differed significantly with plant species on which *P. brassicae* was reared ($t = 8.71$, d.f. = 56, and $P<0.001$; Figure 1B). Individuals were, on average, about 50 mg larger when developing on *B. nigra* than on *S. arvensis*.

Among parasitized larvae of *P. brassicae*, the mass of the host carcass following larval parasitoid emergence was different for the two plant species ($F_{1,104} = 17.9$, $P<0.001$) and co-varied with secondary brood size in *C. glomerata* (with equal slopes $F_{1,104} = 20.0$, $P<0.001$). The statistical model including the size–plant interaction term, which tests for unequal slopes, was not significant; therefore, the interaction term was omitted from the model. For a given brood size, caterpillars reared on *B. nigra* were approximately 25 mg larger than conspecific larvae reared on *S. arvensis* (Figure 2).

Secondary brood size in *C. glomerata* did not vary significantly with the food plant on which the host had been

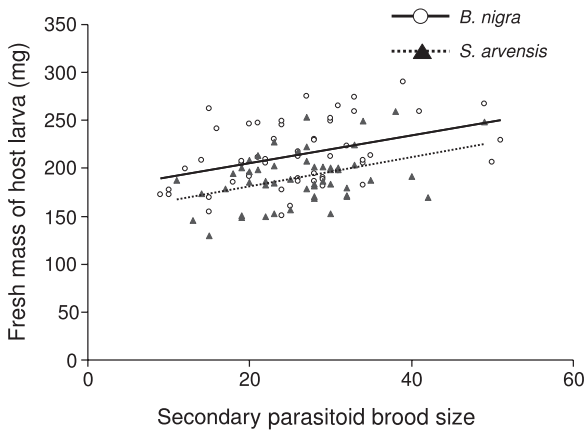

Figure 2 Relation between fresh mass of *Pieris brassicae* caterpillars after emergence of the parasitoid *Cotesia glomerata* and secondary brood size when the host was reared on *Brassica nigra* (open circles) or *Sinapis arvensis* plants (closed triangles).

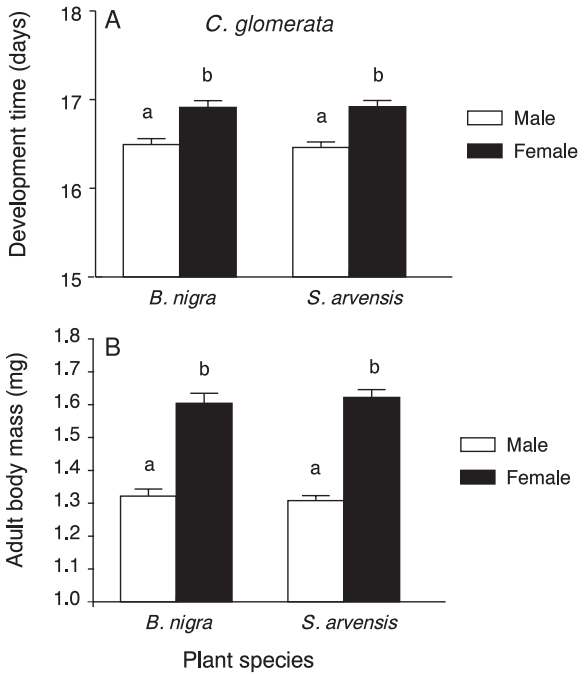

Figure 3 Development of *Cotesia glomerata* from *Pieris brassicae* reared on *Brassica nigra* or *Sinapis arvensis* plants for males (white) and females (black); (A) egg-to-adult development time (mean + SEM, days) and (B) adult fresh mass (mean + SEM, mg). Bars with the same letter are not significantly different (Tukey–Kramer multiple comparisons, P<0.05).

reared (t = 0.14, d.f. = 105, and P = 0.89). The brood size of *C. glomerata* was 26.1 ± 1.3 (mean ± SEM) on *B. nigra* and 26.3 ± 1.0 on *S. arvensis*. Remarkably, 100% (54 out of 54) and 98% (53 out of 54) of parasitized *P. brassicae* larvae on *B. nigra* and *S. arvensis*, respectively, produced parasitoid broods. Also, offspring sex ratio did not differ (t = 0.60, d.f. = 88, and P = 0.55) in broods reared on *B. nigra* (% of males per brood, mean ± SEM: 53.0 ± 4.2) and *S. arvensis* (49.6 ± 3.9). Development time in *C. glomerata* (Figure 3A) varied significantly with offspring sex ($F_{1,193} = 40.4$, P< 0.001), but not between the two crucifer plant species ($F_{1,193} = 0.04$, P = 0.84), nor was the interactive effect of these parameters on development time significant ($F_{1,193} = 0.07$, P = 0.78). *Cotesia glomerata* is protandrous, with male wasps emerging about 12 h earlier than females. Similarly, adult parasitoid mass (Figure 3B) varied significantly with offspring sex ($F_{1,193} = 168$, P<0.001), but not with food-plant species ($F_{1,193} = 0.01$, P = 0.92) and the interactive effect of these parameters also did not significantly affect body mass ($F_{1,193} = 0.47$, P = 0.50). Female wasps were typically some 20% larger than their male counterparts (Figure 3B). As fresh body mass correlated significantly with dry mass in both males ($F_{1,9} = 90.2$, P<0.001; $r^2 = 0.97$) and females ($F_{1,9} = 90.2$, P<0.001; $r^2 = 0.97$), size data were based on measurements of fresh mass.

Glucosinolate analyses

Glucosinolate analysis of leaf tissue revealed qualitative and quantitative differences between the two plant species. In *S. arvensis* plants, sinalbin was the only detectable GS and reached levels of 7.6 ± 2.9 (mean ± SEM) μmol g^{-1} dry weight (DW) (n = 9 plants). In *B. nigra*, sinigrin was the major GS (>98% of total GS content) and was present in levels of 16.9 ± 1.9 μmol g^{-1} DW (n = 8 plants). Other GS detected in *B. nigra* were glucobrassicin (0.045 ± 0.006 μmol g^{-1} DW), neoglucobrassicin (0.003 ± 0.001 μmol g^{-1} DW), 4-methoxy glucobrassicin (0.05 ± 0.02 μmol g^{-1} DW), and 4-hydroxy glucobrassicin (0.037 ± 0.006 μmol g^{-1} DW).

Flight response of *Cotesia glomerata* to *Brassica nigra* and *Sinapsis arvensis* plants infested with *Pieris brassicae*

Cotesia glomerata females, which had an oviposition experience in individual hosts feeding on Brussels sprout, clearly preferred host-damaged plants over clean undamaged plants. In *S. arvensis*, 93% (28 out of 30) of the wasps landed on herbivore-damaged plants, whereas in *B. nigra* 95% (37 out of 39) of the wasps preferred damaged plants over undamaged plants in a two-choice situation. When host-damaged plants of the two species were offered together, *C. glomerata* had a significant preference to land on *B. nigra* plants over *S. arvensis* at day 3 and day 4 since larval feeding was initiated (Figure 4).

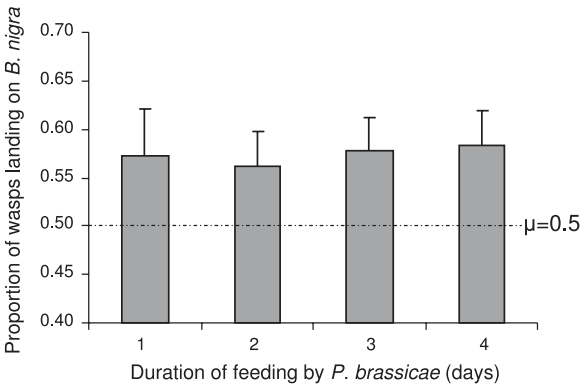

Figure 4 Landing preference of female *Cotesia glomerata* when given the choice between *Brassica nigra* and *Sinapsis arvensis* plants that had been damaged by 20 first-instar *Pieris brassicae* for 1, 2, 3, and 4 days. Each bar represents the mean (+ SEM; n = 18 trials) proportion of wasps landing on *B. nigra* out of a total of 10 wasps. Statistical analysis revealed that preference for *B. nigra* plants was not significant after 1 (t = 1.67, d.f. = 17, and P = 0.11) and 2 days (t = 1.88, d.f. = 17, P = 0.08) of feeding by *P. brassicae*, and significant after 3 (t = 2.22, d.f. = 17, and P = 0.04) and 4 days (t = 2.37, d.f. = 17, and P = 0.03) of feeding.

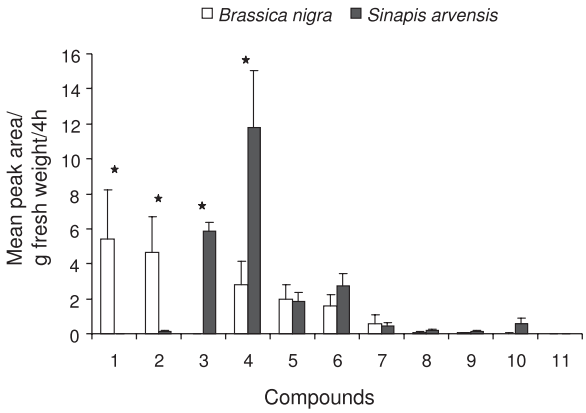

Figure 5 Composition of the volatile blend emitted by *Brassica nigra* (white bars) and *Sinapis arvensis* (black bars) plants after 96 h of feeding damage by *Pieris brassicae*. At t = 0, 20 first instars were placed on the plants, which were left on the plant during sampling. Each bar depicts the mean peak area/g fresh weight of above ground tissue (+ SEM; n = 5). Compounds: (1) allyl isothiocyanate, (2) (*3E*)-4,8-dimethyl-1,3,7-nonatriene (= DMNT), (3) β-caryophyllene, (4) (*Z*)-3-hexen-1-yl acetate, (5) (*Z*)-3-hexen-1-ol, (6) 2-ethyl-1-hexanol, (7) benzothiazole, (8) unknown compound, (9) 3-heptanone, (10) 2-heptanone, and (11) dimethyl trisulfide. Bars with an asterisk denote means that differ significantly between the two plant species.

Feeding duration had no effect on the preference of the wasps ($\chi^2 = 0.21$, d.f. = 3, and P = 0.98). The effect of trial bordered significance ($\chi^2 = 26.8$, d.f. = 17, and P = 0.06). The effect of duration of induction was not influenced by an interaction with trial ($\chi^2 = 60.3$, d.f. = 51, and P = 0.17).

Headspace analyses

In the headspace of both *B. nigra* and *S. arvensis* that had been fed upon by *P. brassicae* larvae for 4 days, 11 compounds were detected, of which nine were present in both plant species (Figure 5). In *S. arvensis*, the major compound was (*Z*)-3-hexen-1-yl-acetate, which formed 48% of the total volatile blend, followed by β-caryophyllene, which contributed 25% to the total blend. (*Z*)-3-hexen-1-yl-acetate was emitted at significantly lower rates by *B. nigra*, whereas β-caryophyllene was not produced by this plant species. Allyl isothiocyanate together with (3*E*)-4,8-dimethyl-1,3,7-nonatriene (= DMNT) were the most dominant compounds in the headspace of *B. nigra* plants, constituting 41 and 33% of the total blend, respectively. These two compounds were either absent (allyl isothiocyanate) or produced in only small amounts (DMNT) by *S. arvensis* plants. Superficial inspection of the plants after 4 days of feeding did not reveal any difference in the amount of tissue that had been consumed from the two plant species.

Discussion

The results of this investigation reveal that some fitness-related parameters (development time and body mass) of the insect herbivore, *P. brassicae*, differed when reared on *B. nigra* or *S. arvensis*. *Pieris brassicae* developed faster and pupae were larger when reared on *B. nigra* than on *S. arvensis*, although survival (to adult) was unaffected. In contrast, the identity of the plant species had little effect on the fitness-related traits of the parasitoid, *C. glomerata*. Although male wasps were smaller and developed faster than female conspecifics, there was little difference in quality between *B. nigra* and *S. arvensis*. Several studies have reported that development of herbivores and/or their parasitoids varies when feeding on different plant species or genotypes (Ohsaki & Sato, 1994; Benrey et al., 1998; Harvey et al., 2003, 2005, 2007; Ode et al., 2004; Gols et al., 2008). This indicates that plant quality varies due to differences in such factors as primary and/or secondary metabolites.

Hydrolysis products of GS, which are produced after cell rupture, have been shown to play a defensive role against insect herbivores (Chew, 1988; Rask et al., 2000). GS analyses of leaf tissues revealed that *B. nigra* contains mainly sinigrin (allyl GS), whereas *S. arvensis* contains only sinalbin. *Pieris brassicae* has evolved an efficient detoxification mechanism, which facilitates the conversion of GS into nitriles, instead of the much more toxic isothiocyanates (Wittstock et al., 2004). However, Agrawal & Kurashige (2003) reported that even highly specialized insect herbivores, such as *P. rapae* (closely related to *P. brassicae*) can be negatively affected by GS, such as sinigrin. A more recent study demonstrated that growth of *P. brassicae* is not negatively influenced by relatively high levels of sinigrin present in *B. nigra* leaf tissue (Smallegange et al., 2007). Moreover, when reaching the second and third instar, many *P. brassicae* caterpillars move to the flowers, which contain significantly higher amounts of sinigrin than leaves, and the larvae attain higher biomass when they selectively feed on flowers (Smallegange et al., 2007). For sinalbin, it has been shown that *P. brassicae* prevents the formation of toxic hydrolysis products by converting sinalbin (4-hydroxybenzyl GS) into 4-hydroxybenzylcyanide sulfate, with the nitrile 4-hydroxybenzylcyanide as intermediate (Agerbirk et al., 2006). Likewise, sinigrin in *B. nigra* may also be converted into less toxic metabolites. Consequently, *P. brassicae* is very efficient in detoxifying sinigrin and sinalbin, and therefore it is unlikely that these GS have a large impact on the performance of *P. brassicae*.

Morphologically, *B. nigra* and *S. arvensis* are quite similar, although *B. nigra* leaves have higher densities of trichomes than *S. arvensis* leaves. As caterpillars performed better on

B. nigra plants, hairiness did not apparently hamper the movement and feeding behaviour of *P. brassicae* caterpillars, or else any negative effects were cancelled out by other positive plant characteristics. The two plant species may have differed in levels of other defense-related compounds such as digestibility reducers. In addition, concentrations of primary metabolites (e.g., amino acids), which are also important factors determining food plant quality for insect herbivores (Slansky, 1993), may have differed between the two plant species.

Although host development was significantly affected by food plant species, parasitoid performance was not. Most importantly, for a given parasitoid brood size, the remaining mass of the host carcass after egression of the parasitoid larvae was significantly smaller in hosts reared on *S. arvensis* than in hosts reared on *B. nigra*. Larvae of *C. glomerata*, like their close relatives in the braconid subfamily Microgastrinae, feed primarily on host hemolymph and fat body during their development and thus consume only a fraction of available host resources (Harvey, 2005). This suggests that *C. glomerata* larvae are able to adjust their feeding behaviour in accordance with differences in host size, and thus consumed a higher proportion of the mass of smaller hosts reared on *S. arvensis* than larger hosts reared on *B. nigra*.

In the flight chamber experiments, *C. glomerata* had a significant preference for *B. nigra* over *S. arvensis* plants and this preference remained constant irrespective of the duration of feeding by *P. brassicae*. Headspace analyses revealed significant quantitative and qualitative differences between the two plant species. Herbivore-damaged *B. nigra* plants produced high levels of allyl isothiocyanate and the terpenoid DMNT, whereas *S. arvensis* produced the sesquiterpenoid β-caryophyllene and significantly higher amounts of the green leaf volatile (*Z*)-3-hexen-yl-acetate. In a time course experiment, Scascighini et al. (2005) found that *C. glomerata* was able to discriminate between infested and uninfested cabbage plants after only 1 h of feeding by *P. brassicae*. The response of the wasp reached a maximum after 3 h of feeding and remained constant for the subsequent 17 h (Scascighini et al., 2005). Our results suggest that the difference in attractiveness between *B. nigra* and *S. arvensis* remains constant for at least 4 days.

Although *C. glomerata* females preferred *B. nigra* plants, many (ca. 45%) still initially alighted on *S. arvensis*. In addition, *C. glomerata* females discriminated between undamaged and host-damaged *S. arvensis* plants. Therefore, the headspace of *S. arvensis* was also highly attractive to this parasitoid. Before the wasps were released in the flight bioassay, they had an oviposition experience in a host feeding on Brussels sprout. Previous work has demonstrated that the volatile blend emitted by Brussels sprout is much more complex (>70 compounds, see Mattiacci et al., 1994) than volatile blends emitted by *B. nigra* and *S. arvensis*. However, we cannot exclude the possibility that previous oviposition experience in larvae feeding on Brussels sprout has enhanced the preference for *B. nigra* over *S. arvensis* through assiociative learning (Vet et al., 1995).

Our results also suggest that the response of *C. glomerata* to herbivore-induced plant volatiles is dynamic. This parasitoid species may use a range of cues in order to recognize the different plant species that are food plants of the host. Alternatively, the parasitoid may use general plant signals to locate host-food plants from a distance, whereas cues from the host itself may play a role in host recognition once the food plant of the host is found. Frass produced by *P. rapae* larvae that had been feeding on cabbage plants emits isothiocyanates, which are breakdown products of GS (Agelopoulos & Keller, 1994; Agelopoulos et al., 1995). In addition *C. glomerata* discriminates between undamaged plants with frass from *P. brassicae* and undamaged plants without frass (Steinberg et al., 1993). If breakdown products of GS play a role in host plant selection of *C. glomerata*, high amounts of allyl isothiocyanate occurring in *B. nigra* plants may explain the enhanced attraction of this plant species to *C. glomerata*. For example, *Diaeretiella rapae*, which is a parasitoid of aphids mainly feeding on *Brassica* plant species, is attracted to synthetic 3-butenyl isothiocyanate (Blande et al., 2007), as well as to *B. napus* plants containing high levels of 3-butenyl isothiocyanate (Bradburne & Mithen, 2000). Low volatility of sinalbin hydrolysis products may be responsible for the absence of these compounds in the headspace of *S. arvensis* and explain why this plant species was less attractive to *C. glomerata*. More detailed studies are necessary to elucidate the importance of GS breakdown products as cues for host-plant selection in parasitoid species that parasitize hosts specialized on brassicaceous plant species.

In summary, we have demonstrated that differences in food plant quality for the host, *P. brassicae*, did not have a negative impact on the development of its larval endoparasitoid, *C. glomerata*. The feeding strategy of the parasitoid larvae, i.e., selectively feeding on hemolymph and fat body, probably enabled the parasitoid to adjust its feeding behavior without affecting the size or development time of its progeny. In the flight-chamber bioassay, female *C. glomerata* preferred damaged over undamaged conspecific plants and also exhibited a preference for herbivore-damaged *B. nigra* over herbivore-damaged *S. arvensis* plants. Although both plant species are annuals, the phenology of the two plant species differs. *Brassica nigra* usually flowers between June and August whereas *S. arvensis* exhibits two flowering peaks, the first in May–June and the second

between September and November. In The Netherlands, *P. brassicae* and *C. glomerata* are primarily bivoltine. The first generation of both the host and the parasitoid coincide with the phenology of *S. arvensis*, whereas the second generation coincides with the phenology of *B. nigra*. It is likely that different generations of *P. brassicae* and *C. glomerata* develop on different brassicaceous plant species. Therefore, it is critically important for *C. glomerata* females to be able to recognize a range of host food plants that may differ considerably in their volatile emission when damaged by hosts. High concentrations of GS breakdown products in the headspace of herbivore-damaged plants may play a role in attracting this parasitoid species. In cultivars of *B. oleracea*, levels of GS are often reduced through the process of artificial selection (Benrey et al., 1998; Harvey et al., 2007; Gols et al., 2008). Because of this, the role of GS in indirect defense may be less clear than would be the case with wild populations where levels of allelochemicals are often much higher. We argue that it is important to use wild brassicaceous species to determine the relative role of GS in plant defense against insect herbivores.

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