Cost of resistance to herbivory in the annual plant *Arabidopsis thaliana*

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Abstract

This study examines the assumption that plant resistance to herbivory has fitness costs. To assess costs, a standard method was used for examining the significant negative genetic correlation between the resistance character and damage in the presence of herbivory and with fitness in the absence of herbivory. Seeds of five plants from four genotypes of *Arabidopsis thaliana* were sown under controlled conditions in a growth chamber. Half of the resulting two months-old rosettes were used for glucosinolate analysis and for herbivory assessment. The other half were transplanted into an enclosure in the natural habitat of this plant and their fitness (fruit number) were measured after harvesting the plants. Caterpillars from *Spodoptera exigua* were obtained from lab culture for herbivory assessment. Two second-instar caterpillars from *S. exigua* were placed on each of the rosettes. Larval weight of caterpillars was measured after 5 days. One hundred mg dry mass of leaves from 5 rosettes of each genotypes were used for HPLC analysis. There were genetic variations in types and quantities of glucosinolate between genotypes. The results from herbivory assessment showed that the larval weight of *S. exigua* fed on some genotypes was significantly lower than others, and therefore there was genetic variation in resistance to herbivore for *A. thaliana* genotypes. The statistical analysis showed that the larval weight of *S. exigua* was negatively correlated with the total glucosinolate concentration and with fruit number. Therefore, under the condition of this experiment, glucosinolates reduced damage by *S. exigua* and exhibited significant fitness costs.

Introduction

The idea that a plant must allocate limited resources among growth, reproduction, and defense has been central to the ecological and evolutionary theories (Coley et al., 1985; Frank, 1993; Herms and Mattson, 1992) and underpins recent ideas about life history trade-offs. Stated simply, if a plant allocates a greater proportion of resources to defense, then less should be available for growth and /or reproduction. If this relationship did not exist, then there would be no cost to counteract the benefit of resistance, and all plants should be resistant. The many examples of polymorphisms in the levels of resistance within and among populations suggest strongly that costs are prevalent (e.g., Parker, 1992; Simms, 1992), and for decades the notion of evolutionary trade-offs associated with resistance has been widely accepted. However, failures to detect costs of resistance to herbivores and pathogens (Agren and Schemske, 1993; Brown, 1988; Simms and Rausher, 1987) have raised questions about whether

such costs exist in plant populations (Simms, 1992; Simms and Triplett, 1994) and thus, about the appropriateness of theories that postulate such cost. Many researchers have attempted to detect costs of resistance to herbivores but they have not observed significant costs. Early attempts, based on nongenetic approaches, initially suggested that costs might be common. However, most of these approaches have not determined whether there is a negative genetic correlation between resistance and fitness. For example, although some investigators have calculated the cost of resistance in the currency of adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), or carbon (Chew and Rodman, 1979), these prove not to be meaningful in an evolutionary context unless the costs can be expressed in the relevant units of plant fitness.

Others have inferred the existence of costs of resistance from the detection of significant negative phenotypic correlations between fitness and resistance (Baldwin et al., 1990; Coley, 1986). Such studies must be viewed with caution because environmental covariances may cause the phenotypic covariances to differ in both sign and magnitude from the underlying genotypic

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covariances (Rausher, 1992). However, the failure may be a result of reasons other than the actual absence of costs of resistance (Charlesworth, 1990). For example, costs may not be revealed as a pairwise negative correlation between resistance and fitness because these two traits are mutually correlated with other characters (Houle, 1991). Costs may also go undetected because they may be manifested only under certain environmental conditions that differ from those used in experiments (Bergelson, 1994).

Arabidopsis thaliana (Brassicaceae) is a predominantly self- fertilizing, annual herb that is native to Europe and now wildly distributed in many parts of north-temperate regions of the world (Baskin and Baskin, 1972; Ratcliffe, 1961). *A. thaliana* is a prime model system of plant molecular genetics, and is currently used to explore the molecular basis of resistance to herbivores (Kroymann et al., 2003). *A. thaliana* produces both secondary compounds (most notably the glucosinolates, a class of secondary compounds characteristic of the Brassicaceae and leaf trichomes that could potentially deter oviposition and insect feeding (Mauricio, 1998).

This paper examines the existence of fitness costs of resistance in populations of the annual plant *Arabidopsis thaliana*. Assessment of costs of resistance requires the presence of genetic variation for the resistance characters. Resistance characters are, by definition, traits that reduce the amount of damage an individual plant experiences. To assess costs, a standard method was used for examining the significant negative genetic correlation between the resistance character and damage in the presence of herbivory and with fitness in the absence of herbivory.

Materials and methods

Seeds of five plants were collected from four populations (genotypes), 2 from dune and 2 from inland area in the Netherlands. Seeds of each population were sown under controlled conditions in a growth chamber (20° C, 18-h light, 70% humidity). Seeds produced by these plants were germinated and half of the resulting two months-old rosettes were used for glucosinolate analysis and for herbivory assessment. To include all environmental condition, the other half was transplanted into an enclosure in the natural habitat of this plant. Rosettes from each of four genotypes in 5 replicates were transplanted into a randomized complete block design. The rosettes were transplanted into small holes with minimal disturbance of the surrounding vegetation. Fitness

(fruit number) was measured after harvesting the plants.

One hundred mg dry mass of leaves of 5 rosettes of each genotype was used for the HPLC analysis. Extraction, purification and glucosinolate measurements were performed following the procedure used by Van Dam et al. (2003) with sinigrin as the external standard. Glucosinolates were extracted with 70% methanol solution, desulphatased with arylsulphatase on a DEAE-Sephadex A25 column and separated on a reversed phase C-18 column on HPLC with an acetonitril-water gradient. The elution program was a linear gradient starting at 0% acetonitrile (ACN) that increases to 35% ACN in water over 30 minutes. Detection was performed with a single wavelength detector set to 229 nm. Glucosinolates that could not be identified were indicated based on their UV absorption spectrum.

To show genetic variation for resistance to the glucosinolstes reduce damage caterpillars from *Spodoptera exigua* were obtained from a lab culture, reared on an artificial diet in a growth chamber at 25°C, 16h/8h L/D photoperiod, 70% RH. Rosettes of 5 plants in 5 replicates from the same rosettes, as used for HPLC analysis, were used for this experiment. Two second-instar (second growth stage) caterpillars from *S. exigua* were placed on each rosette. Larval weight of caterpillars was measured after 5 days. Differences in larval weight show that resistance characters in genotypes are different.

Data were analyzed with SPSS 13. Normality of the data was checked by post-hoc analysis of the residuals using the Kolmogorov-Smirnov test for normality. Differences in larval weight of herbivores and differences in glucosinolate concentration between populations were tested with ANOVA. The correlation between glucosinolates and larval weight of herbivores and with fruit number was analyzed with the Pearson test.

Results

Glucosinolate differences in HPLC analysis

Results indicated a genetic variation in glucosinolates in the studied plants. Twelve principal glucosinolates were found in the leaves of plants grown in the growth room. They were classified into four structural types according to Fahey et al. (2001): indol glucosinolates (I), aliphatic with straight and branched chains glucosinolates or olefins (D), alcohols side chains glucosinolates (E) and sulfur-containing side chains glucosinolates (A). Individual plants and populations (genotypes) differed in glucosinolate

composition. Epiprogoitrin, 4-hydroxy glucobrassicin and an unknown sulfur-containing glucosinolate were found only in one of dune gynotype and gluconapin was found only in dune plants. 3-OH propylglucosinolate and an unknown alkenyl glucosinolate were found only in one of the inland genotype. The concentration of sinigrin was high in dune genotype compared to the inland genotypes. The plants from dune had a significantly higher concentration of total glucosinolates as compared to the inland plants. The aliphatic glucosinolate were found in high concentration on dune genotypes. Concentration in plants from dune was also higher than in inland plants but this was not significant (table 2).

Table 1. Mean (± SE) of larval weight (mg) of *Spodoptera exigua* fed on dune and inland populations.

Herbivore	***Dune 1***	Dune 2	Inland 1	Inland 2
Larval weight of *S. exigua*	20 ± 11.7 a	17.9 ± 8.79 a	128.5 ± 73.4 b	122.2 ± 34 b

The values in each row, followed by a different character are significantly different (ANOVA, Tukey test, *P*<0.05). *n*= 25.

Table 2. Glucosinolate type of leaves for dune and inland plants grown in growth room.

Glucosinolate type	Dune 1	Dune 2	Inland 1	Inland 2
I	1.01 ± 0.16 a	1.26 ± 0.09 a	1.04 ± 0.19 a	0.97 ± 0.07 a
D	14.06 ± 2.41 b	25.80 ± 1.85 a	2.14 ± 0.39 c	8.35 ± 0.79 bc
E	0	0	4.37 ± 0.79	0
A	0.59 ± 0.09 b	1.31 ± 0.07 a	1.01 ± 0.17 ab	1.31 ± 0.07 a
Total glucosinolate concentration	15.66 ± 2.67 b	28.37 ± 1.99 a	2.56 ± 1.53 b	10.63 ± 0.99 b

Mean concentration (±SE) (µmoles/g dry weight) for each type is given, *n*= 5. I = indol glucosinolates; D = aliphatic glucosinolates with straight and branched chains (olefins); E = glucosinolates with alcohols side chains and A = glucosinolates with sulfur-containing side chains. The values in each row, followed by a different character are significantly different (ANOVA, Tukey test, *P*<0.05).

Herbivory Assessment

The results from herbivory assessment showed that the larval weight of *S. exigua* fed on some genotypes was significantly lower than others (*P*<0.001), and indicating genetic variation in resistance to herbivore for *A. thaliana* genotypes (table1).

Herbivory in relation to glucosinolates and fitness

The result showed that the larval weight of generalist herbivore *S. exigua* was negatively correlated with total glucosinolate concentration (r = -0.64, P<0.001) and with the olefin group in the leaves (figure 1). The larval weight of the generalist herbivore *S. exigua* was also negatively correlated with gluconapin, sinigrin and 4 methoxyglucobrassicin (*r* = -0.71, *P*<0.001; *r* = -0.65, *P* = 0.002; *r* = -0.59, *P* = 0.006 respectively).

At the same time there was a significant negative genetic correlation between glucosinolates and fruit numbers (r = -0.45, P = 0.05) (figure 2).
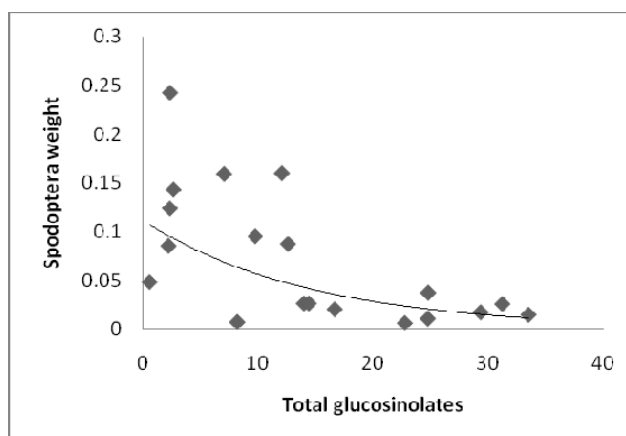

Figure 1. Pattern between total glucosinolates and *Spodoptera* weight.
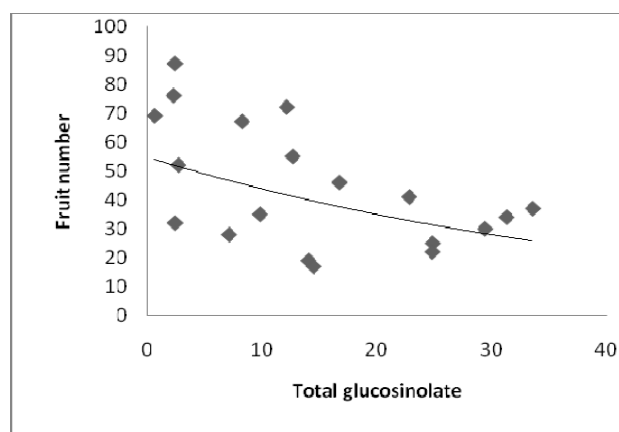
Figure 2. Pattern between total glucosinolates and fruit number.

Discussion

First, this experiment indicated variation in glucosinolate concentration among the studied populations. Significant genetic differences in glucosinolates have already been reported for *Arabidopsis thaliana* (Mosleh Arany, 2009) and for *Brassica oleracea* (Mithen et al., 1995). Second, this experiment demonstrated that the main effect of glucosinolates lies in defense against a generalist herbivore as there was a negative correlation between glucosinolates and *S. exigua* weight. The negative impacts of glucosinolates on generalist herbivore *Trichoplusia ni* also reported by Kliebenstein et al. (2002). In addition to glucosinolate composition, glucosinolate concentration also negatively impacted generalist herbivory for both *S. exigua* and *Trichoplusia ni* (Kroymann et al., 2003). Third, results of this experiment provided evidences that resistance characters to herbivory (glucosinolate here) exhibited fitness costs. Mitchell-Olds et al (1996) demonstrated the cost of resistance to herbivory and disease in *Brassica*. They showed that genetic resistance to the fungal pathogen, *Leptosphaeria maculans* was cost-free, while resistance to *Peronospora parasitica* showed a negative genetic correlation between disease resistance and growth rate. Mauricio (1998) showed cost of resistance to natural enemies in field populations of *Arabidopsis*. The herbivore in his study was not identified, so that it is not clear whether they are specialist or generalist herbivores.

This study shows significant fitness cost for one of two types of resistance characters, glucosinolates. *Arabidopsis* has another resistance character that can reduce damage by herbivores, trichome density. Most plant species possess multiple resistance characters. Based on the assumption that there are costs of resistance,

several authors have suggested that there should be a trade-offs among resistance characters (Bjorkman and Anderson, 1990). There are several evidences for such a relationship. Rehr et al. (1973) found that *Acacia* species possess either cyanogenic glycosides or symbiotic ant-based defense but not both. Bjorkman and Anderson (1990) showed that a morph of a South American blackberry lacking glandular trichomes had significantly tougher leaves than a morph with trichomes. By contrast, Steward and Keeler (1988) found no relationship between indol alkaloide and three physical resistance characters in 19 species of the genus *Ipomoea*. This study did not examined the relationship between two resistance characters in *Arabidopsis* but Mauricio (1998) found a significant positive correlation between trichome density and total glucosinolate concentration in *A. thaliana*.

This study demonstrated that under the condition of this experiment, glucosinolstes reduced damaged by *S. exigua* and exhibited significant fitness costs.

Acknowledgment

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