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ORIGINAL PAPER

Importance of primary metabolites in canola in mediating interactions between a specialist leaf-feeding insect and its specialist solitary endoparasitoid

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Abstract The role of primary plant chemistry on trophic interactions is not well studied. We examined the effect of primary plant metabolites, focusing on nitrogen, on several biological indices of second and third trophic level insects in a model tritrophic system, consisting of two strains of the crucifer, *Brassica napus* (canola) (SLM_{046} and RGS_{003}), the specialist insect herbivore *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and its specialist koinobiont larval-pupal parasitoid *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae). In particular, we measured relative growth rate of the herbivore in relation to an index for plant quality (nitrogen content of leaf tissues), developmental time of the herbivore (sum of second, third, and fourth larval instars durations), and intrinsic rate of increase (r_m) of the herbivore and the parasitoid. Tritrophic studies were conducted on development, survivorship curve analysis, reproductive potential, life history, parasitism, and several other fitness correlates of the parasitoid. The life table parameters of *D. semiclausum* were determined under laboratory conditions. The intrinsic rate of increase (r_m) of the parasitoid was significantly higher on RGS_{003} than SLM_{046}. In this tritrophic model, the results indicated that the bottom-up direct effect on the herbivore population growth rate was marginally as strong as the direct effect of top-down force due to the parasitoid population growth rate; but it was higher than its indirect counterpoint mediated with the parasitoid population growth rate. Consequently, *D. semiclausum* performed better on RGS_{003}, which was the most inferior host to *P. xylostella* in comparison with another plant cultivar and had the lowest content of nitrogen in its leaves.

Keywords Plant quality · Insect fitness · *Plutella xylostella* · *Diadegma semiclausum*

Introduction

Plant quality can affect herbivore fitness directly as food of herbivores and indirectly by affecting foraging cues for natural enemies of herbivores (Awmack and Leather 2002; Walker et al. 2008). Bottom-up effect can further extend to the third and even fourth trophic levels (Harvey et al. 2007). For instance, the rate of parasitism in some lepidoptera can vary considerably depending on the plant species on which they are feeding (Hunter 2003; Lill et al. 2002); this is true even when feeding on different cultivars of one plant species in agro-ecosystems (Eigenbrode et al. 1996). Domestication of brassicaceous plants in some cases has reduced the level of secondary plant metabolites in addition to changes in the strength of indirect plant responses (Gols et al. 2008b). For instance, artificial selection in *Brassica oleracea* (one of the progenitors of *B. napus*) has caused a significant variation in shape and structure of different parts of the plants (Benrey et al. 1998). Artificial selection has also produced different

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concentrations of nitrogen and glucosinolates in several wild and domesticated crucifers (Scriber 1981).

It has been long demonstrated that toxic secondary compounds (allelochemicals) in an herbivore diet may affect their natural enemies' fitness, including development, survival, morphology, and size (Harvey et al. 2007; Ode et al. 2004). This effect of poor-quality plants can thus indirectly lead to poor-quality parasitoids (Hunter and Price 1992; Hunter 2003; Price et al. 1980). However, the strength of this bottom-up force has yet to be measured especially when primary metabolites cascade-up to higher trophic levels.

Much previous research in plant–insect interactions has focused on the role of toxic secondary plant compounds on insect performance; see reviews by Harvey (2005), Ode (2006), and primary studies by Barbosa et al. (1991), Campbell and Duffey (1979), Gols et al. (2008a, b, 2009), Gunasena et al. (1990), Harvey et al. (2003), Kos et al. (2011), Poelman et al. (2009), Soler et al. (2005, 2007); while primary plant metabolites, including nitrogen, which have been shown to strongly affect the feeding and growth of insect herbivores (Johnson 2008; Mattson 1980; Scriber 1979; Smith and Northcott 1951; White 1984) has been paid less attention. Moreover, to manipulate the nitrogen content of plants (i.e., to vary the plants' nutrient quality), studies often apply different levels of synthetic nitrogenous fertilizers; see for example, Aqueel and Leather (2011) and Smith and Northcott (1951). A problem in such studies is that the plant biomass changes as the plants grow faster (Stiling and Moon 2005), so one cannot precisely isolate the effect of plant quality on insect fitness. Here, we selected two *Brassica napus* (canola) cultivars (SLM_{046} and RGS_{003}) to represent high- and low-quality host plants that differ dramatically in nitrogen content and previously were shown to significantly affect the biology and life table parameters of diamondback moth (DBM) (Soufbaf et al. 2010a, b). To quantify the strength of the bottom-up effect, we utilized a tritrophic system consisting of DBM, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), as the herbivore which is among the most important pest of cruciferous crops in the world (Talekar and Shelton 1993), and one of its major parasitoids, *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae), an important natural control agent of DBM (Lohr et al. 2007; Talekar and Shelton 1993).

Our objectives were to determine: (1) whether the performance of DBM, measured by its relative growth rate (*RGR*), the larval development time (sum of the second, third, and fourth larval instars), and larval weight differs when feeding on these two plant cultivars (2) whether the performance of the parasitoid *D. semiclausum*, quantified by the vital rates potentially affecting its fitness, is also affected by the primary metabolite of plant cultivars on which its' host feeds?, and finally, (3) how does the fitness of both DBM and *D. semiclausum* correspond to the primary chemistry of their host plant?

Materials and methods

Study species

Two cultivated *B. napus* cultivars, SLM_{046} and RGS_{003}, were used in the laboratory trials. Seeds were obtained from the Seed and Plant Improvement Institute, Karaj, Iran, and used for raising plants in the greenhouse (27 ± 5°C, 60 ± 10% RH). Plants were raised in a standard potting mix in plastic pots (20 cm height and 15 cm diameter); 4–5 seeds were planted in each pot, without any fertilizer. Leaves from 4-weak-old plants were used in all trials. To evaluate the nitrogen content of plant leaves, we dried leaves of each plant cultivar at 55°C for 12 h to get the dry weights, and then, the nitrogen content of these dried ground leaves was estimated by the *Kjeldahl* method (Karla 1998). Three replicates were run for each host plant; dried leaf samples of each plant cultivar were obtained from ten different plants due to variability in nitrogen quality and quantity among individual plants (Mattson 1980).

A colony of DBM was established and maintained in the laboratory on cabbage, *B. oleracea* var. *capitata* Linnaeus. DBM larvae and pupae were originally collected from cabbage grown in the horticulture fields of the University of Tehran. The stock culture was maintained for about 2 months in the greenhouse. Sub-colonies were established on the two test plant cultivars separately and maintained in a constant environment at 25 ± 2°C on the respective host plants for more than four generations before the trials. At least 120 moths were used to initiate the colony and 20 wild adult males/females, collected from the field, were added to each colony (stock culture and sub-colonies) weekly.

A potted canola plant with one cohort of 200 early-third-instar DBM larvae was placed in a perspex cage (30 cm × 30 cm × 30 cm) with a muslin sleeve on one side of the cage. Ten pairs of 2-d-old mated *D. semiclausum* were introduced into the cage. The wasps were provided with a diet of 10% honey solution. After 24 h, the exposed larvae were removed and placed in ventilated plastic containers (20 cm × 15 cm × 7 cm). Fresh canola leaves were added until pupation. DBM pupa could not form in case of parasitized larva, so the parasitoid pupae were harvested and put in clean plastic containers for adult emergence. The *D. semiclausum* culture was established and maintained in a constant environment at 25 ± 2°C, and adults of the second generation were used in the experiments.

The performance of DBM

To calculate the relative growth rate (*RGR*) of the herbivore, we weighed the larvae every 24 h and defined *RGR* as the difference in wet weights (natural log) between first and last time periods divided by measurement times (Johnson and Zalucki 2007; Kogan and Cope 1974):

$$RGR = (\ln(w_{t1}) - \ln(w_{t0}))/(t_1 - t_0)$$

The *RGR* was measured for ~20 larvae per cultivar. To measure the development times of second, third and fourth larval instars, two cohorts of DBM eggs (50 individual eggs laid on clean plants over a 10-h interval) were reared individually until pupation. Although *D. semiclausum* can parasitize all four larval stages of DBM (Yang et al. 1993), the first instar would not transfer plant quality to the next trophic level. We thus ignored the duration of the first instar in the analysis. The intrinsic rate of increase in the herbivore (r_m) was estimated using the Jackknife simulation model, well described in Maia et al. (2000).

The performance of *D. semiclausum*

Estimation of the development time of the parasitoid was conducted in the laboratory (25 ± 1°C, 55 ± 5% RH). Each of the test plants (~20 cm in average height with eight leaves) was infested with one cohort of 120 second-instar DBM larvae obtained from the respective host plant cultivar. The plants were placed individually in wood cages (120 cm × 80 cm × 80 cm) with a muslin sleeve on all sides of the cage. Ten pairs of 2-d-old mated parasitoids were introduced into the cage through the sleeve and allowed to parasitize hosts. These parasitoids were supplied only with 10% honey solution with no host before the trial. Twenty-four hours later, the plants were removed from the cages. All exposed DBM larvae from each host plant were removed using a soft camel brush and placed individually in plastic Petri dishes (8 cm × 1.5 cm) containing a leaf from the respective host plant. The Petri dishes were covered with a lid with a muslin-covered window for ventilation and placed in an incubator (Binder KBWF 720, Germany). The leaves from respective test plants were changed daily until the larvae pupated. Cocoon spinning was recorded as the start of the pupal period. The cocoons were weighed within 24 h of formation to the nearest 10^{-4}g using a Sartorius electronic balance (Sartorius GMBH, Göttingen, Germany) and then placed individually in plastic vials for adult emergence. Egg–larval period, pupal period, and survivorship of the immature stages of the parasitoid were recorded. When the wasps emerged, the time of eclosion was recorded. Data for superparasitism and dead larvae were discarded.

To assess the effect of different plant cultivars on the reproductive potential of the parasitoid, 20 newly emerged *D. semiclausum* females from each test plant from each cultivar experiment were placed individually in clean plastic vials (2.5 cm × 7.5 cm) and paired with males from the same test plant cultivar and left together for 6 h to ensure mating (most pairs mated in the first few seconds). A leaf from the respective test plant was placed in a ventilated plastic container (15 cm diameter and 20 cm height) and infested with one cohort of 20 early second-instar DBM larvae. To prevent the leaf from wilting, the petiole was wrapped with cotton wool soaked in water. A pair of 1-d-old mated *D. semiclausum* was placed in the container and allowed to oviposit. After every 24 h, parasitoids were transferred to another plastic container with a leaf infested with a cohort of 20 early second-instar DBM larvae, and the process was repeated until death of last female wasp. The exposed larvae were transferred into ventilated plastic containers (10 cm × 6.5 cm) lined with tissue paper to absorb excess moisture. Leaves from respective test plants were added as required until the larvae pupated. Cocoons from individual females were placed separately in clean ventilated plastic containers (5 cm diameter and 4 cm height) and observed daily for the emergence of adults. The number of emerged DBM and parasitoids was recorded. After the wasps had emerged, the cocoons were checked and the number that failed to emerge recorded. Following Carey (2001), we calculated the life table parameters: the intrinsic rate of natural increase (r_m), mean generation time (*T*), finite rate of increase (λ), doubling time (*DT*), and net reproductive rate (R_0).

To calculate successful daily percent parasitism, we used the following equation:

$$\text{Successful Parasitism } (\%) = \left(P_p/L_h\right) \times 100,$$

where P_p stands for numbers of parasitoid pupa that developed successfully, and L_h is total number of DBM larva released daily to each container.

To assess the effect of different plant cultivars on the body size of the parasitoid, 20 newly emerged male and female parasitoids from each test plant cultivar were killed by placing them in a vial containing 70% alcohol. The left forewing and left hind tibia were removed using a pair of forceps under a dissecting microscope. The forewing and hind tibia were placed on a microscope slide using a soft camel hair brush and the wing spread in a drop of alcohol. The lengths were measured to the nearest 10^{-2} mm using a graticule fitted on the dissecting microscope.

Statistical analyses

All data obtained from measurements on two plant cultivars were subjected to Kolmogorov–Smirnov test for normality before analysis, and all pair-wise comparisons were done using Student's *t*-test. The data on fertility were subjected to square root transformation before analysis, and the data on daily parasitism rate and nitrogen contents were subjected to log transformation before analysis. The effect of host plant on the survival of herbivore and parasitoid larvae during the development period was tested using linear regression, and the homogeneity of parasitoid survivorship on the two plant cultivars was tested by the log-rank test (Rosner 2000) (PROC LIFETEST, SAS 2003). Values of $1-l_x$ in the age class of x were used as the mortality on each cultivar in the LIFETEST program. The data on daily parasitism throughout parasitoid age were analyzed using univariate General Linear Model (GLM) to determine the effects of plant cultivar, parasitoid age, and their interactions on daily parasitism (PROC GLM, SAS 2003), and means were compared by Tukey's test at $P < 0.05$ significance level. The life table parameters for the parasitoid were estimated by the Jackknife simulation method (Meyer et al. 1986), and Jackknife pseudo values were subjected to Student's *t*-test for pair-wise comparisons. Pair-wise correlations of pupal weight, fertility, forewing length, and hind tibia were conducted using Pearson product-moment coefficient (r) with a significance level of $\alpha = 0.01$, to identify relationships between structural measurement and weight of parasitoid cocoons. Direct and indirect effects of nitrogen content of leaves on development time of both herbivore and parasitoid, *RGR* of herbivore, and the intrinsic rate of natural increase r_m in both herbivore and parasitoid were assessed using path analysis after Bryman and Cramer (2002). Data transformations, Student's *t*-tests, regressions, path, and Pearson correlation analyses were performed using SPSS 16 (SPSS 2008).

Results

Plant cultivar quality

Nitrogen contents of leaves, as an index for plant quality, were significantly different between the two experimental plant cultivars (5.1 and 1.6% for SLM_{046} and RGS_{003}, respectively). There was 350% more nitrogen in SLM_{046} than in RGS_{003} ($T = -40.86$, $df = 2$, $P = 0.001$).

Effect of plant cultivars on mass growth and development time of DBM

Relative growth rate (*RGR*) of DBM differed between two plant cultivars; for individuals reared on RGS_{003}, the *RGR* was higher compared with those on SLM_{046} (Table 1). Accordingly, weight of larvae at the 4th instar on RGS_{003} was higher than on SLM_{046}. Larval development time (time in second + third + fourth instars) differed between the two plant cultivars (Table 1).

Effect of plant cultivars on the fitness of *D. semiclausum*

Fertility, development, parasitism, and survivorship

The total number of offspring produced by parasitoids was significantly higher on RGS_{003} than SLM_{046} (Table 1), but the overall parasitism rate on the two host plant was not significantly different. The female longevity with host and food was significantly greater on RGS_{003} than on SLM_{046} (Table 1), which probably accounts for the greater number of offspring produced. Data on daily parasitism showed significant differences between the two cultivars and among parasitoid ages (Table 2). The highest daily parasitism was observed on RGS_{003} and on 2nd day as 57.5 ± 4.7% and showed significant difference with daily percent parasitism on 4th day (43.5 ± 12.7%). Also, there was a significant difference between parasitism on days 1 and 4. Daily percent parasitism on 1st day was 2.82% more than its value on 4th day. The age-specific survivorship (l_x) at age of adult emergence of *D. semiclausum* on RGS_{003} and SLM_{046} was 0.82 and 0.69, respectively. Regression analysis showed that larval survivorship declines with a steeper slope in SLM_{046}, *survival = 0.989–0.0289 age* ($R^2 = 95\%$, $p_{intercept} = 0.000$) than in RGS003, that is, *survival = 1.05–0.0166 age* ($R^2 = 87\%$, $p_{intercept} = 0.000$). Log-rank test showed that the survival curve patterns were the same for parasitoids on the two plant cultivars, Deevey's curve type I (Deevey 1947) ($\chi^2 = 3.0248$, $df = 1$, $P = 0.0826$) (Fig. 1). The pupal period of the parasitoid was significantly longer on SLM_{046} (Table 1).

Morphological structures

Forewing length varied significantly between females and males. The lowest and highest values were observed on SLM_{046} and RGS_{003}, respectively (Table 1). Forewing lengths in females varied from 2.7 to 3.67 mm and from 2.87 to 3.4 mm in males on SLM_{046}; these size ranges for females on RGS_{003} were 3.025–3.925 mm and 3–3.9 for males. The lowest and highest pupal weights and lowest and highest adult weights of *D. semiclausum* were observed on SLM_{046} and RGS_{003}, respectively (Table 1). Pupal weights varied from 0.0018 to 0.0039 g and from 0.0020 to 0.0053 g on SLM_{046} and RGS_{003}, respectively. Accordingly, adult body weights varied from 0.0011 to 0.0066 g and from 0.0008 to 0.0065 g on SLM_{046} and RGS_{003}, respectively. There were no significant differences in tibia lengths. Correlations showed significant inverse relationships between pupal weight and female tibia

Table 1 Life history traits of herbivore (*Plutella xylostella*) (DBM) and its larval parasitoid *Diadegma semiclausum* on two cultivated canola cultivars

Plant cultivar	SLM$_{046}$	RGS$_{003}$	*df*	T
Insect performance				
DBM				
RGR (mg mg^{-1} d^{-1})	0.429 ± 0.024	0.509 ± 0.027	44	2.21*
Larval development time (2nd, 3rd, and 4th instars) (days)	5.64 ± 0.14	6.54 ± 0.13	84	4.63***
Larval weight in last instar (4th) (g)	0.005 ± 0.00026	0.006 ± 0.00027	40	−2.32*
D. semiclausum				
Pupal period (days)	6.51 ± 0.09	6.24 ± 0.09	70	−2.18*
Number of offsprings	15.90 ± 1.9	22.30 ± 2.2	17	2.18*
Female longevity with host (with parasitism activity)	3.00 ± 0.24	5.40 ± 0.91	10	2.56*
Pupal weight (mg)	2.54 ± 0.000	3.24 ± 0.000	167	6.03***
Adult body weight (mg)	2.38 ± 0.000	3.22 ± 0.000	63	2.44*
Forewing length (mm)				
Female	3.11 ± 0.05	3.49 ± 0.09	21	−3.42***
Male	3.05 ± 0.03	3.31 ± 0.07	19	−3.08***
Net reproductive rate (R_0)^{a}	5.56 ± 0.67	7.62 ± 0.84	–	ns
Finite rate of increase (λ)	1.16 ± 0.00	1.21 ± 0.16	12	2.69*
Intrinsic rate of increase (r_m)	0.147 ± 0.07	0.188 ± 0.01	13	2.72*
Mean generation time (T)	20.25 ± 5.43	14.00 ± 1.75	–	ns
Doubling time (DT)	4.67 ± 0.18	3.66 ± 0.27	15	−3.06**

Population growth parameters of *Diadegma semiclausum* obtained using Jackknife simulation method on canola cultivars

The values are Means ± SE of insect performances measurements after Student's *t*-test

[a] This parameter showed difference between cultivars at $P = 0.07$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and $^{ns}P > 0.05$

Table 2 Analysis from the daily percent parasitism of *Diadegma semiclausum*, parasitoid of *Plutella xylostella,* on two cultivated canola cultivars

Source	Type III sum of squares	*df*	F
Model	9.83283772	48	3.40***
Age	2.84042867	4	11.78***
Cultivar	0.78640240	1	13.04**
Replication	2.14410660	9	3.95**
Age × cultivar	1.05702778	3	5.84**
Replication × age	4.84948351	23	3.50**
Error	0.90440362	15	

A linear model approach was used to assay changes in percent parasitism between cultivars with age of parasitoid

The only significant main effects and their interactions were showed here. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ^{ns} $P > 0.05$

lengths on the two plant cultivar ($r = -0.642$, $P < 0.01$ on SLM$_{046}$; $r = -0.598$, $P < 0.05$ on RGS$_{003}$), and positive correlation between pupal weight and female forewing length for parasitoids reared on RGS$_{003}$ ($r = 0.712$, $P < 0.01$).

Life table parameters

The intrinsic rate of increase, r_m, in *D. semiclausum* was significantly different between cultivars as the cohort reared on RGS$_{003}$ had the higher r_m (Table 1), but the net reproductive rate (R_0) and mean generation time (T) were not significantly different between cultivars. The cohort reared on RGS$_{003}$ had the smallest doubling time (DT) and those on SLM$_{046}$ had the smallest finite rate of increase (λ) (Table 1).

Relationship between plant quality and insect fitness

The total standardized direct effect of nitrogen on parasitoid r_m was negative and strong, more powerful than the standardized indirect effect mediated with *RGR*

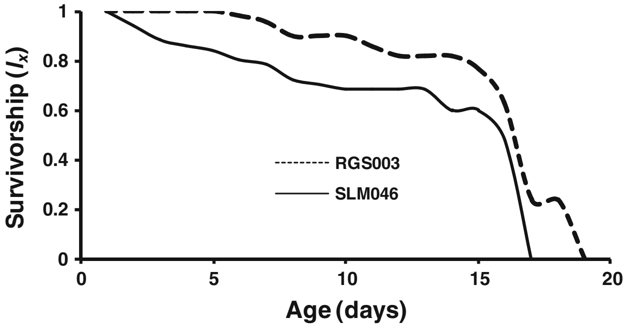

Fig. 1 Survivorship patterns of *Diadegma semiclausum* reared on two plant cultivars under laboratory conditions. This figure is prepared using Microsoft Office Excel 2007, copied and then pasted as picture (enhanced metafile) to this file

(Nitrogen → *RGR* → Parasitoid r_m, $\beta = -0.196$). The direct effect of nitrogen on the herbivore r_m was positive and stronger than its effect on parasitoid r_m (Fig. 2). The indirect path Nitrogen → *RGR* → Herbivore r_m was relatively effective ($\beta = +0.307$), but lower than its direct counterpoint. There was a negative effect of herbivore development time on parasitoid development time; but the effect in question on parasitoid r_m was weak. The effect of *RGR* on herbivore r_m was negative and relatively strong. The direct effect defined as Nitrogen → Herbivore r_m was the strongest bottom-up force at the first place and Nitrogen → Parasitoid r_m at the second place as demonstrated here. As a strong top–down force, there was a direct negative effect of parasitoid r_m on herbivore r_m (Fig. 2, Table 3).

Discussion

There are many studies regarding the development of fitness strategies in both herbivore and parasitoid but most of them have focused on secondary chemistry of plants, while ignoring the potentially opposite (or countering) effect of primary plant metabolites. Furthermore, many studies are available which examine the effects of synthetic nitrogenous fertilizers as an index for plant quality on insect fitness. Here, we compare our results with some of these studies to compare primary and secondary metabolites' effects on the performance of insects in the second and third trophic level and, to explore the potential differences between natural plant quality and chemically enhanced plant quality using fertilizers.

DBM performance

DBMs grew slower (lower *RGR*), completed development in a shorter time at a smaller weight on SLM_{046}, which is the most suitable host plant for DBM, in terms of r_m (Soufbaf et al. 2010a). This cultivar has a significantly higher value of nitrogen in its leaves than another host plant, RGS_{003}. On the latter, development time for DBM

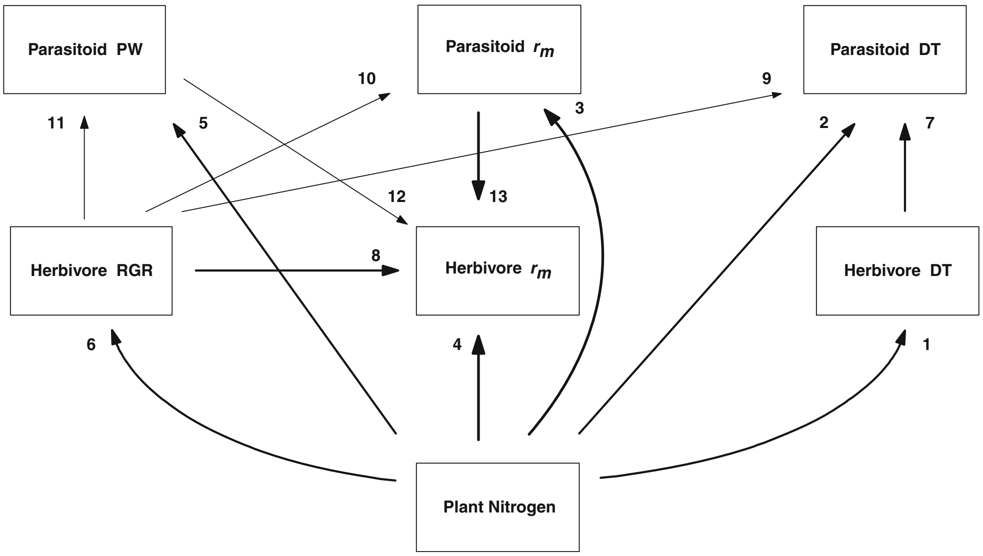

Fig. 2 Output diagram of causal relationships in the plant nitrogen survey on the fitness of second (DBM), and third (*Diadegma semiclausum*) trophic levels after path analysis. *Arrows* represent standardized direct and indirect effects of trophic levels components on each other. Thickness of arrows is proportional to the size of the effect. Path coefficients (β), errors ($e = 1 - R^2$), and *P*-values represented in Table 3 (the numbers close to the head of arrows represent the path number). DT represents larval development time (2nd + 3rd + 4th instars) for herbivore and egg + larvae + pupa duration for parasitoid, PW represents pupal weight. *Upward* and *downward arrows* represent bottom-up and top-down forces directions in the tritrophic model. This figure is prepared using Flow Charting 6, copied and then pasted as picture (enhanced metafile) to this file

Table 3 Standardized path coefficients (β), errors ($e = 1 - R^2$), and *P*-values of causal relationships in the plant nitrogen survey on the fitness of second (DBM), and third (*Diadegma semiclausum*) trophic levels after path analysis

Path	β	$e = (1-R^2)$	*F*	*P* value
1	−0.476	0.773	1.174	0.348
2	+0.431	0.815	0.910	0.394
3	−0.895	0.199	16.052	0.016
4	+0.959	0.08	46.047	0.002
5	−0.495	0.755	1.295	0.319
6	−0.661	0.563	3.110	0.153
7	−0.303	0.908	7.162	0.009
8	−0.465	0.784	7.983	0.008
9	−0.126	0.984	0.760	0.388
10	+0.296	0.913	1.725	0.206
11	+0.239	0.943	2.114	0.155
12	−0.318	0.899	3.267	0.081
13	−0.960	0.079	46.716	0.002

was longer, but larvae gained weight faster (high *RGR*) and were heavier at the end of the larval period. One interpretation could be related to the high level of nitrogen available in the plant cultivar SLM_{046} and so DBM satisfies nitrogen needs with feeding in shorter periods and a lower growth rate. Larvae on the low-nitrogen plant has to feed longer to gain the same or similar amount of nitrogen and end up larger but not necessarily fitter; see Raubenheimer and Simpson (1997) on the C/N balance hypothesis. Using a different method to define *RGR*, Coley et al. (2006) showed that caterpillar's *RGR* was faster on young leaves, which had higher values of nitrogen than the older leaves. Contrary to our findings, many workers have found that caterpillars developed more slowly on low-nitrogen plants than on high-nitrogen plants (e.g., Loader and Damman 1991; Lou and Baldwin 2004). However, there are reports that larval performance (e.g., larval growth) is relatively independent of plant nitrogen (Karowe and Martin 1989; Prudic et al. 2005). We observed a negative and relatively strong effect of *RGR* on the herbivore r_m; as an implicit result, higher population growth rates could not have resulted from higher *RGR*s.

High level of secondary metabolites, glucosinolate (GS) as the major secondary metabolite in crucifers, reduce the performance of DBM (Gols et al. 2008a; Li et al. 2000), and this reduction is usually less pronounced than in generalist herbivores. Gols et al. (2008a) reported that DBM developed most poorly on the wild population of *Brassica* which had higher values of GS in their leaf tissues. We encountered a similar scenario, as nitrogen showed a negative and relatively strong effect on *RGR* ($\beta = -0.661$, $P = 0.153$). This negative effect is confirmed according to slower *RGR* on SLM_{046}, which had the higher contents of nitrogen in its leaves than the other plant cultivar. Low nitrogen results in a total consumption increase through prolonged developments of herbivores (Coley et al. 2006; Mattson 1980), as we found.

Agrawal (2004) suggested that nitrogen content is a good predictor of r_m, which will have a strong influence on the population dynamics of herbivores, and our result supports this suggestion. Also, larval development time (feeding window) of herbivores could be a good predictor of parasitoid development time in the current study. A positive causal relationship between nitrogen and intrinsic growth rate of herbivore was observed, which is in spite of Johnson (2008) who reported negative correlations between leaf nitrogen and herbivore r_m, but is similar to many studies, that report a positive relationship (e.g., Agrawal 2004, Aqueel and Leather 2011, Winter and Rostás 2010).

Parasitoid performance

Immature survival of parasitoid showed faster declines on SLM_{046} than on the other plant cultivars. Despite our result, some workers report that immature parasitoid survival was higher on plants with higher nitrogen (fertilizer), which DBM fed on (e.g., Sarfraz et al. 2009). Winter and Rostás (2010) working on soybean—*Spodoptera frugiperda*—*Cotesia marginiventris* system, reported similar findings to ours for parasitoid pupal weight, development time, and longevity; although they used synthetic N fertilizers in plant cultures. Coley et al. (2006) suggested that species feeding on mature leaves (with lower nitrogen contents) were the most defended against natural enemy (ants). Nitrogen had a strong negative effect on parasitoid r_m; accordingly, RGS_{003} that had lower values of nitrogen supported fitter parasitoids in our system. Despite our results, there is a positive relationship between nitrogenous fertilizer applications and parasitoid abundance (De Kraker et al. 2000; Loader and Damman 1991). We found lower progeny and r_m of parasitoid when its host was reared on the plant cultivar that contained a higher level of nitrogen. However, it is frequently reported that performance of parasitoids declined when caterpillars fed on plants raised in nitrogen-poor cultures (e.g., Winter and Rostás 2010).

Gols et al. (2008a; 2009) examining the effect of plant populations with different GS values on parasitoid performance showed that *D. semiclausum* developed more slowly on the cultivated and wild populations of *Brassica* than on the feral population, while plant population had no significant effect on the survival rates of the parasitoid. However, parasitoid development time did not differ significantly between plant cultivars in our study (data not shown), while survival of immature parasitoids declined faster on the plant cultivar with higher value of nitrogen. Gols et al. (2008b) showed that development time and adult body mass in *Cotesia glomerata* did not vary significantly with plant species, while adults and pupa of parasitoid were heavier on RGS_{003} (with lower level of nitrogen) than on another cultivar in the current study.

Our results on the performance of herbivore and its parasitoid under the effect of nitrogen differ from many studies that have examined the effect of secondary plant metabolites on herbivore and its parasitoid performance. We found that performance of DBM increased on the plant cultivar that had a higher content of nitrogen in its leaf tissues, but the story was reverse for parasitoid, as the most fitness correlates of *D. semiclausum* were higher when its host reared on the plant cultivar with lower content of nitrogen. Most often, GSs enhanced the parasioids performance in different ways, but the herbivore performance under GSs effects showed more inconsistent results (see above).

Applied implications and study concerns

We observed a significant effect of both plant cultivar and parasitoid age on daily percent parasitism, which is similar to many other studies (e.g., Moreau et al. 2009). The highest daily percent parasitism occurred on the second day of parasitism on RGS_{003}, which was reported as the most inferior host plant for DBM (Soufbaf et al. 2010a, b), and had the lowest nitrogen content in the current study. Moreover, there was a strong negative and significant effect of nitrogen on the parasitoid growth rate. Host plant resistance and biological control with parasitoids are two important tactics, which are utilized in many management programs (Ode 2006); interestingly, RGS_{003} showed both compatibility to parasitoid and unsuitability to herbivores in our artificial system. In general, it is thought that plant resistance and biological control are incompatible. So, using some plant cultivars like RGS_{003}, which naturally represent low qualities for the herbivore and inversely improve the parasitoid fitness could have implications for managers to achieve safer agro-ecosystems and potentially lessen management costs. We have yet to address the question of how plant palatability and yield correlate with primary metabolites in plant cultivars, and we leave this interesting question for future research.

Even though nitrogen is one of the most important plant components that strongly affect insect fitness, DBM has shown to be influenced considerably by sulfur contents in leaf tissues of its host plant. Gupta and Thorsteinson (1960) argued that sulfur could enhance the attractiveness of host plant as oviposition substrate for DBM, but McHugh and Foster (1996) suggested that sulfur applications on cabbage foliage caused a significant decrease in DBM oviposition. However, besides sulfur or nitrogen on their own, nitrogen/sulfur ratio has been shown to affect GS content and subsequently plant–insect interactions (Badenes-Prez et al. 2010). Plant quality tends to decrease with leaf age (Travers-Martin and Müller 2008), and this decline in quality can affect insect fitness through different ways, but continuous usage of fertilizers keeps nutritious elements at accessible levels for insect, which is one of the aspects of anthropogenic influences on the ecosystems, and the outcome can divert us from improving our understanding of reality. However, in some ecosystems nutrient enrichments increased primary consumer production but not predator's counterpoint (Davis et al. 2010) and can cause instability in a system (Roy and Chattopadhyay 2007).

It is well known that plant allelochemicals can affect the behavior and performance of parasitoids (Harvey et al. 2005), but scenarios are different depending on plant condition during experiments. When plants are living, they can interact with herbivores, and it will be expected that this interaction differs throughout an herbivores feeding window, as both interacting species are varying in performance. But when leaves are excised from plants, their interactions with herbivores may be disrupted, as the leaves are just dead materials with the same quality of their plant at cutting time. In this case, the quality of the host plant is fixed during herbivore feeding time and the amount of the food will only decrease. In such studies that used excised leaves as host plant material, the herbivores do not encounter with living plant reactions (to herbivore feeding activity) and so are expected to suffer less from their host defense strategies. But the effect on parasitoid performance is less well predicted, as their host (herbivore) shows two faces, one as a high-quality host and another as well defended against natural enemies. However, results of such studies should be interpreted with caution.

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