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# EFFECTS OF QUANTITATIVE VARIATION IN ALLELOCHEMICALS IN *Plantago lanceolata* ON DEVELOPMENT OF A GENERALIST AND A SPECIALIST HERBIVORE AND THEIR ENDOPARASITOIDS

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**Abstract—**Studies in crop species show that the effect of plant allelochemicals is not necessarily restricted to herbivores, but can extend to (positive as well as negative) effects on performance at higher trophic levels, including the predators and parasitoids of herbivores. We examined how quantitative variation in allelochemicals (iridoid glycosides) in ribwort plantain, *Plantago lanceolata*, affects the development of a specialist and a generalist herbivore and their respective specialist and generalist endoparasitoids. Plants were grown from two selection lines that differed ca. 5-fold in the concentration of leaf iridoid glycosides. Development time of the specialist herbivore, *Melitaea cinxia*, and its solitary endoparasitoid, *Hyposoter horticola*, proceeded most rapidly when reared on the high iridoid line, whereas pupal mass in *M. cinxia* and adult mass in *H. horticola* were unaffected by plant line. *Cotesia melitaearum*, a gregarious endoparasitoid of *M. cinxia*, performed equally well on hosts feeding on the two lines of *P. lanceolata*. In contrast, the pupal mass of the generalist herbivore, *Spodoptera exigua*, and the emerging adult mass of its solitary endoparasitoid, *C. marginiventris*, were significantly lower when reared on the high line, whereas development time was unaffected. The results are discussed with regards to (1) differences between specialist and generalist herbivores and

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their natural enemies to quantitative variation in plant secondary chemistry, and (2) potentially differing selection pressures on plant defense.

**Key Words—**Chemical defense, iridoid glycosides, *Melitaea cinxia*, multitrophic interactions, *Plantago lanceolata*, *Spodoptera exigua*.

## INTRODUCTION

Many plants produce a range of toxic secondary compounds (allelochemicals) that are either constitutively expressed, or induced in response to herbivory (Karban and Baldwin, 1997). These chemicals may act as feeding deterrents or alter the physiology and development of herbivores, resulting in reduced rates of growth, smaller adult size, and increased mortality. However, plant allelochemicals not only affect the behavior and performance of herbivores feeding on the plant, but also the behavior and performance of organisms at higher trophic levels (Hare, 2002). Such effects have implications for the evolution of plant chemical defense. In some cases, higher-trophic level effects are beneficial for the plant and enhance the selective advantage of producing high levels of an allelochemical in the presence of parasitoids or predators of herbivores. For instance, allelochemicals that slow down the rate of herbivore development increase the exposure time or window of their vulnerability to parasitoids and predators (Turlings and Benrey, 1998). In other cases, these effects may be detrimental, especially if herbivores use plant allelochemicals for their own defense against parasitoids and predators (e.g., Campbell and Duffey, 1979). Many insect herbivores are specialized on plants producing particular groups of allelochemicals. For example, larvae of many species of checkerspot (fritillary) butterflies (Nymphalidae) are restricted to plants producing iridoid glycosides (Wahlberg, 2001). Specialized herbivores can often deal with high levels of specific phytotoxins in their diet (Nishida, 2002), even though their performance may be lower on plants containing high rather than low amounts of these phytotoxins (Adler et al., 1995). Although some herbivore species break down and excrete ingested allelochemicals during development, many others use them to their own advantage by sequestering them in hemolymph and other tissues, or concentrating them in the gut (Rimpler, 1991; Nishida, 2002).

A number of studies have reported that allelochemicals in herbivores reduce the performance of less well-adapted predators and parasitoids (e.g., Duffey et al., 1986; Gunasena et al., 1990; Barbosa et al., 1991). In contrast, in the handful of studies addressing the performance of specialized natural enemies, such as parasitoids that attack one or only a few related hosts in nature, development appears to be less affected by differences in allelochemicals (Barbosa et al., 1986; Sznajder and Harvey, 2003). The higher-tropic-level effects of allelochemicals are, thus, likely to depend on the level of specialization of the herbivores and parasitoids or predators involved.

We examined the developmental responses of a generalist and a specialist insect herbivore and their endoparasitoids to quantitative variation in iridoid glycosides (IGs) in ribwort plantain, *Plantago lanceolata* L. (Plantaginaceae), a perennial plant species with a worldwide distribution and a large ecological amplitude. IGs are a group of monoterpene-derived compounds that have been recorded in over 50 plant families (Jensen, 1991). The main IGs found in *P. lanceolata* are catalpol and its precursor aucubin (Bowers, 1991). Concentrations of IGs in natural populations of *P. lanceolata* range from undetectable to ca. 9% of plant dry weight, and vary both among populations and individuals within populations (Bowers, 1991; Nieminen et al., 2003). IG concentrations in *P. lanceolata* are partly under genetic control (Adler et al., 1995; Marak et al., 2000), but also vary in response to plant attributes such as leaf and plant age (Bowers and Stamp, 1993) and abiotic factors such as light and nutrient levels (Marak et al., 2003), and can be induced by both herbivores and pathogens (Darrow and Bowers, 1999; Marak et al., 2002a). In artificial diet studies, these compounds reduce the growth of generalist, but not specialist, insect herbivores (Bowers and Puttick, 1988; Puttick and Bowers, 1988). Specialist insects use these compounds as oviposition and feeding stimulants (Pereyra and Bowers, 1988; Nieminen et al., 2003) and sequester them for their own defense (Bowers and Collinge, 1992; Camara, 1997; Suomi et al., 2001).

The insect species used in this study were (i) the specialist herbivore, *Melitaea cinxia* L. (Lepidoptera: Nymphalidae) and its endoparasitoids, *Cotesia melitaearum* Wilkinson (Hymenoptera: Braconidae) and *Hyposoter horticola* Gravenhorst (Hymenoptera: Ichneumonidae) and (ii) the generalist herbivore, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) and its endoparasitoid, *C. marginiventris* L. (Hymenoptera: Bracondiae).

*M. cinxia*, the Glanville fritillary, is a specialist feeder of plants containing iridoid glycosides. Larvae use IGs as feeding stimulants, whereas adults use them as oviposition cues (Nieminen et al., 2003; van Nouhuys and Kumar, unpublished data). The first five instars develop within a large silken web during the first year. The following spring, after diapause, larvae complete the final two instars. In Northern Europe, *M. cinxia* is univoltine. A detailed description of the life cycle is provided by Kuussaari et al. (2004). *C. melitaearum* is a specialist parasitoid of butterfly larvae in the genus *Melitaea* (van Nouhuys and Hanski, 2004). It attacks early and late instars of *M. cinxia*, ovipositing into the host hemocoel. Larvae feed primarily on host hemolymph and fat body. It may complete up to three generations in a single year. *H. horticola* is also a specialist parasitoid of *M. cinxia*. It produces only one generation per year and its development is closely synchronized with that of its host. It lays a single egg in first instar host larvae just before they hatch from the egg (van Nouhuys and Hanski, 2004; van Nouhuys and Ehrnsten, unpublished results). Although the eggs hatch within a few days, development is suspended as a first instar until the host has reached its fifth instar

and has broken diapause. Once the host has entered its final instar, the parasitoid larva starts attacking all tissues, ultimately consuming the entire host except for its cuticle.

*S. exigua*, the southern beet armyworm, is a highly polyphagous insect herbivore (Greenberg et al., 2001). It is endemic to South East Asia, but has been introduced over much of the world. Larvae complete five instars during development. In warm regions, it produces several generations per year. *C. marginiventris* parasitizes larvae of several species in the family Noctuidae. It parasitizes first to fourth instars of *S. exigua* and oviposits a single egg in the host hemocoel. Like the closely related species *C. melitaearum*, larvae of *C. marginiventris* feed primarily on host hemolymph and fat body.

In this study, we compare fitness correlates (development time and pupal or adult body mass) of the herbivores and their parasitoids reared on two lines of *P. lanceolata* containing different levels of iridoid glycosides. The results are discussed in terms of the role that generalist and specialist herbivores and their natural enemies play in selection for direct chemical defense.

## METHODS AND MATERIALS

*Plants. P. lanceolata* used in this experiment was derived from an artificial selection experiment (for details see Marak et al., 2000), in which plants were selected on the basis of high and low concentrations of total leaf iridoid glycosides for four generations. Selection resulted in an average 3-fold difference in leaf iridoid glycoside concentration between upward and downward selected lines (Marak et al., 2000, 2003). Within each of these selection lines, iridoid glycoside concentrations vary considerably among maternal half-sib families, so that much larger, up to 20-fold, differences are present between extreme families from these selection lines. For the current experiment, seeds from the six most extreme female half-sib families from each selection line were used. Ten seeds from each family were germinated in a growth cabinet (14/10 hr and 25/15°C L/D), and transplanted individually into plastic 2.2 l pots with a mixture of potting soil and sand (4:1 v:v). Plants were grown in a greenhouse (16/8 hr and 22/18°C L/D) and regularly fertilized with half strength Hoagland's nutrient solution. Two extra plants per family were grown and harvested 5 wk after transplantation for chemical analysis. Of each plant, 50 mg from the freeze-dried and fine-ground leaf material were extracted overnight in 10 ml of 70% methanol for analysis of iridoid glycosides (aucubin and catalpol) using HPLC (methods in Marak et al., 2002b). Another 300 mg of each plant were used for analysis of total nitrogen and phosphorus using a Technicon Traacs 800 autoanalyzer (Technicon Instruments Corp., Tarrytown, NY) following methods described in Novozamsky et al. (1983).

*Specialist Insects*. Unless indicated otherwise, cultures of insect species were maintained in climate rooms under 16:8 hr L/D photoperiod under a constant temperature of 25 ± 0.5°C and 50 ± 2% RH. *M. cinxia* used in the experiments all originated from the Åland islands of SW Finland. Two different groups of *M. cinxia* larvae were used, one group (reared in 2000–2001) of which a subset was parasitized by *C. melitaearum* and another group (reared in 2001–2002) of which a subset was parasitized by *H. horticola*.

The first group of *M. cinxia* larvae consisted of approximately 200 offspring of field-caught butterflies that were reared at ambient temperatures in the laboratory from June to August 2000 (diapause as L5) on *P. lanceolata* plants that had been collected randomly from natural populations in the Åland islands. Larvae were maintained in diapause at 2°C under 6:16 hr L/D photoperiod until April 2001. At this time, diapause was broken by gently spraying water on the larvae. Upon resuming activity, larvae were randomly separated into two cohorts of approximately 100 individuals and placed into plastic boxes containing moistened paper towel and excised leaves of *P. lanceolata* containing either high or low levels of iridoid glycosides (see below for a quantitative measure). Leaves were selected from both plant lines, which were of approximately the same age and location on the plant, and were refreshed on a daily basis.

Approximately 100 cocoons of *C. melitaearum* were obtained from overwintering Åland laboratory populations in April 2001. Upon emergence, wasps were housed collectively in plastic Petri dishes (20 cm diam) at 10°C and were constantly supplied with honey and water. The remaining insects were reared according to standard protocol for cultures (top). Approximately half of the *M. cinxia* larvae reared on each line of *P. lanceolata* were randomly selected from culture as L6, and were presented individually to an adult female of *C. melitaearum* in small plastic vials. Oviposition was verified by a single insertion and removal of the ovipositor. Parasitized caterpillars were reared separately on leaves of *P. lanceolata* from either the high or low IG line, continuing the treatment prior to parasitism. Upon larval egression from the host, parasitoid brood sizes were determined, and following adult parasitoid emergence, wasps were killed by freezing. Egg-to-adult development time in days was determined, and adult (wet weight) body mass was measured using a Sartorius microbalance (accuracy 1 $\mu$g).

The second group of *M. cinxia* larvae was directly collected from the field. In contrast to *C. melitaearum*, experimental parasitation of *M. cinxia* by *H. horticola*, which parasitizes larvae before hatching, is extremely difficult. Therefore, eight larval groups of *M. cinxia* that had been observed being naturally parasitized by *H. horticola* (S. van Nouhuys, personal observation) were obtained from Åland populations in July 2001. *H. horticola* parasitizes 1/4 to 1/3 of the larvae in each larval group, so approximately 200 of the 800 larvae were parasitized. From L3, each larval group was split into two, and one half was reared (under standard rearing conditions, top) on intact plants from the high IG line and the other half on

plants from the low IG line. At the end of August, L5 larvae were removed from food-plants and maintained under diapause conditions at 2°C under 6:16 hr L/D photoperiod until April 2002. Thereafter, the experimental protocol was the same as for *C. melitaearum*.

The parasitized and the unparasitized larvae were reared together because they are indistinguishable from each other until just prior to pupation of the parasitoid. The development of healthy (unparasitized) larvae of *M. cinxia* reared on each line of *P. lanceolata* was monitored along with that of the parasitized larvae. At pupation, the development time (calculated in days from the breaking of disapause in L5 to adult butterfly emergence) and pupal wet weight (mg) of *M. cinxia* were measured from larvae reared on each line of *P. lanceolata*.

*Generalist Insects.* The host moth *S. exigua* was maintained on artificial diet as described by Vickerman and Trumble (1999). Moths were housed in 1 l plastic beakers containing a vermiculite base and were provided *ad libitum* with 20% sugar solution absorbed into cotton wool. Adult females oviposited onto filter paper placed inside the beaker. Newly hatched eggs were placed onto artificial diet. In rearing *C. marginiventris*, approximately 50 L1 larvae were placed into Petri dishes (20 cm diam) in which five mated female wasps were added. Wasps were allowed to parasitize hosts for several hours, after which they were returned to separate Petri dishes containing drops of honey and water. Cocoons of *C. marginiventris* were collected periodically, and wasps were allowed to emerge in Petri dishes (above).

For experiments, newly hatched larvae of *S. exigua* were reared on the two lines of *P. lanceolata* in April 2002. Newly molted L2 larvae of *S. exigua* were individually presented to female *C. marginiventris* in small plastic vials. Parasitism was verified by a single insertion and removal of the ovipositor. Parasitized larvae were reared using the same methods described for *M. cinxia*. At adult eclosion, wasps were killed by freezing, and wasps were weighed on a Sartorius microbalance. Egg-to-adult development time in days was also measured. The development of control (unparasitized) larvae was measured as for *M. cinxia*.

*Statistical Analyses.* Differences in weight and development time between hosts reared on high and low iridoid glycoside plants and between parasitoids developing on these two types of hosts were analyzed with independent $t$-tests if conditions for parametric analyses were met (Levene's test for homogeneity of variances and Kolmogorov–Smirnov's test for normality) or with Mann–Whitney $U$-tests otherwise. Differences in leaf concentrations of nitrogen, phosphorus, and the iridoid glycosides aucubin and catalpol between selection lines were analyzed with ANOVA. Line effects were tested over effects of maternal half-sib family within selection lines. Data for iridoid glycosides were square-root transformed prior to analysis to meet assumptions for parametric analysis. All analyses were performed using Statistica version 6.1 (StatSoft, Inc., Tulsa OK, USA).

## RESULTS

*Differences in Iridoid Glycosides, Nitrogen, and Phosphorus Among P. lanceolata Lines.* Leaf iridoid glycoside levels differed 5-fold between plants from the low and from the high selection line (Table 1). The higher total iridoid glycoside level in the leaves was due to both higher levels of aucubin and of catalpol (Table 1). Nitrogen concentrations did not differ between selection lines (Table 1, $P = 0.10$). Phosphorus concentration varied among families within lines, but no consistent difference between selection lines was observed (Table 1, $P = 0.56$).

*Development of Specialist Insects.* Development time of *M. cinxia* postdiapause larvae until adult in 2001 was affected by plant line ($t_{17} = 2.49$, $P = 0.024$); butterflies developed more rapidly on plants containing high levels of iridoid glycosides (Figure 1a). However, host pupal weight was not affected by plant line ($t_{19} = 1.29$, $P = 0.21$), although there was a tendency for pupae to be slightly larger when reared on lines containing higher levels of iridoid glycosides (Figure 1b).

In line with 2001 results, development time in *M. cinxia* in 2002 was affected by plant line (Mann–Whitney $U$-test, $U_{30,24} = 502.5$, $P = 0.006$). Development proceeded more rapidly when larvae were reared on lines containing high levels of iridoid glycosides (Figure 1c). In both years, development was completed about 2 days earlier for cohorts reared on the line of *P. lanceolata* containing high levels of iridoid glycosides. As in 2001, the pupal weight of unparasitized individuals was not affected by plant line ($t_{68} = 0.70$, $P = 0.49$) although pupae tended to be somewhat larger than in the previous year (Figure 1d).

Development time ($t_{46} = 1.45$, $P = 0.15$) and adult body mass ($t_{46} = 0.97$, $P = 0.34$) of *C. melitaearum* were not affected by plant line on which their host had been reared (Figure 1e and f). Also, secondary clutch size of *C. melitaearum*

TABLE 1. LEAF CONCENTRATIONS OF THE IRIDOID GLYCOSIDES AUCUBIN AND CATALPOL, TOTAL *N*, AND TOTAL *P*, IN SIX MATERNAL HALF-SIB FAMILIES FROM EACH OF TWO *Plantago lanceolata* LINES SELECTED FOR LOW AND HIGH LEVELS OF IRIDOID GLYCOSIDES[a]

| | Low line Mean (SE) | High line Mean (SE) | Line $F$[1,10] | Fam(Line) $F$[10,12] |
|---|---|---|---|---|
| Iridoid glycosides (% dw) | 0.56 (0.09) | 2.98 (0.33) | 70.58*** | 1.26 |
| Aucubin (% dw) | 0.33 (0.07) | 1.73 (0.16) | 88.20*** | 1.31 |
| Catalpol (% dw) | 0.22 (0.03) | 1.25 (0.33) | 34.13*** | 1.03 |
| Nitrogen (% dw) | 4.02 (0.29) | 3.41 (0.19) | 3.23 | 1.08 |
| Phosphorus (% dw) | 0.49 (0.03) | 0.48 (0.01) | 0.36 | 7.72*** |

[a]Significance of differences between lines and between families nested within lines (ANOVA) are indicated (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$); sample size $N = 24$.

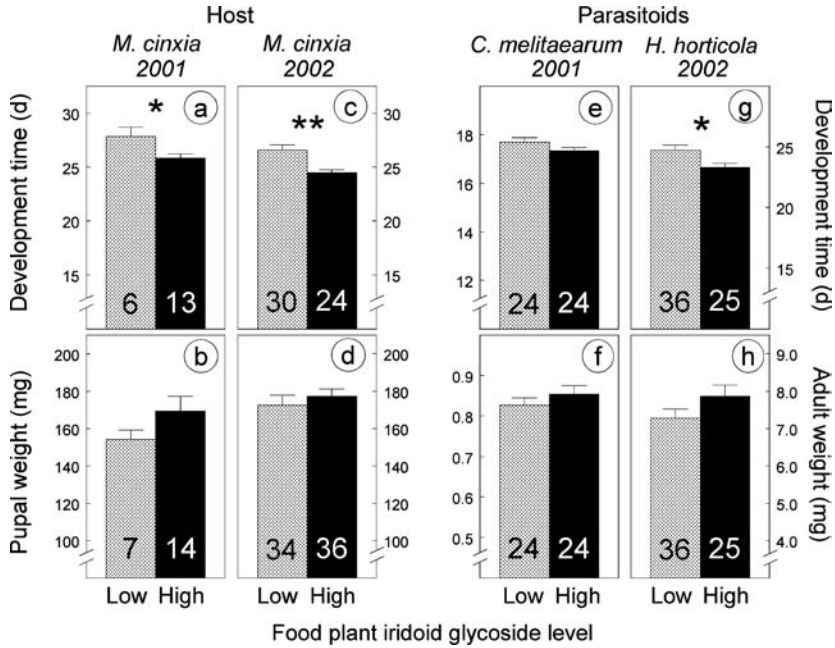


FIG. 1. Development of unparasitized caterpillars of the specialist herbivore *M. cinxia* feeding on *P. lanceolata* selected for low (gray bars) and high (black bars) levels of iridoid glycosides in 2001 (a and b) and 2002 (c and d) and development of its endoparasitoids *C. melitaearum* (e and f) and *H. horticola* (g and h) in parasitized caterpillars from the larval groups raised in 2001 and 2002, respectively. Numbers within bars represent sample sizes. Bars represent 1 SE of the mean. Asterisks indicate significant effects of plant selection line ($^{*}P < 0.05$; $^{**}P < 0.01$).

did not vary among wasps reared on *M. cinxia* that were fed *P. lanceolata* leaves containing high or low levels of iridoid glycosides ($t_{27} = 0.31$, $P = 0.76$). Brood sizes were on average only slightly higher for wasps reared from high (mean $\pm$ SE: $7.13 \pm 0.69$) than from low iridoid plants ($6.71 \pm 0.69$).

As with the host *M. cinxia*, development time in *H. horticola* was significantly different when reared on hosts from the two lines of *P. lanceolata* ($t_{59} = 2.42$, $P = 0.019$). Parasitoids completed their development approximately 1–2 days earlier when originating from high iridoid lines (Figure 1g). However, plant line did not affect emerging adult parasitoid size ($t_{59} = 1.49$, $P = 0.14$) even though wasps tended be larger on high lines (Figure 1h).

*Development of Generalist Insects.* Egg-to-adult development time of *S. exigua* did not vary with the diet ($t_{19} = 0.78$, $P = 0.45$). Irrespective of iridoid glycoside content, larvae of *S. exigua* completed their development in

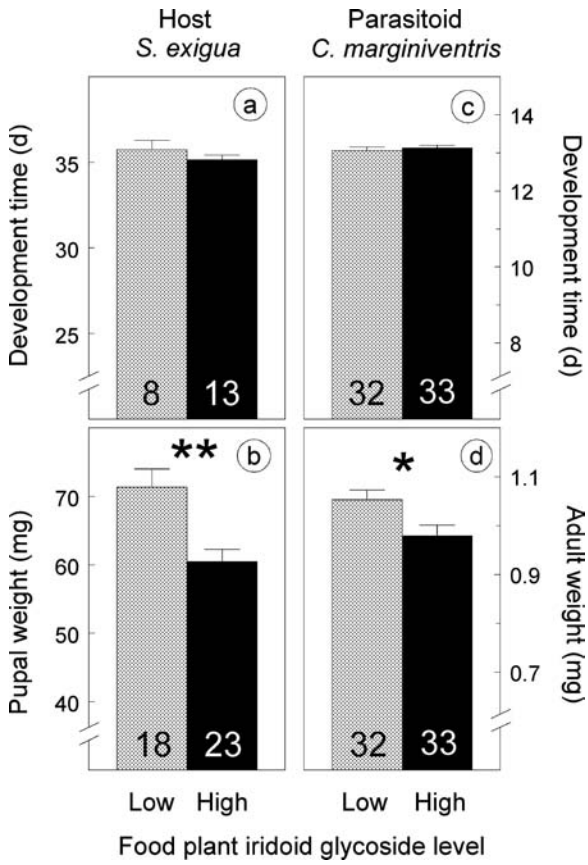


FIG. 2. Development of unparasitized caterpillars of the generalist herbivore *S. exigua* feeding on *P. lanceolata* selected for low (gray bars) and high (black bars) levels of iridoid glycosides (a and b), and development of its endoparasitoid *C. marginiventris* in parasitized caterpillars (c and d). Numbers within bars represent sample sizes. Bars represent 1 SE of the mean. Asterisks indicate significant effects of plant selection line ($^{*}P < 0.05$; $^{**}P < 0.01$).

approximately 35–36 days (Figure 2a). In contrast, pupal weight in *S. exigua* was strongly affected by the line of *P. lanceolata* upon which the larvae had developed ($t_{39} = 3.49$, $P < 0.001$). Pupae of *S. exigua* reared on the low iridoid line were some 15% larger than conspecifics reared on the high iridoid line (Figure 2b).

Similarly, development time in *C. marginiventris* was uniform when reared from *S. exigua* on both lines of *P. lanceolata* ($t_{63} = 0.51$, $P = 0.61$). Parasitoids typically took just over 13 days to complete their development in host caterpillars on the two iridoid lines (Figure 2c). However, like *S. exigua*, adult parasitoid size

differed with the plant line upon which their hosts had been reared ($t_{63} = 2.48$, $P = 0.016$). Wasps originating from the high iridoid line were 7% smaller than those originating from the low iridoid line (Figure 2d).

## DISCUSSION

Our results show that the effect of genotypic variation in IG content in *P. lanceolata* on the development of herbivores differed between the generalist and specialist species used. High levels of IGs reduced the pupal weight of the generalist *S. exigua*. In contrast, IG levels did not affect the pupal weight of the specialist *M. cinxia*, and larvae of this specialist were able to complete their development more quickly on plants with higher levels of these allelochemicals. Levels of nitrogen and phosphorus did not consistently differ between the IG lines, suggesting that effects of allelochemical differences were not confounded by differences in primary metabolites. Our results are in agreement with previous studies on the effects of IGs from *P. lanceolata* on other generalist and specialist herbivores using artificial diet (Bowers and Puttick, 1988; Puttick and Bowers, 1988; Bowers, 1991); addition of aucubin and catalpol to artificial diet reduced larval growth rate of the generalists *Lymantria dispar* and *S. eridania*, but not of the specialist *Junonia coenia,* that developed more quickly on diets with higher amounts of IGs (but see Adler et al., 1995 for *in vivo* effects on *J. coenia*). This suggests that specialized mono- or oligophagous herbivores are better able to cope with plant allelochemicals than polyphagous herbivores. We note, however, that the generalist herbivore was derived from a lab-strain that did not necessarily have previous experience with *P. lanceolata*. As a consequence, it might have had limited opportunity to adapt to the chemistry of this host plant.

Recent studies provide insight into the effects of IGs on the performance of *S. exigua* and *M. cinxia*. Using the same selection lines, it was found (Biere et al., 2004) that the reduced growth of L4 *S. exigua* caterpillars on the high IG line was due to a reduced ingestion rate and not to a reduced digestibility or lower efficiency of conversion of ingested food. Thus, for the generalist, IGs act as feeding deterrents without additional post-ingestive effects, at least at this larval stage. In contrast, L4 caterpillars of *M. cinxia* had higher consumption rates on the high IG line (S. van Nouhuys and S. Kumar, unpublished results). Hence, for the specialist, IGs act as feeding stimulants. Moreover, L4 *M. cinxia* larvae ingested food from the high IG line more efficiently, and had a higher growth rate than caterpillars feeding on the low IG line. This contrasts with artificial diet studies of the specialist *J. coenia*, where lower efficiency of using high IG diets was observed due to reduced digestibility (Camara, 1997). As we did not observe a significantly higher pupal weight of *M. cinxia* reared on the high-IG plants in the present experiment, the higher growth rate of L4 *M. cinxia* may be instar-specific

or may not be sufficiently large to translate into a significant effect on weight at the pupal stage.

The development of endoparasitoids was also affected by the level of allelochemicals in the food plant of their herbivore hosts. For two of the parasitoids, developmental responses paralleled those of their hosts. High IG levels in host plants resulted in a more rapid development of both the specialist *M. cinxia* and its parasitoid *H. horticola* and in reduced weight in both the generalist herbivore *S. exigua* and its parasitoid *C. marginiventris*. In the only other study addressing effects of IGs on herbivore-parasitoid development (Mallampalli et al., 1996), a similar pattern, i.e., parallel responses of herbivore and parasitoid, was found. Early stages of the generalist herbivore *L. dispar* suffered reduced growth on diets with high levels of catalpol, but total larval development was not significantly affected, nor was that of the tachinid parasitoid *Compsilura concinnata* (Mallampalli et al., 1996). In the present experiment, only the parasitoid *C. melitaearum* was not affected by the IG level in the diet of its host, while its host showed a more rapid development.

A number of studies have reported detrimental effects of allelochemicals in the diet of host or prey on the growth and development of parasitoids and predators (Barbosa et al., 1986; Duffey et al., 1986; Gunasena et al., 1990; Havill and Raffa, 2000). In general, toxic phytochemicals in the plant tend to have more negative effects on the development of polyphagous herbivores and their natural enemies than on oligophages and their antagonists, presumably because the former are less-well adapted to cope with them (Gunasena et al., 1990; Barbosa et al., 1991; Sznajder and Harvey, 2003). Consequently, beneficial effects of the production of allelochemicals to the plant in terms of reduced plant damage by generalist herbivores may be partly mitigated by harmful effects of these allelochemicals on the natural enemies of the herbivore. On the other hand, slower development of generalist herbivores on more toxic host plants may increase their "window of vulnerability" to parasitoid or predator attack (the "slow-growth-high-mortality hypothesis," sensu Turlings and Benrey, 1998), enhancing the efficacy of natural enemies through higher parasitation rates, even if they have a slower development on these hosts. Since the development rate of neither *S. exigua* nor *C. marginiventris* was affected by IG level, such a mechanism does not seem to operate in the *P. lanceolata*—*S. exigua*—*C. marginiventris* system.

Positive responses (accelerated development while attaining similar size) to increased levels of IGs were observed for *M. cinxia* and the parasitoid *H. horticola*. This suggests adaptation of the parasitoid to the presence of IGs in its herbivorous host. Like other butterfly larvae that are restricted to feeding on IG producing host plants (Bowers and Collinge, 1992; Bowers, 2003), caterpillars of *M. cinxia* sequester IGs from their food plants (Suomi et al., 2001; Nieminen et al., 2003). Catalpol seems to be a more effective deterrent to predators than aucubin, and is also sequestered in higher proportions than aucubin in *J. coenia* (Bowers and

Collinge, 1992), *Ceratomia catalpae* (Bowers, 2003), and *M. cinxia* (Nieminen et al., 2003). Endoparasitoids must cope with sequestered or concentrated phytochemicals during their entire larval development because ingested allelochemicals cannot be excreted outside the host environment until after egression (Quicke, 1997). If we assume that *M. cinxia* caterpillars raised on food plants from the high-IG line sequester higher levels of IGs than caterpillars raised on the low line, then *H. horticola* does not appear to suffer from the higher levels of sequestered IGs in its host. On the contrary, like its host, *H. horticola* showed accelerated development while reaching similar weight, suggesting increased consumption or more efficient use of hemolymph, and later fat and other ingested tissues, from hosts raised on high-IG plants.

In contrast to *H. horticola*, the development of *C. melitaearum* was not affected by differences in IG levels in the diet of its host. In the field, parasitism of *M. cinxia* by *C. melitaearum* significantly decreased with increasing average level of catalpol in the *P. lanceolata* plant that larval groups of caterpillars had been feeding on (Nieminen et al., 2003). This trend could be the consequence of adult parasitoids avoiding oviposition into larvae that were feeding on plants with high levels of catalpol, or differential mortality of parasitoids developing in larvae feeding on high and low catalpol containing plants. Our results appear to support the first hypothesis.

The differential developmental responses of the two parasitoids may be due to the different host exploitation strategies they exhibit (Harvey and Strand, 2002). *C. melitaearum* is gregarious and passes through several generations during a single host generation. It completes development in a relatively short time, feeding primarily on host hemolymph until the last instar during which it feeds on a portion of the host fat body. In contrast, the development of *H. horticola* is more synchronized physiologically with that of the host. It inhabits hosts from early in the first host instar until just before the time that an unparasitized host would pupate. During most of this time, the parasitoid remains small and ingests little host hemolymph, but at the end of the final stage it consumes all host tissues except for the cuticle (E. Punju and S. van Nouhuys unpublished results; Lei et al., 1997). The positive effect of IGs on *H. horticola* development rate may be a direct response to an increase in host development rate mediated by allelochemicals ingested from its food plant.

Since the seminal paper by Price et al. (1980), many studies have assessed the effects of plant traits on the interaction between herbivores and their natural enemies (for reviews, see e.g., Bottrell et al., 1998; Turlings and Benrey, 1998; Cortesero et al., 2000), mostly by using agricultural, artificially assembled, biological control systems. Of great concern in classical biological control programs are cases where the combined effects of plant resistance and natural enemy impact on herbivores are less than the additive effect of each these components separately ("antagonistic" interactions sensu Hare, 2002). In

extreme cases, where natural enemies are more susceptible to plant resistance mechanisms than herbivores (e.g., Campbell and Duffey, 1979), chemical defense may prevent herbivore control by natural enemies ("disruptive interactions" sensu Hare, 2002). In agricultural systems, additive effects seem to predominate, and only few examples of disruptive interactions have been found (Hare, 2002).

In contrast to agricultural systems, knowledge of indirect effects of plant secondary metabolites on the natural enemies of herbivores in natural systems is scarce despite the fact that plant secondary chemistry has evolved under natural conditions. Non-additive effects might have a large impact on the evolution of levels of plant secondary metabolites ("direct defense") in natural populations. For instance, antagonistic effects may result in selection for lower levels of plant allelochemicals in the presence than in the absence of natural enemies, and we would not be able to understand patterns of selection in natural populations when studied in the traditional bitrophic plant-herbivore or plant-pathogen context (Hare, 1992, 2002).

Thus far, few studies have assessed the effects of quantitative allelochemical variation in non-agricultural plants on the development of higher trophic level organisms (e.g., Harvey et al., 2003; Sznajder and Harvey, 2003). As far as we know, our study is one of the first to document effects of genetic variation in secondary metabolites within a single natural plant species on the parasitoids of its herbivores. We suggest that there might be antagonistic effects of IGs on the generalist parasitoid of the generalist herbivore, but not on the specialist parasitoids of the specialist herbivore of *P. lanceolata*. Conclusive evidence for effects of natural enemies of herbivores on the evolution of concentrations of plant allelochemicals in natural populations still awaits studies that exploit natural study systems (i) in which there is evidence for top-down control of herbivores by parasitoids or predators, (ii) the pattern of selection on plant chemical defense differs in the presence and absence of natural enemies of the herbivores, and (iii) the evolutionary response to selection is investigated.

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