

Lack of correlation between constitutive and induced resistance to a herbivore in crucifer plants: real or flawed by experimental methods?

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Abstract

The correlation between constitutive and induced resistance to herbivores in plants has long been of interest to evolutionary biologists, and various approaches to determining levels of resistance have been used in this field of research. In this study, we examined the relationship between constitutive and induced resistance to the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in 11 closely related species of wild crucifers. We assessed the survival, development, and reproduction of the test insects and calculated their intrinsic rate of increase as an indicator of constitutive and induced resistance for the plants. We used larvae of *P. xylostella* and jasmonic acid as elicitors of the induced response. We failed to find a correlation between constitutive and induced resistance in these crucifer plants when the induction of resistance was initiated by either herbivory or jasmonic acid application. Analysis of the results suggests that the failure to detect a relationship between the two types of resistance could be caused by flaws in measuring constitutive resistance, which was apparently confounded with induced resistance. We discuss the difficulties and pitfalls in measuring constitutive resistance and ways to improve the methodology in investigating the relationships between constitutive and induced resistance in plants.

Introduction

Plants have evolved a variety of resistance traits to herbivores, including morphological barriers, the synthesis of toxic secondary metabolites and defensive proteins, and the release of herbivore-induced volatiles that attract natural enemies of herbivores or directly affect herbivore performance. Plant resistances can be divided into constitutive resistance, expressed independent of attack, and induced resistance, which is activated after the plant is attacked or otherwise injured (Karban & Baldwin, 1997). The possession of several resistance mechanisms may be costly for a plant, because expression of resistance traits reduces the resources available for growth and reproduction (Herms & Mattson, 1992; Strauss et al., 2002), and thus a negative correlation between constitutive and inducible

resistance in plants has often been hypothesized (Karban & Myers, 1989; Herms & Mattson, 1992). However, many case studies to date, using either plant traits or herbivore performance, have given mixed results. A negative correlation between constitutive and induced resistance was found in gymnosperm species (Lewinsohn et al., 1991), clones of silver birch (Keinänen et al., 1999), a population of *Brassica nigra* Koch (Traw, 2002), and wheat seedlings (Gianoli, 2002). But a lack of correlation between the two types of resistance was reported for 10 varieties of cotton (Brody & Karban, 1992), 21 species of *Gossypium* (Thaler & Karban, 1997), nine varieties of grapes (English-Loeb et al., 1998), and 14 genotypes of soybean (Underwood et al., 2000). However, a positive correlation between constitutive and induced resistance was found in parsnip (Zangerl & Berenbaum, 1990) and in *Brassica rapa* L. (Siemens & Mitchell-Olds, 1998). As many of the studies on the relationships between constitutive and induced resistance were conducted using cultivated plant cultivars that have been subjected to artificial selection, their results may offer

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limited relevance to the evolution of plant resistance in nature (Brody & Karban, 1992). Therefore, it is necessary to investigate the relationships between constitutive and induced resistance by experimentation using a range of wild plants (Lu et al., 2004).

Plant resistance has been clearly shown to affect both behavior and performance of herbivores. However, most studies cited above aimed to explore the relationships between constitutive and induced resistance by measuring plant resistance traits, such as secondary metabolites or trichomes of plants (Zangerl & Berenbaum, 1990; Lewinsohn et al., 1991; Siemsen & Mitchell-Olds, 1998; Keinänen et al., 1999; Gianoli, 2002; Traw, 2002). Few studies focused on the effect of plant resistance on herbivore performance (Brody & Karban, 1992; Thaler & Karban, 1997) or behavior (Underwood et al., 2000; Lu et al., 2004). As the concordance between plant resistance traits and biological effects on herbivores may not be straightforward, measuring the behavior and performance of herbivores may more directly reflect the level of plant resistance. Up to now, no study has considered both the oviposition behavior of adults and the performance of all life stages of an insect on plants in exploring correlation between constitutive and induced resistance.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is known to be the most destructive insect of cruciferous plants throughout the world (Talekar & Shelton, 1993). *Plutella xylostella* can also exploit various wild crucifers. Talekar & Shelton (1993) reported that wild crucifers play an important role in maintaining *P. xylostella* populations in temperate regions, especially in the winter and early spring seasons before cruciferous crops are widely cultivated. Some studies show that the performance of *P. xylostella* on wild crucifers is comparable to that on cultivated cruciferous crops (Begum et al., 1996). These findings suggest that wild crucifers are important hosts for *P. xylostella* in natural habitats. Although *P. xylostella* is believed to have originated in the Mediterranean area, it now occurs wherever crucifers are grown. Moreover, *P. xylostella* and crucifers have been widely used to examine the interactions between herbivores and plants (Talekar & Shelton, 1993; Verkerk & Wright, 1996).

In an attempt to investigate whether there is a trade-off between constitutive and induced resistance, we used a range of closely related species of wild crucifers as model plants and measured both the oviposition preference of adults as well as the performance of all life stages of their common specialist herbivore, *P. xylostella*, to determine their resistance level (Thompson, 1988). When we analyzed the levels of resistance as measured by oviposition preference, we detected a significant negative correlation between constitutive and induced resistance when the plants were

induced by insect feeding, but a positive correlation between the two types of resistance when the plants were treated by exogenous application of jasmonic acid (JA) (Zhang et al., 2008). In this article, we analyze the levels of resistance as measured by the survival, development, and reproduction of the insect through its life stages. In contrast to the results with oviposition preference as measure for plant resistance (Zhang et al., 2008), we failed to find a correlation between constitutive and induced resistance in these crucifer plants when the induction of resistance was initiated by either herbivory or JA application. We discuss the contrast between these findings and in particular the difficulties and pitfalls in experimentation to determine constitutive and induced resistance in plants.

Materials and methods

Insects and plants

The *P. xylostella* culture was established in October 2004 from a field collection on a cabbage farm in a suburb of Hangzhou, China (30°15'N, 120°10'E), and maintained on common cabbage, *Brassica oleracea* L. var. *capitata* (Brassicaceae), cultivar Jing-feng No. 1, in a temperature-controlled room at 26 ± 1 °C, 60–70% r.h., and L14:D10 photoperiod. The culture has been replenished once a year with field-collected materials to avoid inbreeding effects of continuous rearing in the laboratory on the population.

Seeds of 11 species of wild crucifers were collected in May 2005 in the suburbs and nearby areas of Hangzhou (from 120°15' to 119°0'E and from 30°10' to 30°45'N; Table 1). This indicates that these plants phenologically co-occur in nature. Infestation of each of the plant species by *P. xylostella* in the field was encountered on at least one occasion during the field collection. All experiments utilized wild crucifers that were grown from seeds in pots (10.5 cm high, 13 cm diameter at the top, and 7.5 cm at the base) in a mixture of peat moss, vermiculite, organic fertilizer, and perlite, in a 10:10:10:1 volume ratio. The plants for measuring constitutive resistance were seeded at the same time, and those for measuring resistance induced either by herbivory or by JA application were also seeded at the same time. The plants were maintained in a greenhouse under ambient temperature, humidity, and light. When plants had grown to the vegetative stage with 6–8 true leaves, plants with similar leaf area were used for experiments.

Measuring constitutive resistance

The level of constitutive resistance of the various plant species was determined by the performance of *P. xylostella*, including development, survival, and reproduction. All the experiments were conducted in temperature-controlled rooms at 25 ± 1 °C, 60–70% r.h., and L14:D10 photoperiod.

Table 1 Performance parameters (mean \pm SE) of *Plutella xylostella* reared on intact plants of 11 species of wild crucifers. On each of the plant species, observations were initiated with 100 *P. xylostella* eggs on 10 plants (10 eggs/plant)

| Plants | Survival from egg to pupa (%) ¹ | Development time from egg to pupation (days) ² | Pupal weight (mg) ² | Fecundity (no. eggs/♀) ³ | Intrinsic rate of increase (♀/♀/ day) |
|--|--|---|--------------------------------|-------------------------------------|---------------------------------------|
| <i>Cardamine hirsuta</i> L. | 75.7 \pm 2.3cd | 10.0 \pm 0.1e | 6.2 \pm 0.2ab | 184 \pm 13b | 0.3046 |
| <i>Rorippa dubia</i> (Pers.) Hara | 86.0 \pm 2.1a | 9.8 \pm 0.1e | 5.3 \pm 0.1e | 126 \pm 17cde | 0.2963 |
| <i>Cardamine flexuosa</i> With. | 77.6 \pm 2.2bc | 11.0 \pm 0.2d | 6.3 \pm 0.2a | 245 \pm 13a | 0.2894 |
| <i>Cardamine limprichtiana</i> Pax | 68.6 \pm 2.0e | 11.2 \pm 0.2d | 5.7 \pm 0.1bcd | 135 \pm 13cd | 0.2695 |
| <i>Cardamine zhejiangensis</i> TY Cheo et RC Fang | 83.3 \pm 2.6ab | 11.1 \pm 0.1d | 5.6 \pm 0.1cde | 154 \pm 15bc | 0.2688 |
| <i>Cardamine</i> var. <i>fallax</i> TY Cheo et RC Fang | 81.7 \pm 2.0abc | 11.1 \pm 0.2d | 6.1 \pm 0.1abc | 105 \pm 11de | 0.2675 |
| <i>Rorippa indica</i> (L.) Hiern | 70.0 \pm 2.5de | 9.8 \pm 0.1e | 6.3 \pm 0.2a | 126 \pm 14cde | 0.2601 |
| <i>Cardamine urbaniana</i> OE Schulz | 80.0 \pm 3.1abc | 12.7 \pm 0.2a | 5.4 \pm 0.1de | 98 \pm 11ef | 0.2576 |
| <i>Lepidium virginicum</i> L. | 78.5 \pm 2.0ab | 12.0 \pm 0.1c | 5.5 \pm 0.1de | 134 \pm 16cde | 0.2540 |
| <i>Cardamine impatiens</i> L. | 56.0 \pm 4.2f | 12.2 \pm 0.2bc | 6.2 \pm 0.1a | 161 \pm 16bc | 0.2362 |
| <i>Capsella bursa-pastoris</i> (L.) Medic | 66.3 \pm 3.0e | 12.5 \pm 0.2ab | 4.8 \pm 0.1f | 67 \pm 5f | 0.2022 |

Mean values in the same column followed by different letters differ significantly (Fisher's protected least significant difference test: $P < 0.05$).

¹For each of the 11 plant species, 10 replicates (10 eggs in each replicate) were conducted.

²For each of the 11 plant species, 10 independent samples were taken from 10 individual plants. The number of insects in each independent sample varied from 4 to 10 depending on the survival of the test insects in a given sample (plant).

³For each of the 11 plant species, 15 females were observed.

The following procedure was used to assess the performance of *P. xylostella*. First, 1 100 *P. xylostella* eggs, deposited on plastic sheets, were taken from the culture. Ten eggs were added close to the top leaves of each of 10 individual plants of each of the 11 plant species. When the eggs had developed into the 4th instar, daily observations were made on survival and development until pupation. Pupae were collected within 24 h of pupation and weighed individually to the nearest 0.1 mg (using a Mettler-Toledo AE200 electronic balance; Shanghai, China) as an index of body size. Pupae were then placed individually in glass tubes (8 cm long \times 2 cm in diameter) covered with a cotton plug until adult emergence. We recorded the following variables: (1) survival of eggs, larvae, and pupae, (2) development duration from egg to pupation, and (3) pupal weight.

Fecundity and longevity of adults were assessed using detached leaves of cabbage as an oviposition substrate in small rearing cages. The cage consisted of a clear, cylindrical and ventilated plastic container (14.5 cm high, 9 cm diameter at the top, and 13 cm diameter at the base). A cabbage leaf was fixed in the cage by inserting its petiole through a hole in the center of the base. The cage was placed onto a 500-ml glass bottle containing tap water and the petiole of the cabbage leaf was inserted in the water to maintain freshness. The use of detached cabbage leaves provided a uniform substrate for the adults throughout their life time and thus the differences in performance between adults reared from different plants would reflect the levels of plant resistance.

Fifteen replicates were conducted for assessing fecundity and longevity on each plant species. For each replicate, one female and one male (<24 h post-emergence) were placed into a rearing cage for mating and oviposition (only water was provided). Eggs deposited on the leaf and cage walls were counted every 2 days and adults were transferred at each observation to a new leaf in a clean cage until their death. We assessed two variables: (1) number of eggs laid, and (2) longevity of adults.

Measuring induced resistance

We selected six plant species (*Cardamine hirsuta* L., *Cardamine limprichtiana* Pax, *Rorippa indica* (L.) Hiern, *Cardamine impatiens* L., *Capsella bursa-pastoris* (L.) Medic, and *Lepidium virginicum* L.) that covered the whole range (i.e., low, medium, and high levels) of constitutive resistance in the 11 plant species as determined by the above experiments, and tested these six species for induced resistance.

Eliciting induced resistance. We elicited induced resistance in the plants by (1) herbivory, or (2) application of JA. (1) We infested each plant with 10 *P. xylostella* third instars and allowed them to feed freely on the plants for 24 h. The larvae were then removed and the damaged plants were used in various tests. Intact plants were used as controls. (2) JA (Sigma-Aldrich, Steinheim, Germany) was dissolved in 2 ml of acetone and dispersed in water to achieve a 1-mM JA solution, which has been demonstrated to be effective in

eliciting induced resistance in crucifers (Lu & Liu, 2005). We liberally sprayed the foliage of each plant with 5 ml of JA solution (containing 0.5% Tween-20) with a hand-sprayer. Treated plants were used in the various tests 24 h after JA application. We sprayed intact plants with 5 ml water (containing 2% acetone and 0.5% Tween-20) and used these plants as controls.

Effects of induced resistance on performance of Plutella xylostella. The bioassays for the performance of *P. xylostella* on plants in the herbivory or JA treatments were the same as the assays for constitutive resistance (above).

Data analysis

The percentage of survival of the 10 insects in each replicate was taken as one observation to calculate the mean survival in each treatment, whereas mean values of development time, pupal weight, and fecundity for the various treatments were calculated from individual insects. Percentages of survival were transformed by arcsine \sqrt{x} before analysis. To compare the performance of *P. xylostella* on the 11 plant species (constitutive resistance), mean values of survival and fecundity were analyzed by a one-way analysis of variance (ANOVA), and the mean values of development time and pupal weight were analyzed by a nested ANOVA, with the plant individual in each species as the nested term in the analysis. When an ANOVA indicated significant effects of plant species, the means were compared pair-wise using the Fisher protected least significant difference procedure. To compare the performance of *P. xylostella* between control and herbivore- or JA-treated plants of a given species (induced resistance), any two mean values of a parameter, including survival, development time, pupal weight, or adult fecundity, on control and treated plants were analyzed using a Student's t-test.

With the data of development time, survival, and reproduction, age-specific life (l_x) and fertility (m_x) tables were compiled on a daily basis for each of the cohorts reared in different treatments. Sex ratio of offspring was assumed to be 1:1. The population parameters net reproductive rate (R_0) and intrinsic rate of increase (r_m) were computed as follows: $R_0 = \sum(l_x m_x)$, and $r_m = R_0 \ln R_0 / \sum(x l_x m_x)$ (Krebs, 2001).

We used the r_m values of cohorts reared on initially intact plants as an indicator for the relative levels of constitutive resistance. Plants associated with higher r_m values were assumed to have lower levels of constitutive resistance and vice versa. The level of induced resistance was estimated by the difference in r_m values between treated plants and control plants, that is, $d = r_{m,t} - r_{m,c}$, where a positive d indicates induced susceptibility and a negative d indicates induced resistance. Pearson's correlation coefficient was used as a measure of the relationship between constitutive and

induced resistance (Cipollini et al., 2005). Statistical analyses were done using the statistical software package STATISTICA® (version 6.1).

Results

Level of constitutive resistance

Survival, development time, pupal weight, and fecundity. The means of each of the four parameters differed significantly between the 11 plant species (Table 1; survival: $F_{10,99} = 17.6$, $P < 0.001$; development time: $F_{10,713} = 40.2$, $P < 0.001$; pupal weight: $F_{10,713} = 9.7$, $P < 0.001$; and fecundity: $F_{10,154} = 12.4$, $P < 0.001$). For development time and pupal weight, the variations between the larval development on individual plants in a plant species were also significant (development time: $F_{99,713} = 2.56$, $P < 0.01$; pupal weight: $F_{99,713} = 2.12$, $P < 0.01$). Assuming that lower survival, slower development, lower body mass, and lower fecundity are associated with higher levels of resistance, the changes of the four parameters with different plant species did not follow the same pattern. For example, compared to the performance of other cohorts, the cohort reared on *C. impatiens* had the lowest survival and relatively slow development (i.e., longer development time), but relatively high pupal weight and fecundity. Likewise the cohort reared on *R. dubia* had the highest survival and fastest development but the lowest pupal weight and a mid-level fecundity (Table 1).

Intrinsic rate of increase (r_m). Substantial variation in r_m occurred between the cohorts reared on different plant species (Table 1). The cohort reared on *C. hirsuta* showed the highest r_m , that reared on *C. bursa-pastoris* the lowest (Table 1).

Level of induced resistance

Induced resistance elicited by herbivory: survival, development time, pupal weight, and fecundity. A significant difference was recorded in one or more parameters between the cohorts reared on treated and control plants for all plant species, except *L. virginicum* (Table 2). Reduced performance associated with previous herbivory treatment of the plants occurred in *C. impatiens*, *C. hirsuta*, and *R. indica*, whereas elevated performance occurred in *C. bursa-pastoris*. Interestingly, in *C. limprichtiana* the cohort reared on herbivore-treated plants showed a reduction in pupal weight but an increase in fecundity compared to its counterpart on control plants (Table 2).

Induced resistance elicited by herbivory: intrinsic rate of increase (r_m). The cohorts reared on herbivore-treated plants of *C. bursa-pastoris* and *C. limprichtiana* exhibited a higher r_m than their counterparts on control plants, whereas

Table 2 Performance parameters (mean \pm SE) of *Plutella xylostella* reared on control and herbivore-treated plants of each of six species of wild crucifers. The observations on each of the two treatments for each of the six plant species were initiated with 100 *P. xylostella* eggs on 10 plants (10 eggs/plant)

| Plants | Treatment | Survival from egg to pupa (%) ¹ | Development time from egg to pupation (days) ¹ | Pupal weight (mg) ¹ | Fecundity (no. eggs/♀) ² | Intrinsic rate of increase (♀/♀/ day) |
|--------------------------------|-----------|--|---|--------------------------------|-------------------------------------|---------------------------------------|
| <i>Capsella bursa-pastoris</i> | Control | 65.5 \pm 2.0 | 11.5 \pm 0.1 | 4.9 \pm 0.1 | 136 \pm 24b | 0.2296 |
| | Herbivore | 64.6 \pm 2.6 | 11.2 \pm 0.1 | 4.6 \pm 0.1 | 201 \pm 13a | 0.2460 |
| <i>Lepidium virginicum</i> | Control | 68.3 \pm 3.0 | 11.2 \pm 0.2 | 5.1 \pm 0.1 | 153 \pm 17 | 0.2700 |
| | Herbivore | 64.8 \pm 2.5 | 11.1 \pm 0.2 | 5.1 \pm 0.1 | 112 \pm 13 | 0.2327 |
| <i>Cardamine impatiens</i> | Control | 58.5 \pm 3.0 | 10.5 \pm 0.1b | 4.7 \pm 0.1 | 138 \pm 13 | 0.2591 |
| | Herbivore | 65.7 \pm 4.5 | 11.0 \pm 0.1a | 4.9 \pm 0.1 | 105 \pm 15 | 0.2556 |
| <i>Cardamine hirsuta</i> | Control | 64.5 \pm 3.1a | 9.6 \pm 0.1 | 5.5 \pm 0.1a | 161 \pm 13 | 0.2847 |
| | Herbivore | 48.0 \pm 4.2b | 9.7 \pm 0.2 | 5.1 \pm 0.2b | 141 \pm 10 | 0.2750 |
| <i>Cardamine limprichtiana</i> | Control | 63.2 \pm 2.8 | 11.0 \pm 0.2 | 5.3 \pm 0.1a | 113 \pm 12b | 0.2660 |
| | Herbivore | 60.5 \pm 3.0 | 11.5 \pm 0.4 | 4.5 \pm 0.2b | 161 \pm 14a | 0.2770 |
| <i>Rorippa indica</i> | Control | 77.0 \pm 2.1a | 9.3 \pm 0.1b | 5.9 \pm 0.1a | 187 \pm 20 | 0.2866 |
| | Herbivore | 54.4 \pm 3.2b | 10.5 \pm 0.2a | 5.6 \pm 0.1b | 195 \pm 26 | 0.2460 |

Mean values within a column and within a plant species followed by different letters differ significantly (Student's t-test: $P < 0.05$).

¹For each of the two treatments in each of the six plant species, 10 replicates were conducted (for survival 10 eggs were observed in each replicate, and for development time and pupal weight the number of insects observed in each replicate varied from 4 to 10 depending on the survival of the test insects in a given replicate).

²For each of the two treatments in each of the six plant species, 15 females were observed.

those reared on herbivore-treated plants of *L. virginicum*, *C. hirsuta*, and *R. indica* showed a lower r_m than on the control, although the cohort reared on herbivore-treated plants of *L. virginicum* did not exhibit a significant change in any of the individual parameters. In *C. impatiens*, the r_m value for *P. xylostella* on herbivore-treated plants was similar to that on control plants (Table 2). No significant

correlation was found between levels of constitutive and induced resistance in the six plant species in the herbivore treatment (Pearson coefficient = -0.70 , $P = 0.12$; Figure 1A).

Induced resistance elicited by JA: survival, development time, pupal weight, and fecundity. A significant difference was recorded in one or more parameters between the cohorts

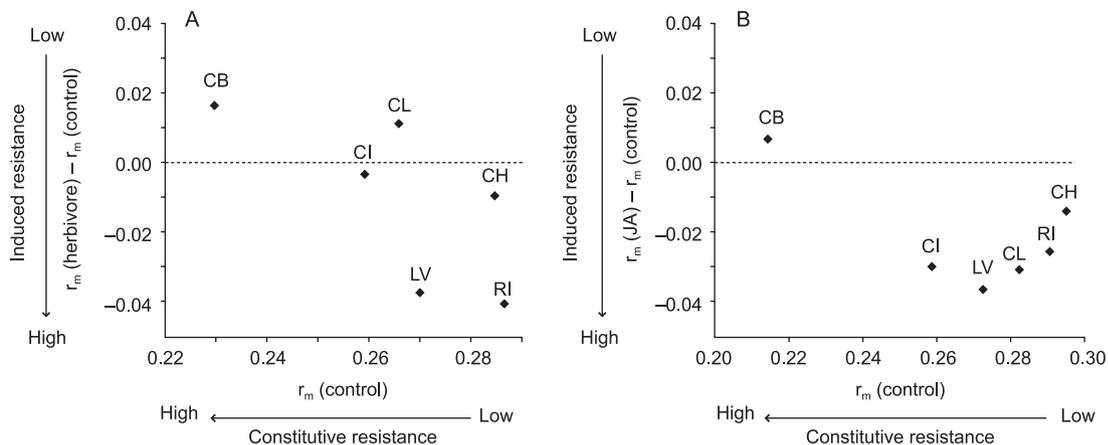


Figure 1 Relationship between levels of constitutive and induced resistance, elicited either (A) by herbivore damage or (B) by jasmonic acid (JA), in six species of wild crucifers (RI, *Rorippa indica*; CL, *Cardamine limprichtiana*; CH, *Cardamine hirsuta*; CI, *Cardamine impatiens*; LV, *Lepidium virginicum*; CB, *Capsella bursa-pastoris*). The intrinsic rate of increase (r_m) values of *Plutella xylostella* cohorts on plants of control or induced treatments were taken as a measure of resistance of the plants to the herbivore, and plants associated with higher r_m values were assumed to have lower levels of resistance and vice versa. See text for details.

Table 3 Performance parameters (mean \pm SE) of *Plutella xylostella* reared on control and jasmonic acid (JA)-treated plants of each of six species of wild crucifers. The observations on each of the two treatments for each of the six plant species were initiated with 100 *P. xylostella* eggs on 10 plants (10 eggs/plant)

| Plants | Treatment | Survival from egg to pupa (%) ¹ | Development time from egg to pupation (days) ¹ | Pupal weight (mg) ¹ | Fecundity (no. eggs/♀) ² | Intrinsic rate of increase (♀/♀/ day) |
|--------------------------------|-----------|--|---|--------------------------------|-------------------------------------|---------------------------------------|
| <i>Capsella bursa-pastoris</i> | Control | 54.6 \pm 4.0 | 11.3 \pm 0.1b | 4.8 \pm 0.2 | 111 \pm 23 | 0.2143 |
| | JA | 60.5 \pm 3.5 | 11.9 \pm 0.1a | 4.6 \pm 0.1 | 141 \pm 27 | 0.2212 |
| <i>Lepidium virginicum</i> | Control | 64.2 \pm 2.5a | 10.1 \pm 0.1b | 5.6 \pm 0.2a | 175 \pm 20a | 0.2727 |
| | JA | 46.2 \pm 3.1b | 10.7 \pm 0.1a | 5.2 \pm 0.1b | 109 \pm 12b | 0.2355 |
| <i>Cardamine impatiens</i> | Control | 56.3 \pm 2.7 | 10.4 \pm 0.1b | 4.7 \pm 0.1 | 135 \pm 14a | 0.2587 |
| | JA | 60.0 \pm 2.0 | 12.1 \pm 0.2a | 4.5 \pm 0.1 | 94 \pm 14b | 0.2290 |
| <i>Cardamine hirsuta</i> | Control | 69.5 \pm 4.5 | 9.5 \pm 0.2b | 5.7 \pm 0.2 | 154 \pm 7 | 0.2953 |
| | JA | 62.3 \pm 3.2 | 10.0 \pm 0.2a | 4.9 \pm 0.1 | 139 \pm 8 | 0.2819 |
| <i>Cardamine limprichtiana</i> | Control | 64.5 \pm 3.5 | 11.1 \pm 0.2 | 4.9 \pm 0.1 | 143 \pm 14 | 0.2825 |
| | JA | 58.4 \pm 2.8 | 11.1 \pm 0.2 | 4.7 \pm 0.1 | 125 \pm 10 | 0.2511 |
| <i>Rorippa indica</i> | Control | 68.6 \pm 3.1 | 9.3 \pm 0.2b | 6.1 \pm 0.1a | 222 \pm 17 | 0.2907 |
| | JA | 60.0 \pm 3.8 | 10.3 \pm 0.2a | 5.8 \pm 0.1b | 170 \pm 10 | 0.2646 |

Mean values within a column and within a plant species followed by different letters differ significantly (Student's t-test: $P < 0.05$).

¹For each of the two treatments in each of the six plant species, 10 replicates were conducted (for survival 10 eggs were observed in each replicate, and for development time and pupal weight the number of insects observed in each replicate varied from 4 to 10 depending on the survival of the test insects in a given replicate).

²For each of the two treatments in each of the six plant species, 15 females were observed.

reared on treated vs. control plants of all plant species, except *C. limprichtiana*, and these differences were all associated with reduced performance on JA-treated plants (Table 3).

Induced resistance elicited by JA: intrinsic rate of increase (r_m). Except for *C. bursa-pastoris*, for which the cohort reared on JA-treated plants exhibited a slightly higher r_m value, on each of the remaining plant species the cohort reared on JA-treated plants showed a lower r_m than its counterpart on control plants (Table 3). No significant correlation was found between levels of constitutive and induced resistance in the six plant species in the JA-treatment experiment (Pearson coefficient = -0.64 , $P = 0.17$; Figure 1A).

Discussion

We quantified the survival, development time, body size, and fecundity of the herbivore to assess resistance in the plants. There appeared to be complex relationships between the four parameters and many of the measurements seemed to contradict with each other in terms of reflecting plant resistance (Thompson, 1988, Table 1). Therefore, the intrinsic rate of increase (r_m), a population parameter based on age-specific survivorship and fertility (Krebs, 2001), was taken to describe the levels of plant resistance. We found substantial variation in constitutive resistance to *P. xylostella* in the 11 plant species (Table 1). However, we

failed to detect a significant correlation between constitutive and induced resistance in the six plant species that covered the range of constitutive resistance in these plant species, when the induction of resistance was initiated by either insect feeding or JA application (Figure 1).

In *P. xylostella*, the development of the egg takes approximately one-fourth of the whole duration from egg to pupation (Liu et al., 2002). Accordingly, the development of eggs in various cohorts would take approximately 2.5 to 3 days and development of larvae would take 7–9 days (Tables 1–3). Thus, in all treatments to determine constitutive resistance, insect larvae were feeding on plants for 7–9 days. It has been demonstrated that induced changes in the plants occur well within 24 h after the initiation of insect feeding, as indicated by the analysis of volatile emission or gene expression (Scascighini et al., 2005; Zheng et al., 2007; Zhang et al., 2008), and continues to occur through the period of larval feeding. It is likely, therefore, that the performance of the test insects reflects the levels of not only constitutive resistance but a mixture of both constitutive and induced resistance. As the 'constitutive resistance' we measured was a mix of constitutive and induced resistance, further analysis on the correlation between the two types of resistance is no longer warranted.

In contrast, when we used oviposition preference of *P. xylostella* as an indicator of plant resistance, we detected a significant negative correlation between constitutive and

induced resistance in this group of closely related plant species when the induction of resistance was initiated by insect feeding (Zhang et al., 2008). Because oviposition preference for intact plants by adult females reflects one aspect of constitutive resistance and does not suffer from the problem of mixing up both types of resistance, the negative correlation detected could be taken as evidence of a trade-off between constitutive and induced resistance (Zhang et al., 2008). It is likely that the failure of finding a negative correlation between constitutive and induced resistance in this study was due to the problems associated with measuring constitutive resistance, as stated above. On the other hand, even if a negative correlation between the two types of resistance was detected statistically, the correlation would be deemed spurious, because the 'constitutive resistance' was mixed up with induced resistance. This pitfall of mixing up the two types of resistance in measuring constitutive resistance is inherent to quantifying induced resistance for intact plants in terms of herbivore performance. It has most likely also occurred in several previous studies in which the performance or population increase over a period of many days was taken as a proxy of constitutive resistance (e.g., Brody & Karban, 1992; Thaler & Karban, 1997; English-Loeb et al., 1998). However, if the time intervals between the initiation of feeding and the occurrence of significant induction responses in the plants are known, one may avoid this pitfall by allowing the larvae to feed on the plants only for the lengths of the defined time periods and moving the larvae to new plants at regular intervals (Müller & Sieling, 2006).

Our results bring up the issue with regard to the methodology of measuring constitutive resistance of plants (Morris et al., 2006). By definition, constitutive resistance must be measured in the absence of herbivore feeding. Meanwhile the performance of an insect throughout its development probably is the best estimation of the actual level of resistance of the plant on which it feeds. Several authors have used changes in secondary metabolites or morphological characters in plants as indicators of resistance (see Introduction). Such an approach could be used to help avoid the problem of mixing up both types of resistance in measuring constitutive resistance, but could become valid only if a dose-dependent relationship is known for the metabolites or characters with the actual level of resistance of the plants. However, such a dose-dependent relationship has rarely been documented, and it is likely that the changes of resistance in many plants are associated in a complex manner with a range of chemical, physiological, and morphological characters. Several studies have found that induced resistance increased with increasing intensity of insect feeding or dose of exogenous JA (Underwood, 2000; Thaler et al., 2001; Zheng et al., 2007), indicating

that induced resistance is a graded response rather than an on/off process. Furthermore, spatial and temporal difference in sampling may offer divergent results. When the seventh leaves of *B. nigra* were chosen for chemical analysis of glucosinolates (Traw, 2002), a negative correlation between constitutive and induced resistance was found. However, a positive correlation between the two types of resistance was found by analyzing glucosinolates in cotyledons of *B. rapa* (Siemens & Mitchell-Olds, 1998). Despite these complexities, direct measurements of resistance traits in plants appear to be a feasible approach to measure constitutive resistance to the feeding stages of a herbivore. A quantitative measurement of constitutive resistance becomes possible when dose-dependent relationships between several key resistance traits and the actual level of resistance are known and a series of measurements for the various resistance traits are taken at diverse time-points.

Jasmonic acid is known as an essential regulatory compound for the expression of direct and indirect resistances against herbivores (Farmer & Ryan, 1990; Baldwin, 1998; Dicke et al., 1999), and exogenous application of JA has been shown to negatively impact the performance of many insect species (e.g., Thaler et al., 2001). In the six plant species used in investigating induced resistance in this study, all plant species but *C. bursa-pastoris* showed JA-induced resistance to *P. xylostella* (Table 3, Figure 1B). Despite the problems associated with measuring constitutive resistance, our results seem to agree in general with earlier studies in which exogenous application of JA onto crucifer plants has been shown to exert an adverse effect on the performance of *P. xylostella* (Lu & Liu, 2005), *Spodoptera exigua* (Hübner) (Cipollini et al., 2004), and cabbage white butterflies (Bruinsma et al., 2007). Because JA or methyl jasmonate can be easily applied to plants and their effects are often dose-dependent (Farmer & Ryan, 1990), they have clear advantages in terms of precision and ease of manipulation. Many ecologists have utilized JA or methyl jasmonate to mimic herbivore attack to examine the effects of induced responses on herbivores or their natural enemies (Dicke et al., 1999; Thaler et al., 2001, 2002; Bruinsma et al., 2007) and to estimate fitness or cost of induced response (Baldwin, 1998, van Dam & Baldwin, 1998; Cipollini, 2007). However, our study shows that JA-induced responses may differ from herbivore-induced responses in terms of the effects on herbivores (Figure 1). Differences between JA-induced and herbivore-induced responses have also been reported in a few earlier studies (Halitschke et al., 2000; Schmelz et al., 2003; Zhang et al., 2008). Thus, patterns of induced resistance revealed by JA treatment of plants are unlikely to reflect the relationships between constitutive and induced resistance in nature.

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