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Differential effects of jasmonic acid treatment of *Brassica nigra* on the attraction of pollinators, parasitoids, and butterflies

Maaike Bruinsma*, Harm IJdema, Joop J.A. van Loon & Marcel Dicke
Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands

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Abstract

Herbivore-induced plant defences influence the behaviour of herbivores as well as that of their natural enemies. Jasmonic acid is one of the key hormones involved in both these direct and indirect induced defences. Jasmonic acid treatment of plants changes the composition of defence chemicals in the plants, induces volatile emission, and increases the production of extrafloral nectar. However, few studies have addressed the potential influence of induced defences on flower nectar chemistry and pollinator behaviour. These have shown that herbivore damage can affect pollination rates and plant fitness. Here, we have investigated the effect of jasmonic acid treatment on floral nectar production and the attraction of pollinators, as well as the effect on the behaviour of an herbivore and its natural enemy. The study system consisted of black mustard plants, *Brassica nigra* L. (Brassicaceae), pollinators of *Brassica nigra* (i.e., honeybees and syrphid flies), a specialist herbivore, *Pieris rapae* L. (Lepidoptera: Pieridae), and a parasitoid wasp that uses *Pieris* larvae as hosts, *Cotesia glomerata* L. (Hymenoptera: Braconidae). We show that different trophic levels are differentially affected by jasmonic acid-induced changes. While the herbivore prefers control leaves over jasmonic acid-treated leaves for oviposition, the parasitoid *C. glomerata* is more attracted to jasmonic acid-treated plants than to control plants. We did not observe differences in pollinator preference, the rates of flower visitation by honeybees and syrphid flies were similar for control and jasmonic acid-treated plants. Plants treated with jasmonic acid secreted less nectar than control plants and the concentrations of glucose and fructose tended to be lower than in nectar from control plants. Jasmonic acid treatment resulted in a lower nectar production than actual feeding damage by *P. rapae* caterpillars.

Introduction

Induction of defence responses in plants can alter the behaviour of associated insects. This is well studied for foliar herbivores (folivores) and their natural enemies associated with vegetative plants (e.g., Dicke et al., 1990; Turlings et al., 1990; Shiojiri et al., 2002; Bruinsma & Dicke 2008). However, the effects of induced defences on flowering plants, and consequently pollinator behaviour, have received much less attention. Root and foliar herbivory may indirectly affect plant fitness by reducing resources for reproduction through reduction of root volume or photosynthetic area. Floral herbivory (florivory) directly affects plant fitness by reducing the number of gametes (Poveda et al., 2003). Observed effects of foliar herbivory on flowering plants include a decrease in pollinator visitation, an increase in secondary metabolites in leaves, flowers, or nectar, fewer and smaller flowers, and decreased pollen production (Strauss et al., 1996, 2004; Lehtilä & Strauss, 1997; Mothershead & Marquis, 2000; Hambäck, 2001; Smallegange et al., 2007). Root herbivory can increase pollinator visitation (Poveda et al., 2003, 2005) and florivory can decrease the number of flowers, nectar production, seed set, and pollinator visitation (Krupnick et al., 1999; Adler et al., 2001).

Although these examples show that herbivory can increase or decrease pollinator visitation, depending on type of damage, plant and pollinator species, the mechanisms

*Correspondence: E-mail: maaike.bruinsma@wur.nl

causing this change in pollinator attraction have not been elucidated (Kessler & Halitschke, 2007). For example, in wild radish both bees and syrphid flies preferred undamaged plants to plants with leaf herbivory (Lehtilä & Strauss, 1997). The preference of the bees could be explained by reductions in number and size of flowers on infested plants. Preference of the syrphid flies for undamaged plants remained when the plants were controlled for these characteristics, indicating another, possibly chemical, basis for differential syrphid fly attraction.

Flowers are not only visited by mutualistic pollinating species. Herbivore adults such as *Pieris rapae* L. (Lepidoptera: Pieridae) butterflies may also forage on *Brassica nigra* L. (Brassicaceae) flowers. Herbivores can be attracted or repelled by volatiles emitted by vegetative parts of the plants; moreover, flower volatiles can influence their foraging behaviour (Honda et al., 1998; Wäckers et al., 2007). Changes in nectar secretion or flower volatile production could therefore not only affect pollinators but also herbivores and natural enemies of the herbivore that forage on the same flowers. This implies that direct effects on plant fitness due to insect–flower interactions will not only depend on pollination rates, but also on attraction of herbivores and their natural enemies to flowers.

In the present study, we investigated the effect of herbivore-induced defences on herbivores, parasitoids, and pollinators. We explicitly excluded the effect of physical feeding damage by using a plant hormone to induce plant defence responses. This method of induction does not remove any tissue and reduces variability due to uncontrollable differences in the amount of feeding damage. This allowed us to compare the response of associated insects to the chemical changes of the plant without any visual cues or indirect effects resulting from tissue removal, which in itself may influence insect behaviour. In response to herbivore infestation, several signal-transduction pathways are induced that result in the production of defensive chemicals (Dicke & van Poecke, 2002). For both direct and indirect defence against caterpillars, the octadecanoid pathway plays an important role in the induction. Treatment of plants with jasmonic acid, a central phytohormone in the octadecanoid pathway, has been shown to affect herbivore and carnivore behaviour, volatile and extrafloral nectar production, as well as plant toxin accumulation. Jasmonic acid-treatment increases extrafloral nectar production in several plant species (Heil et al., 2001; Heil, 2004). Whereas, for instance, oviposition by herbivores decreases, carnivores prefer jasmonic acid-induced plants over non-induced plants (Thaler, 1999; Thaler et al., 2001). However, the effects of jasmonic acid treatment on floral nectar production and on mutualists, such as pollinators, have not been studied so far. We address the following questions in this study: (i) does jasmonic acid treatment of *B. nigra* affect herbivore and parasitoid behaviour as it does in several other brassicaceous plants?, (ii) does jasmonic acid treatment affect nectar secretion, quantitatively or qualitatively?, and (iii) does jasmonic acid treatment influence the number or duration of visits of pollinators to flowers?

Materials and methods

Plants and insects

All experiments were performed with flowering *B. nigra* (black mustard) plants of approximately 7 weeks old (ca. 1.5 m in height), growth stage 4.2 (Harper & Berkenkamp, 1975), that were grown in a greenhouse at 22–26 °C, 50–70% r.h., and a L16:D8 photoperiod. The plants were grown from seeds collected in the field in 2005 from *B. nigra* accession CGN06619 open-pollinated plants (obtained from the Centre for Genetic Resources, Wageningen, The Netherlands).

A colony of honeybees, *Apis mellifera* L. (Hymenoptera: Apidae), was provided by a commercial beekeeper (Inbuzz, Wageningen, The Netherlands). The hive consisted of three frames with brood of all stages plus the laying queen. The colony was placed in the greenhouse compartment during the days that the experiments were performed (2 days per week). The remaining days of the week, the hive was moved to a field outside the greenhouse. In the greenhouse, the bees could forage on *B. nigra* flowers. Outside the greenhouse, they foraged on a range of other plant species present in the field.

Small cabbage white butterflies, *P. rapae* were reared on Brussels sprouts, *Brassica oleracea* var. *gemmifera* L. cv. Cyrus at 22–26 °C, 50–70% r.h., and a L16:D8 photoperiod. Adult butterflies were fed a 10% (wt/vol) sucrose solution and allowed to oviposit on Brussels sprouts plants until the day before the experiment. *Cotesia glomerata* L. (Hymenoptera: Braconidae) was reared on caterpillars of the large cabbage white butterfly, *Pieris brassicae* L., at 22–26 °C, 50–70% r.h., and a L16:D8 photoperiod. Female wasps, eclosed 3–7 days before the experiment, and which had no previous experience with plant material, were used for the experiments.

Plant treatments

For the jasmonic acid treatment, *B. nigra* leaves were sprayed with a 0.5 mM jasmonic acid (purity>97%; Sigma-Aldrich, St. Louis, MO, USA) solution with 0.1% Tween-20 as a surfactant. Both sides of all leaves were sprayed until run-off. The control plants were sprayed with 0.1% Tween-20 solution. On average, the leaves were sprayed with 12 µl solution cm^{-2}. The jasmonic acid-treated plants

and the control plants were selected for the same height and number of open flowers. Herbivore-infested plants were infested with second instar *P. rapae* caterpillars on the middle three fully expanded leaves, 10 caterpillars per leaf. The plants were used for experiments 48 h after treatment.

Butterfly behaviour

The butterfly bioassay was similar to the one described by Bruinsma et al. (2007). One male and one female butterfly were placed in a cage (67 × 50 × 75 cm), in a greenhouse compartment at 22–24 °C and 50–70% r.h., 1 day before the experiment. The next morning, one freshly excised control and one jasmonic acid-treated leaf were introduced into the cages. The middle three fully expanded leaves of a plant were used for these experiments. Butterflies were allowed to oviposit for approximately 4 h. Subsequently, the number of eggs on each leaf was counted. Apart from natural daylight, the cages were illuminated by sodium vapour lamps (type SON-T, 500 W; Philips, Eindhoven, The Netherlands) from 8:00 until 14:00 hours.

Parasitoid behaviour

Parasitoid choice experiments took place in a flight chamber in a greenhouse compartment at 24 ± 2 °C and 50–70% r.h. with additional illumination provided by six lamps of the same type as used in the butterfly experiments. In the flight chamber that consisted of a gauze tent of 293 × 200 cm and 230 cm in height, stood a table (90 cm high) on which a glass cylinder, a jasmonic acid-treated plant, and a control plant were placed. The female parasitoids were released from a glass cylinder (50 cm above the surface of the table, at a distance of 50 cm from the two plants) on a piece of caterpillar-damaged leaf from which the caterpillars and their faeces had been removed. The jasmonic acid-treated and control plant were positioned at 50 cm distance from each other. The plant on which the first landing was made within 10 min after release was recorded; no landing on a plant within 10 min was recorded as 'no choice'.

Honeybee behaviour

In order to test whether there are differences in the attraction of pollinators between jasmonic acid-induced plants and control plants, flower visitation by honeybees was observed in the greenhouse. The experiments were performed between 13:00 and 17:00 hours in the same flight chamber as used for the parasitoid choice experiments, at 22 ± 2 °C and 50–70% r.h. Four plants, two jasmonic acid-treated and two control plants, were placed in a square on a table with a surface of 123 cm × 91 cm and a height of 90 cm. Plants of the same treatment were placed diagonally. The distance between plants was approximately 80 cm. A single honeybee at a time was released into the flight chamber with the four plants. Its behaviour was recorded using a handheld computer (Psion Workabout; Psion Teklogix Inc., Mississauga, ON, Canada), programmed with The Observer (version 4.1; Noldus Information Technology, Wageningen, The Netherlands). Two parameters of flower visitation behaviour of the bee were recorded for 10 min: the plants it visited and the time it spent on the plants. After this time, the bee was caught and released outside the tent and a new bee was released inside the tent. Bees to be introduced into the setup were caught when they were leaving the hive because they are most likely to be motivated to collect nectar. After two bees had been observed, the plants were rotated to exclude positional effects and after 10 bees the set of four plants was replaced with a new set of plants. In total, 17 sets of plants were observed.

Field observations

Flower visitation by naturally occurring pollinators was observed in field experiments from late June until mid-August 2006. The different species and number of insects that visited the jasmonic acid-treated plants were observed and compared to the species and number of insects that visited the control plants. Approximately 48 h after jasmonic acid treatment, the plants were transported from the greenhouse to an agricultural field in the vicinity of Wageningen, The Netherlands. There, they were planted (without pot) in a square (two jasmonic acid-treated and two control plants per experimental day) 150 cm apart. Other flowering plants in the plot were removed as much as possible.

Every plant was observed twice a day for 5 min to record the pollinators that visited the plant. Not every individual pollinator that visited the plants could be identified to species. The most common species present in the field belonged to four important pollinator groups: honeybees (*A. mellifera*), solitary bees, bumblebees (*Bombus* spp.), and syrphid flies (Syrphidae). The pollinators that belong to these categories are relatively easy to discriminate and when an individual entered the plot, it could rapidly be classified in one of these four groups. The pollinators were observed using the same handheld computer with The Observer software as for the honeybee observations in the greenhouse, which was now programmed to record both the arrival and the departure of every single individual of these species groups that entered the plot. The first series of observations took place approximately 48 h after treatment, on the same day the plants had been planted in the field. The second series of observations took place ca. 96 h after treatment. Sometimes, due to heavy rainfall, a planned series of observations was not possible and

then the first observations were made 72 h after treatment when possible.

Nectar collection

To test the effect of jasmonic acid treatment on the nectar secretion of *B. nigra*, the quantity and sugar composition of the nectar of jasmonic acid-treated and control plants were measured. Nectar was collected from the plants 47 ± 1 h after treatment. Around 08:00 hours, 1 h before collecting the nectar, the air humidity was increased to approximately 80% r.h. using a humidifier (Defensor 3001; Aircare Systems, Slough, UK). The nectar was collected with a capillary from three flowering branches of the inflorescence of every plant: the central, third, and fifth flowering branch counted from the top of the plant. Nectar was collected from the five distal flowers of every branch. Hence, nectar was collected from 15 flowers of every plant. After the nectar had been collected, the number of open flowers of every plant was counted.

To reach the nectaries more easily, the capillaries were adjusted by heating the end of a 5-μl glass capillary tube (Blaubrand Intramark, Wertheim, Germany) and elongating it until a thin pointed end was formed using a vertical pipette puller (PB Puller, Narishige, Tokyo, Japan). The amount of nectar collected from each plant was determined by measuring the number of millimetres (1.48 mm corresponded to 1 μl) of nectar in a capillary (total amount of nectar collected from 15 flowers per plant). The nectar obtained for each plant was stored in an Eppendorf tube with 10 μl 70% ethanol and kept at –20 °C until further analysis. The sugar composition in the obtained nectar was determined using high-performance liquid chromatography analysis. Samples from each experimental day were paired, yielding 24 series (24 control samples and 24 jasmonic acid-treated samples). The samples were diluted 10 times and injected in a Dionex BioLC system, equipped with a GS50 gradient pump, a CarboPac PA1 analytical column 4 × 250 mm with a CarboPac guard column 4 × 50 mm, and an ED_{50} electrochemical detector (Dionex, Breda, The Netherlands). The column was eluted with 100 mM NaOH at 1 ml min^{-1} and kept at 25 °C. The amount of glucose, fructose, and sucrose was determined in grams per litre using Chromeleon software version 6.60 (Dionex Corporation, Sunnyvale, CA, USA).

Data analysis

The number of eggs laid by the butterflies on control and jasmonic acid-treated plants was compared using a paired t-test using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The parasitoid data of the different experimental days were pooled and compared using a binomial test in Microsoft Excel. The number of open flowers was tested for differences between treatments using the Mann–Whitney U-test. These statistical analyses were performed with SPSS 15.0. Differences in the amount and composition of the nectar were analysed with a general linear model (GLM; SAS 8.2®; SAS Institute, Cary, NC, USA), for which the volume of nectar was normalized by natural-log transformation. The duration of the plant visits of honeybees to jasmonic acid-treated and control plants were natural-log transformed and compared for effects of treatment, number of visits, number of flowers, and amount of nectar using a mixed model with random coefficients (MIXED; SAS 8.2®). Each bee visited the same plant more than once; the times spent on a plant were considered repeated observations carried out on independent subjects (bees). It was assumed that the time spent on a plant was linearly related to the number of previous visits to that plant, where the deviations from the intercept and slope were random, possibly correlated and drawn from a Gaussian distribution. Because the dataset was unbalanced, the Satterthwaite's estimation of the degrees of freedom was used. Non-significant interactions were omitted from the model. In addition, the effect of the order of bees per series was tested. Each bee was classified as number 1–10 per series and these were compared separately with a t-test, with Bonferroni correction. The data were also tested for correlation between the amount of nectar produced or number of flowers and the flower visitation with a Pearson's correlation test. Only the visits of syrphid flies to the plants were tested, as other pollinator species did not visit the plots frequently enough to allow statistical analysis. A Kruskal–Wallis test was performed to assess differences between the plant treatments, and a Mann–Whitney U-test to test for differences between the different observation times. A Spearman's rank correlation test was performed in order to test for a correlation between amount of secreted nectar and number of syrphid fly visits per observation (SPSS 15.0).

Results

Butterfly oviposition behaviour

The oviposition preference of *P. rapae* females was compared between control and jasmonic acid-treated leaves. We observed that *P. rapae* females oviposited more on control leaves than on jasmonic acid-treated leaves (paired t-test: $t = 2.112$, $P = 0.046$, $n = 24$; Figure 1).

Parasitoid behaviour

The parasitoid wasps showed a preference that was the reverse of that of *P. rapae*. *Cotesia glomerata* females were more attracted to jasmonic acid-treated plants than to control plants in a dual-choice experiment (binomial test:

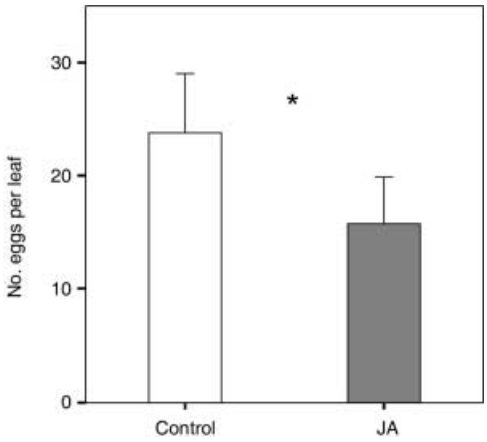

Figure 1 Mean (+ SE) number of *Pieris rapae* eggs on control leaves and jasmonic acid (JA)-treated leaves of *Brassica nigra* (paired t-test: *P<0.05; n = 24).

P = 0.006, n = 48; Figure 2). However, herbivore-infested plants were preferred both over control (binomial test: P = 0.002, n = 43) and over jasmonic acid-treated plants (binomial test: P = 0.003, n = 39) by *C. glomerata*.

Pollinator behaviour

We did not detect differences in attraction of honeybees to control and jasmonic acid-treated plants. Moreover, the duration of a visit to a plant did not differ between treatments ($F_{1,369} = 0.61$, P = 0.44). The only factor (negatively) influencing the duration of a visit was the number of visits of a bee to a plant; other factors such as the number of flowers of a plant and the amount of nectar secretion did not influence the duration of a visit (number of plant visits: $F_{1,533} = 33.29$, P<0.0001; number of flowers: $F_{1,204} = 2.34$, P = 0.13; nectar: $F_{1,195} = 0.01$, P = 0.91). Furthermore, the number of visits did not correlate with

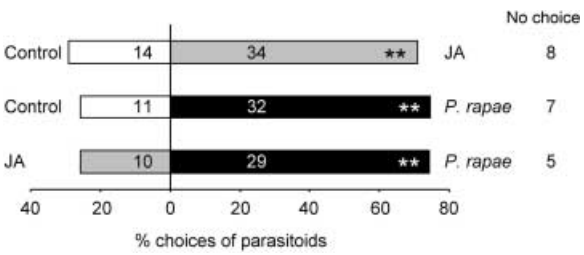

Figure 2 Preference of *Cotesia glomerata* parasitoids for control (white), jasmonic acid (JA)-treated (grey) or *Pieris rapae*-infested (black) *Brassica nigra* plants in two-choice bioassays in a flight chamber; numbers in bars indicate numbers of parasitoids, asterisks indicate statistical differences (binomial test: **P<0.01).

the amount of secreted nectar or number of flowers per plant (number of visits–flowers: Pearson: r = –0.081, P = 0.22, n = 236; number of visits–nectar: r = –0.035, P = 0.60, n = 236). The number of flowers did not differ between treatments (t-test: t = –0.245, d.f. = 148, P = 0.81). However, looking separately at plant visitation by the first bee of each series, we observed a longer visit duration for control plants than for jasmonic acid-treated plants (t-test: t = 3.977, d.f. = 16.8, P = 0.01). This difference was not observed for any of the subsequent bees (all P>0.05).

In the field, the plants were mostly visited by syrphid flies (Table 1), primarily drone flies [*Eristalis tenax* L. (Diptera: Syrphidae)], a commonly occurring member of the Syrphidae family. None of the three time intervals (48, 72, and 96 h) between plant treatment and pollinator observation was associated with a difference between the number of visits to control and jasmonic acid-treated plants (Mann–Whitney U-test: 48 h: Z = –1.138, P = 0.26, n = 82; 72 h: Z = –0.199, P = 0.84, n = 32; 96 h: Z = –0.623, P = 0.53, n = 96).

Table 1 Total number of pollinators visiting control and jasmonic acid (JA)-treated *Brassica nigra* plants in the field. Each plant was observed for 5 min in an agricultural field near Wageningen, The Netherlands, from late June to early August.

	Number of observed individuals	
Species	Control	JA
Honeybees	1	2
Solitary bees	27	24
Syrphid flies	379	337
Bumblebees	5	1

Nectar analysis

The amount of nectar secreted by *B. nigra* plants that were used for the pollinator experiments was lower for jasmonic acid-treated plants than for control plants (GLM: treatment: $F_{1,149} = 4.91$, P = 0.029). Because several samples were collected on every experimental day (i.e., same batch of plants and same abiotic conditions), we included this possible sampling effect in the analysis; and although the amount of nectar differed significantly between experimental days, there was no interaction between experiment and treatment effect (experiment: $F_{23,102} = 4.19$, P<0.001; interaction: $F_{23,102} = 0.76$, P = 0.768; Figure 3A). In the nectar analysis, three compounds were detected: glucose, fructose, and sucrose. Sucrose was only detected in 3 out of 48 samples and therefore not included in the statistical analyses. The average concentrations of both

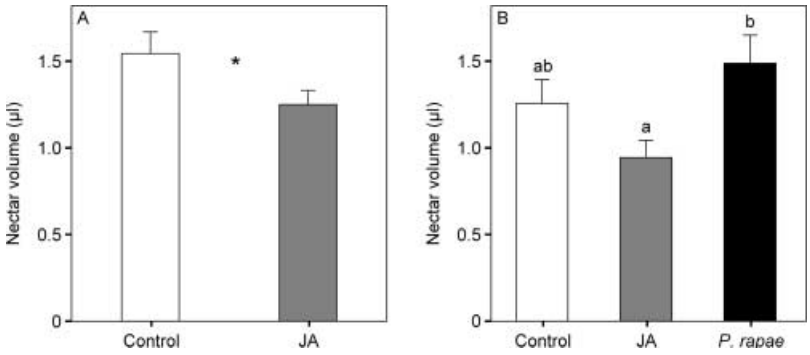

Figure 3 Nectar quantity 48 h after treatment of *Brassica nigra* with Tween-20 (Control), jasmonic acid (JA), or herbivore–infestation (*Pieris rapae*). (A) Mean (+ SE) of nectar collected during pollinator experiments (GLM: *P<0.05; n = 75). (B) Mean (+ SE) of nectar collected in period of parasitoid and butterfly experiments (GLM: $n_{control} = 25$, $n_{JA} = 22$, $n_{P.\ rapae} = 18$). Significant differences are indicated with different letters.

glucose and fructose tended to be higher in control samples than in samples from jasmonic acid-treated plants, the differences being close to significance at the 5% level (GLM: glucose: $F_{1,42} = 3.72$, $P = 0.060$; fructose: $F_{1,42} = 3.97$, $P = 0.053$; Figure 4). We included the amount of nectar as a covariate in the analysis and found that the glucose and fructose concentrations were negatively correlated with the amount of nectar (glucose: $F_{1,42} = 11.67$, $P = 0.001$; fructose: $F_{1,42} = 12.51$, $P = 0.001$).

In a second series of nectar collection, from the plants that were used for the butterfly and parasitoid experiments, the amount of secreted nectar differed between treatments (GLM: $F_{2,62} = 3.73$, $P = 0.030$). In this experiment, we included herbivore-induced plants. Jasmonic acid-treated plants secreted less nectar than control and herbivore-infested plants. However, this difference was only statistically significant for jasmonic acid-treated vs. herbivore-infested plants (least-squares means: jasmonic acid–herbivore-infested: $P = 0.023$; jasmonic acid–control: $P = 0.248$; control–herbivore-infested: $P = 0.422$; Figure 3B).

Discussion

Treatment of *B. nigra* with jasmonic acid affected herbivore and parasitoid behaviour. The observation that jasmonic acid treatment of plants affects *Pieris* spp. as well as *Cotesia* spp. is consistent with observations made for several other brassicaceous plants such as Brussels sprouts (Bruinsma et al., 2007; M Bruinsma, MA Posthumus, JJA van Loon & M Dicke, unpubl.) and *Arabidopsis thaliana* (van Poecke & Dicke, 2002). These studies observed similar effects of jasmonic acid dosages comparable to those applied in this study. Although most plants were tested 24 h after treatment, a time series test indicated that a single 1 mM jasmonic acid application to Brussels sprouts can attract parasitoids for at least 5 days (M Bruinsma, MA Posthumus, JJA van Loon & M Dicke, unpubl.). These previous studies compared induced and uninduced vegetative plants. In the present study, however, we tested flowering plants. Although we only treated the vegetative parts of the flowering *B. nigra* plants, the flowers could influence the attraction of the parasitoids to the plants, as nectar is an important food source for the parasitoids. However, because the results for vegetative and flowering plants are similar, the presence of flowers does not seem to change parasitoid host location behaviour. In the present study, satiated parasitoids were used, as starvation of parasitoids may change their searching behaviour and flowers may be more important to starved parasitoids. Food-deprived *Cotesia rubecula*, a species that is closely related to *C. glomerata*, prefer flowers over leaves with feeding hosts, while satiated parasitoids prefer the latter

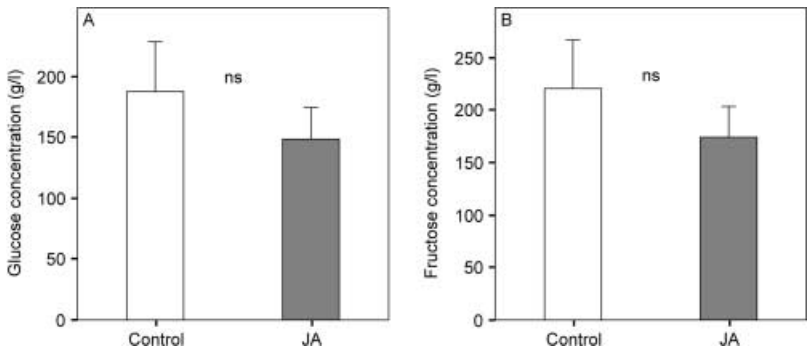

Figure 4 Nectar quality measured in nectar samples collected 48 h after treatment of *Brassica nigra* with Tween-20 (Control) or jasmonic acid (JA). (A) Mean (+ SE) concentration of glucose, GLM: $P = 0.060$; $n = 46$. (B) Mean (+ SE) concentration of fructose, GLM: $P = 0.053$; $n = 46$.

over flowers (Wäckers, 1994). Although excised leaves may differ chemically from attached leaves, practical limitations of the setup did not allow testing intact plants with the butterflies. However, because the butterflies discriminated between leaves freshly excised from either jasmonic acid-induced or control plants, jasmonic acid treatment is likely the causal factor (see also control experiments for another *Brassica* species in Bruinsma et al., 2007).

Although nectar volume in jasmonic acid-treated plants was lower than in control plants, we did not observe any differences in pollinator preference behaviour, except for the first bees of each series. The first bee, of a series of 10, visited control plants longer than jasmonic acid-treated plants. As this difference disappeared already with the second bee it is unlikely to be of great importance in field situations. It remains to be investigated whether pollen or nectar removal by the first bee changed the attractiveness. Several studies have shown that herbivory can influence pollinator behaviour. In some of these studies, the observed differences could be explained by the difference between the number of flowers of herbivore-induced and non-induced plants (Lehtilä & Strauss, 1997). In our setup, the time interval between spraying and behavioural assays was too short to cause significant differences in flower numbers resulting from treatment. Lehtilä & Strauss (1997) applied the herbivore treatment approximately 2 weeks before flowering. When sprayed in an earlier developmental stage, jasmonic acid may affect time of flowering as well as the number of flowers (Maciejewska et al., 2004), and therefore may cause differences in pollinator visitation of the plants.

The time interval between induction treatment and behavioural observations was sufficient to allow changes in the amount of nectar secretion. We observed a difference between nectar production in control and jasmonic acid-induced plants, and between jasmonic acid-treated and herbivore-infested plants at the herbivore density tested. This means that jasmonic acid treatment seems to have a different effect than herbivore infestation on nectar secretion and might therefore not be entirely suitable to simulate herbivore infestation in ecological studies. The effect of jasmonic acid treatment on parasitoid attraction is, however, similar to that of feeding damage. Moreover, extrafloral nectar production in, for example, *Macaranga tanarius* (L.) Müll. Arg. increased both after jasmonic acid application and herbivore infestation (Heil et al., 2001). Whether herbivore infestation would result in changes in the behaviour of pollinators of *B. nigra* plants remains an interesting issue to be investigated.

Besides nectar quantity, sugar concentrations in nectar may possibly influence pollinator visitation rates (reviewed in Mitchell, 2004; Schoonhoven et al., 2005). In the present study, the concentrations of both fructose and glucose tended to be higher, but not significantly so, in nectar from control plants than from jasmonic acid-treated plants. Besides nectar sugars and quantity, other compounds in flowers that may influence pollinator behaviour, such as secondary metabolites, may change in response to stress. Concentrations of secondary metabolites, such as alkaloids, in nectar or flower tissue can increase after herbivory (Euler & Baldwin, 1996; Adler et al., 2006) and result in changes in flower visitation (Gegear et al., 2007; Kessler & Baldwin, 2007). A study on *B. nigra* glucosinolate levels reported a higher level of the dominant compound sinigrin in flower tissues after leaf herbivory (Smallegange et al., 2007). However, we do not know yet whether herbivore damage causes changes in flower visitation or in secondary metabolites in nectar and floral volatile emission in *B. nigra*. Further studies should clarify the effects of herbivore damage on these plant characteristics, and subsequently elucidate the role of jasmonic acid in these processes. Timing of induction is also an important factor; treatment of plants in an early stage of development may have a more severe effect on future flower development than when the plants are already flowering.

For plants that depend on pollination for reproduction, it is important to know the effect of induction on pollinator visitation. Enhanced pollinator visitation rates have been shown to increase pollen removal and increase the probability of pollen grains reaching mates (e.g., Galen, 1992) thereby increasing plant fitness, which stresses the importance of investigating the effect of infochemicals on pollinator visitation. In this study, jasmonic acid-induced *B. nigra* plants are avoided by *P. rapae* butterflies for oviposition, are preferred by the parasitoid wasp *C. glomerata* over uninduced plants, and although jasmonic acid treatment reduced the amount of secreted nectar, it did not influence pollinator visitation, suggesting that it is suitable as a crop protectant in this seed crop.

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