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## VARIABLE IMPACT OF DIVERSE INSECT HERBIVORES ON DIMORPHIC *DATURA WRIGHTII*

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**Abstract.** Traits that confer plant resistance to some herbivore species but increase plant susceptibility to other herbivore species are said to carry an “ecological cost” of herbivore resistance. The native perennial, *Datura wrightii*, is dimorphic for leaf trichome type, and the production of glandular trichomes carries a potential ecological cost over the production of nonglandular trichomes. Glandular trichomes provide resistance to at least six herbivore species, but they also confer susceptibility to *Tupiocoris notatus*, an abundant mirid bug with special adaptations to glandular trichomes. We estimated herbivore-specific damage to plants of each trichome type and measured plant seed production, a component of fitness, in a common garden over three years. Plant seed production increased with the size and persistence of leaf canopies and occasionally declined with increasing damage caused by some of the defoliating insects. Plant seed production was never reduced by *T. notatus* damage, however. Even though the ecological cost of glandular trichome production was not apparent in *D. wrightii*, the trait still was not beneficial. The fitness of plants with glandular trichomes never exceeded that of plants with nonglandular trichomes despite variation in both the composition of the herbivore community and the total level of damage inflicted by herbivores over three years of study. The persistence of the allele coding for glandular trichomes cannot be explained solely on the basis of the herbivore resistance that those trichomes provide.

**Key words:** *Datura wrightii*; ecological cost; herbivore community; plant defense; plant fitness; trichomes; *Tupiocoris notatus*; water availability.

### INTRODUCTION

Herbivory can reduce plant fitness, so herbivores should select for plant genotypes that are resistant to herbivore attack (Crawley 1983, Marquis 1992). Consequently, frequencies of genes conferring resistance to herbivores should increase in response to herbivory. Yet, genetic variation for resistance traits is commonly found in plant populations (e.g., Berenbaum et al. 1986, Maddox and Root 1987, Simms and Rausher 1987, Fritz and Price 1988, Marquis 1990, Karban 1992, Ågren and Schemske 1994, Mauricio 1998, Elle and Hare 2000). The existence of such variation has stimulated substantial interest in questions concerning the evolution of plant resistance to herbivores.

Most plant species are attacked by multiple insect herbivores, and particular plant traits may not provide equivalent resistance to all herbivores. Many classes of plant allelochemicals, for example, are known to have opposing effects on adapted and nonadapted insect species, (Feeny 1992, Futuyma and Keese 1992); the value of such compounds in protecting plants should vary with the proportion of adapted and nonadapted species in the herbivore community. In cru-

cifers, for example, natural selection might favor the production of glucosinolates when poorly adapted species predominate in the herbivore community but disfavor the production of these compounds when the herbivore community is dominated by specialists that exploit glucosinolates or their break-down products for host recognition (Chew 1988). Traits that confer resistance to some herbivore species but susceptibility to others are said to carry an “ecological cost” (Simms 1992), for the benefits of providing resistance to some herbivores are offset to some extent by the costs of conferring susceptibility to others.

Recently, we identified a potential ecological cost of glandular trichome production in the native perennial plant, *Datura wrightii* Regel (Solanaceae). Plants either produce mostly (>95%) glandular trichomes and feel sticky when touched (hereafter “sticky” plants), or they produce a similar percentage of nonglandular trichomes and feel velvety when touched (“velvety” plants). A single dominant gene largely governs trichome type (van Dam et al. 1999). The primary difference between sticky and velvety plants is the secretion of acyl glucose esters from glandular trichomes of sticky plants (van Dam and Hare 1998a). Sticky and velvety plants may also differ in other ways, but they do not differ in water content, total nitrogen content, soluble sugar content, total alkaloid content, or total phenolic content (van Dam and Hare 1998a). In laboratory studies, sticky plants were a less suitable food

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for tobacco hornworms, *Manduca sexta*, than velvety plants and were entirely resistant to whiteflies (van Dam and Hare 1998a, b). In the same studies, sticky plants were more susceptible to, and a preferred food source for, the mirid bug, *Tupiocoris notatus* (van Dam and Hare 1998b), a species with particular morphological adaptations to its tarsi to cope with glandular trichomes (Southwood 1986, Schuh and Slater 1995).

Previously, we investigated the potential genetic and allocation costs of glandular trichomes. We found a surprisingly high genetic cost for glandular trichome production, in that first-year seed production of sticky plants grown in the absence of herbivores was 45% less than velvety plants, although sticky plants grew to a larger size. We suggested that sticky plants were relatively more growth dominated while velvety plants were relatively more reproduction dominated, and that these life history differences may be due either to pleiotropic effects of the trichome locus or other loci linked to the trichome locus (Elle et al. 1999).

The costs as well as the magnitude of herbivore resistance may vary with the environment. Allocation costs, for example, are most likely to be seen at low rather than high resource availability (Simms 1992). Although a multitude of studies show that the level of resistance or suitability of plants to herbivores varies with the magnitude of nutrient, water, or other abiotic stresses (reviewed by Waring and Cobb 1992, Koricheva et al. 1998), relatively few such studies address genotypic variation among plants in their responses to those stresses. Those that do suggest that genotype-by-environment interactions may be common, and that both the magnitude and the direction of insect responses to plant stress may vary with plant genotype (Waring and Cobb, 1992, Koricheva et al. 1998).

Such a genotype-by-environment interaction may exist in *D. wrightii* involving water availability. We previously hypothesized that the abundance of water might benefit sticky plants more than velvety plants as the frequency of sticky plants increases with annual rainfall (Hare and Elle 2001). We postulated that sticky plants may have an additional water demand for the production of the water-based glandular exudate. In the presence of herbivores, irrigation increases seed production, but more so for sticky than velvety plants, suggesting that the addition of water allows sticky plants to better compensate for herbivore damage (Elle et al. 1999). Forkner and Hare (2000) showed that irrigation actually caused a reduction in the quantity of glucose esters per unit of leaf area but a general increase in leaf size and leaf protein content. Glucose ester production was uncorrelated with trichome density, suggesting that factors in addition to the total number of trichomes govern glucose ester production (Forkner and Hare 2000). Water appears to be more beneficial to functions related to growth of sticky *D. wrightii* than to defense, and additional water may allow sticky plants to regrow more than velvety plants

following damage by herbivores. If so, then the effect of herbivore damage on seed production of the two plant types may vary with the availability of water in different environments.

Here we investigate in detail the impact of herbivory on seed production of *D. wrightii* to evaluate the potential ecological costs of glandular trichome production. Ecological costs would be highest if equivalent damage by differentially adapted herbivores caused equal reductions in plant fitness. Ecological costs of glandular trichomes would be minimized, however, if the damage caused by *T. notatus* were less debilitating than the damage caused by other insect species attacking velvety plants, as suggested by Elle and Hare (2000). Our approach toward evaluating the potential ecological costs of glandular trichome production was to quantify the impact of feeding by populations of the different herbivores on seed production of the two plant types with or without added water for two years beyond our initial results in 1997 (Elle et al. 1999). We were particularly interested in determining if irrigation differentially affected the abilities of the two plant types to compensate for herbivore damage. Ultimately, our goal is to evaluate the roles of herbivore species that are differentially adapted to glandular trichomes as multiple agents of selection for trichome morphology.

## METHODS

### *The study system*

*Datura wrightii* is a perennial plant species found in sandy or gravelly dry places in the southwestern U.S. and Mexico (Avery et al. 1959, Munz 1973). Aspects of the plant's morphology, reproductive biology, and trichome development and inheritance have been reviewed elsewhere (Elle et al. 1999, van Dam et al. 1999, Elle and Hare 2002). Sticky and velvety plants grow adjacently in populations, often with their branches interdigitated, indicating that microsite partitioning of the available habitat by the two types is unlikely. *Datura wrightii* is attacked by several herbivores, most of which are specialists on the Solanaceae. Each herbivore produces a characteristic type of damage that is easily recognized (see Plate 1 and Table 1). We have observed seasonal defoliation to range between 24% and 58% in natural populations and some plants can be 98% defoliated at certain times of the season (Elle and Hare 2000).

### *Experimental design*

These experiments were carried out as a portion of a long-term project, and the experimental design has been fully described elsewhere (Elle et al. 1999). Only the most relevant points and new procedures will be described in this section.

Seeds were originally collected from five natural populations of *D. wrightii* described in van Dam et al. (1999). These were primarily roadside populations in

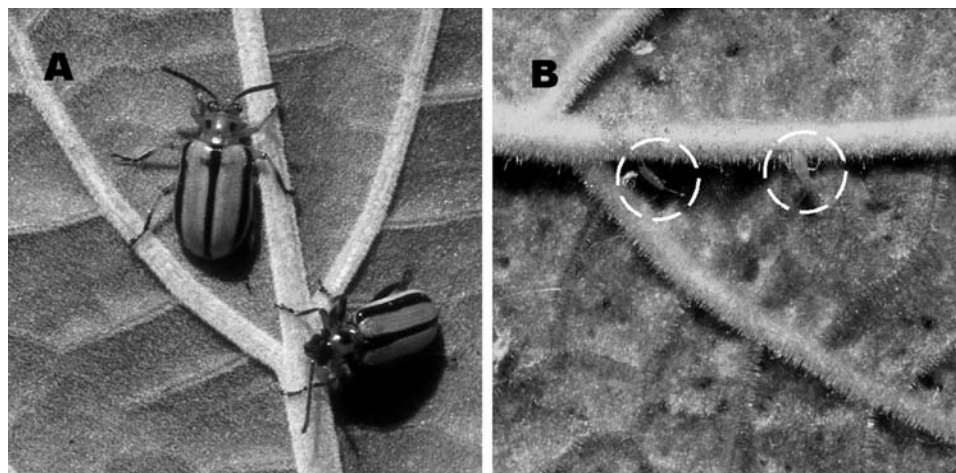


PLATE 1. (A) Adult *Lema trilineata daturaphila* on a *Datura wrightii* leaf with nonglandular trichomes, and (B) nymphs of *Tupiocoris notatus* (circled) and exuviae on a *D. wrightii* leaf with glandular trichomes. Each image shows a  $2 \times 2$  cm section of leaf area, and (B) also shows the characteristic mottling of leaves following extensive injury by *T. notatus*. Photo by J. D. Hare.

dry washes and canyons, in environments ranging from the mountains to the inland valleys of southern California. Experimental families were created by performing crosses both within and between populations for each trichome type, so any effects due to heterosis or inbreeding depression are expected to be similar for the two types (Elle et al. 1999).

At three weeks of age (25 March 1997), seedlings were transplanted to the experimental garden at Agricultural Operations, University of California, Riverside, as described in Elle et al. 1999. Plants were arranged in four replicate blocks with each block assigned to one of two irrigation treatments (twice-weekly irrigation vs. no irrigation) alternately. Each block was a rectangle consisting of five furrows and 30 rows, and the spacing of adult plants was  $\sim 3$  m between plants and between rows, with plants from families randomly assigned to positions within furrows.

All transplants were irrigated twice weekly for the first 6 wk in 1997 to aid establishment, after which the

formal irrigation treatments were imposed for the remainder of 1997, and 1998 and 1999. The designated unirrigated blocks were left dry, while the remaining two blocks continued to be furrow irrigated twice weekly for 8 h each day. Twice-weekly irrigation in each year was imposed until the onset of winter rains on 17 November 1997, from 3 April to 2 November 1998, and from 30 March to 1 November 1999. Unirrigated blocks only received natural rainfall from May 1997 through December 1999.

#### *Plant growth and herbivore damage*

Two estimates of herbivore densities and herbivory were made in 1997, and these data have been presented earlier (Elle et al. 1999). Data on plant size and herbivory were collected weekly from 27 March to 1 December 1998 and 2 March to 30 November 1999 using methods similar to those in Elle and Hare (2000). On each date, a branch with 10–20 leaves was chosen at random to estimate the size of the leaf canopy and the

TABLE 1. Insect herbivores that attack *Datura wrightii*.

Species	Order, Family	Feeding mode
<i>Lema trilineata daturaphila</i> Kogan and Goeden	Coleoptera, Chrysomelidae	removes leaf blade in irregular holes, avoiding major veins
<i>Manduca sexta</i> (Johansson)	Lepidoptera, Sphingidae	removes all or part of entire leaves, including veins
<i>Trichobaris compacta</i> (Casey)	Coleoptera, Curculionidae	small holes, primarily at base of leaf
<i>Epitrix hirtipennis</i> (Melsheimer)	Coleoptera, Chrysomelidae	small scattered pits, does not usually perforate leaf
and <i>E. subcrinita</i> (LeConte)		
<i>Tupiocoris notatus</i> (Distant)	Hemiptera, Miridae	sucking insect, removes cell contents
<i>Empoasca</i> sp.	Homoptera, Cicadellidae	punctures and kills cells, causes necrotic areas to form
Whiteflies, <i>Bemesia argentifolii</i> Bellows and Perring and <i>Trialeurodes</i> sp.	Homoptera, Aleyrodidae	punctures and kills cells, removes cell contents
<i>Liriomyza trifolii</i> (Burgess)	Diptera, Agromyzidae	larvae feed on leaf cells between epidermal layers

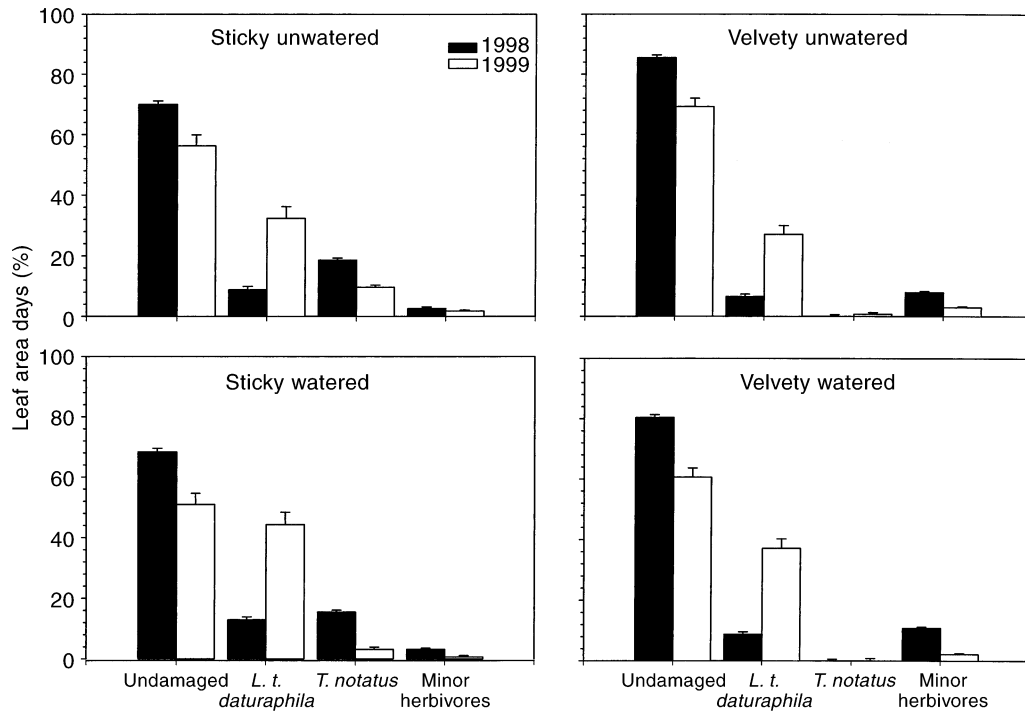


FIG. 1. Mean (+1 SE) percentage of leaf area days undamaged, or damaged by *L. t. daturaphila*, *T. notatus*, or all minor herbivores combined, from unirrigated and irrigated sticky and velvety plants in 1998 and 1999.

damage inflicted by each herbivore species for the entire plant. The number of leaves on the selected branch was recorded, as well as the total number of branches on the plant. The product of these two values estimates the total leaf number on the plant. Total leaf number was converted to total leaf area by multiplying the total number of leaves by a family-specific conversion factor. This factor was developed empirically from the relationship between leaf length and leaf area for plants from all families grown in the field under herbivore-free conditions, then used to estimate leaf area from the mean length of leaves from each family in the herbivore-attacked plot. Leaf area was measured with a Li-Cor model 3000 leaf area meter (Li-Cor, Lincoln, Nebraska, USA). The proportion of variance in leaf area explained by leaf length averaged 0.86 and ranged from 0.64 to 0.98 among families and irrigation treatments ( $n = 12\text{--}20$  leaves per family).

We estimated herbivore-specific damage as the proportion of the leaf removed or damaged in the characteristic way for each herbivore species (Table 1). The total leaf area per plant that was removed or damaged by each herbivore species was estimated for each date as the product of total leaf area and the herbivore-specific proportion removed/damaged from the randomly sampled branch.

We combined the effects of variation in both the size and the persistence of leaf canopies by integrating the weekly leaf area estimates over time, in units of leaf area days. We also calculated season-long cumulative

total removal of leaf area days by individual herbivore species by integrating their weekly estimates of damage over time. We expect the variation in leaf area days to be a function not only of current defoliation, but also reduction in future growth caused by current defoliation, as well as any inherent variation in plant growth rates irrespective of defoliation.

#### Plant reproductive output

We estimated total viable seed production per plant per year as described previously (Elle et al. 1999). Total fruit production for each plant was easily assessed at the end of the growing season because ripe seed capsules remain on the plant after releasing their seeds. Seed production per capsule was determined by enclosing developing capsules in ventilated plastic bags to collect the seeds released as capsules opened. Up to three randomly chosen capsules were bagged per plant on 2 August and 15 October 1998. Not all plants had enough seed capsules to bag on 2 August, however. Because of the high levels of herbivore damage in 1999, capsules were produced relatively late in the growing season and were bagged starting on 11 October.

After bagged capsules opened, they were brought to the laboratory and seed number was estimated by weighing 10 seeds per capsule and then dividing the total seed weight per capsule by mean seed weight (correlation between estimated and actual seed number per capsule = 0.998,  $P < 0.0001$ ,  $N = 12$ ). In all

analyses, seed number per capsule and the weight of individual seeds for each plant represent means determined from 3–6 seed capsules per individual.

We assessed seed viability within 3 wk of the collection of the last capsule. Fifty seeds from one randomly chosen capsule per individual were stratified on wet filter paper in Petri plates at 10°C for 10 d, and then placed in a germination chamber with alternating light and temperature regimes (16 h light : 8 h dark and 30°/20° light and temperature cycles). Germination was scored twice a week for 3 wk as emergence of the radicle. Germination rate is the mean proportion of seeds that germinated for an individual during two germination trials. The total viable seeds for each plant were calculated as the product of mean seeds per capsule, total capsule number, and the mean proportion of seeds that germinated. For eight plants in 1999, we were unable to collect capsules to obtain mean seed number per capsule, although they produced a few ripe capsules later in the season. For these plants we used the mean number of seeds per capsule and germination rates calculated from the other plants in the same family and irrigation treatment.

#### Statistical analyses

Repeated-measures analyses of variance (ANOVA) were used to analyze the variation in herbivore damage as a function of trichome type, irrigation treatment, and their interaction for those herbivore species that damaged plants in both 1998 and 1999. Univariate ANOVAs were used similarly for damage by whiteflies and leafminers, which were present only in 1999. We used the PROC MIXED procedure of SAS (SAS Institute 1996) for all repeated measures and univariate ANOVAs because our design contained both fixed and random factors. PROC MIXED is more robust for the analysis of mixed models with uneven sample sizes than is PROC GLM (SAS Institute 1996). Family within type was specified to be a random factor, whereas type, irrigation, and their interaction were fixed. The Satterthwaite method was used to estimate degrees of freedom. Block effects were not significant in an initial analysis and were deleted from the final model presented here.

The dependent variable was the proportion of leaf area days removed by each herbivore species because absolute leaf canopy losses were confounded by variation in plant size. Larger plants had a larger quantity of leaf area remaining, but larger plants also lost a larger absolute quantity of leaf area to herbivores. The relative losses of leaf area days per plant to each herbivore species accurately measures the relative susceptibility of that plant to different herbivores when plants differ in size. Data were angular transformed to better conform to the assumptions of ANOVA. We also used repeated measures ANOVA (PROC MIXED) to analyze the variation in absolute undamaged leaf area and total viable seed production after applying the cube

root transformation to ensure normality, independence of mean and variance, and homogeneity of variances.

We used multiple regression (PROC REG of SAS; SAS Institute 1996) to determine the relationship between herbivore damage and viable seed production for each plant type separately pooled over irrigation treatments. Regressors were undamaged leaf area days and absolute herbivore-specific leaf area losses, and the response variable was total viable seed production. We applied the cube-root transformation to all variables to ensure normality, independence of mean and variance, and homogeneity of variances. Regression analyses were carried out after combining the relatively minor herbivores, *Trichobaris compacta* (a seed weevil), *Epitrix* spp. (flea beetles), *Empoasca* sp. (leafhoppers), *M. sexta*, the three species of whiteflies, and *Liriomyza trifolii* (leaf miners), into a single group to simplify the analysis.

## RESULTS

### Plant growth and herbivore damage

Only one significant type-by-water interaction occurred overall (Table 2A), and this interaction probably is an artifact because the herbivore, *T. notatus*, is limited to only one plant type; *T. notatus* differentially damaged irrigated and unirrigated sticky plants, but it was virtually excluded from velvety plants under both irrigation treatments (Fig. 1). Season-long damage by *T. notatus* was significantly higher on sticky plants than velvety plants ( $P < 0.0001$ , 17.1% vs. 0.0% in 1998 and 6.4% vs. 0.4% in 1999 over irrigation treatments pooled; Table 2A, Fig. 1). Losses from *Lema trilineata daturaphila* did not differ between types in either year ( $P = 0.28$ , 10.9% on sticky plants vs. 7.6% on velvety plants in 1998 and 38.3% vs. 32.1% in 1999 over irrigation treatments pooled; Table 2A, Fig. 1). Leaf losses from three of the remaining six herbivores (*T. compacta*, *Epitrix* spp., and *Empoasca* sp.) were significantly higher on velvety plants than sticky plants (all  $P \leq 0.05$ ; Table 2A), whereas damage by *L. trifolii* was significantly higher on sticky plants than velvety plants ( $P = 0.027$ , 0.55 vs. 0.3%; Table 2A). Although all whitefly damage was confined to velvety plants, the overall damage level (0.2%) was too low to produce a statistically significant difference due to type (Table 2A). As a group, the herbivores, *T. compacta*, *Epitrix* spp., *Empoasca* sp., *L. trifolii*, *M. sexta*, and whiteflies, (hereafter the “minor” herbivores) imposed smaller losses than either *T. notatus* or *L. t. daturaphila* did individually. The group of minor herbivores consistently imposed significantly more damage on velvety plants than sticky plants ( $P < 0.0001$ , 9.4% on velvety plants vs. 2.9% on sticky plants pooled over irrigation treatments in 1998 and 2.6% vs. 1.7% in 1999; Table 2A, Fig. 1).

Losses from *L. t. daturaphila* were significantly greater on irrigated plants than unirrigated plants over

TABLE 2. Analyses of variance of proportional leaf area losses for each herbivore, proportional and absolute undamaged leaf area, and total viable seed production.

A) Between-subjects effects												
Source	<i>T. notatus</i> losses		<i>L. t. daturaphila</i> losses		<i>T. compacta</i> losses		<i>Epitrix</i> spp. losses		<i>Empoasca</i> sp. losses		<i>M. sexta</i> losses	
	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
Trichome type	1, 8.17	<b>210.08</b>	1, 9.26	1.31	1, 13.7	<b>4.67</b>	1, 6.99	<b>13.45</b>	1, 8.19	<b>119.36</b>	1, 10.1	3.57
Irrigation	1, 52.8	<b>21.58</b>	1, 69.5	<b>13.57</b>	1, 81.5	0.01	1, 82.7	<b>7.48</b>	1, 73.4	3.08	1, 70.5	1.13
Type × irrigation	1, 52.8	<b>10.39</b>	1, 69.5	0.10	1, 81.5	2.52	1, 82.7	0.52	1, 73.4	3.08	1, 70.5	0.61
B) Within-subjects effects												
Source	<i>T. notatus</i> losses		<i>L. t. daturaphila</i> losses		<i>T. compacta</i> losses		<i>Epitrix</i> sp. losses		<i>Empoasca</i> sp. losses		<i>M. sexta</i> losses	
	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
Year	1, 67.1	<b>66.09</b>	1, 76.4	<b>195.27</b>	1, 81.5	<b>8.30</b>	1, 84.2	<b>48.15</b>	1, 80.8	<b>157.77</b>	1, 72.3	<b>285.85</b>
Year × type	1, 67.1	<b>109.3</b>	1, 76.4	0.07	1, 81.5	1.07	1, 84.2	0.87	1, 80.8	<b>157.77</b>	1, 72.3	<b>9.84</b>
Year × irrigation	1, 67.5	5.36	1, 76.8	0.66	1, 81.6	0.00	1, 84.3	<b>4.94</b>	1, 80.6	3.20	1, 72.2	2.61
Year × type × irrigation	1, 67.5	1.40	1, 76.8	0.07	1, 81.6	1.14	1, 84.3	0.02	1, 80.6	3.20	1, 72.2	0.10

Notes: The degrees of freedom and *F* statistics are shown for each analysis. Repeated-measures analyses were used for all herbivores that were present in both 1998 and 1999 and for total viable seed production, while univariate analyses were used for whitefly and *L. trifolii* damage (present only in 1999). "Minor herbivores" includes damage by all species except *T. notatus* and *L. t. daturaphila*. *F* statistics for significant effects ( $P \leq 0.05$ ) are shown in bold.

both types pooled ( $P < 0.0001$ , 10.9% vs. 7.7% in 1998 and 40.7% vs. 29.7% in 1999; Table 2A, Fig. 1). Losses from *T. notatus*, however, were significantly greater on unirrigated than irrigated plants ( $P < 0.0001$ , 9.3% vs. 7.8% in 1998 and 5.1% vs. 1.6% in 1999; Table 2A, Fig. 1). Flea beetle losses also were greater on unirrigated than irrigated plants ( $P = 0.008$ ; Table 2A), especially in 1999 (0.5% vs. 0.2%).

Herbivore damage differed significantly between years (all  $P \leq 0.005$ ; Table 2B). Losses were greater in 1998 than in 1999 for *T. notatus* and the minor herbivores, but the opposite was true for *L. t. daturaphila* (Fig. 1). Relatively few year × type interactions were statistically significant (Table 2B); those that were reflect quantitative differences in the magnitude of the type effect between years and not a change in direction.

We calculated Pearson product-moment correlation coefficients to determine if there was any consistent pattern of correlation of damage by different herbivore species. Of the 40 possible correlation coefficients between damage by different pairs of herbivores that attacked the same trichome type, 26 did not differ significantly from zero, and none were significantly negative in both years (data not shown). Only the damage by *Epitrix* spp. and *T. compacta* were significantly and positively correlated in both years of the study.

Sticky plants suffered greater overall proportional damage than velvety plants ( $P < 0.0001$ , 30.9% vs. 17.0% in 1998 over irrigation treatments pooled and 46.5% vs. 35.1% in 1999; Table 2A, Fig. 1), and irrigated plants were significantly more damaged than unirrigated plants ( $P = 0.007$ , 25.7% vs. 22.2% total damage in 1998 and 44.2% vs. 37.3% in 1999; Table

2A, Fig. 1). Although sticky plants suffered greater proportional damage than velvety plants, the absolute size and duration of undamaged leaf canopies did not differ significantly between plant types, nor was there a type by irrigation interaction (Table 2A, Fig. 2). This probably reflected the larger size of sticky plants such that the greater proportional damage was offset by greater total leaf production.

Irrigation was associated with a reduced absolute quantity of leaf area remaining ( $P = 0.05$ ; Table 2A, Fig. 2) because of the greater leaf area removed by *L. t. daturaphila* on irrigated plants. The absolute quantity of remaining leaf area also differed significantly between years and was smaller in 1999 than in 1998 ( $P < 0.0001$ ; Table 2B, Fig. 2).

#### Plant reproductive output and relationships with herbivore damage

Total viable seed production differed significantly between irrigation treatments, and, unexpectedly, was lower for irrigated than unirrigated plants ( $P < 0.0001$ ; Table 2A, Fig. 2). Mean seed production differed significantly between years ( $P < 0.0001$ ; Table 2A) and ranged from ~10 000 to 40 000 viable seeds per plant in 1998 but, except for unirrigated velvety plants, did not exceed 1000 seeds in 1999 (Fig. 2). The irrigation effect also differed significantly between years (year × irrigation interaction,  $P = 0.0015$ ; Table 2B) and was greater in 1998 than in 1999 (Fig. 2).

Total viable seed production increased with increasing leaf area days of both plant types in both years (Table 3). Leaf area days also was the single strongest predictor of total viable seed production, accounting for 37–74% of the variation in total viable seed pro-

TABLE 2. Extended.

Whitefly losses		<i>L. trifolii</i> losses		Minor herbivores pooled		Proportional undamaged leaf area		Absolute undamaged leaf area		Viable seed production	
df	F	df	F	df	F	df	F	df	F	df	F
1, 7.65	3.86	1, 81	<b>5.07</b>	1, 8.0	<b>65.76</b>	1, 15.0	<b>25.89</b>	1, 7.14	0.08	1, 9.86	1.06
1, 79.0	1.28	1, 81	2.92	1, 77.3	2.60	1, 72.5	<b>7.67</b>	1, 78.7	<b>3.96</b>	1, 86.4	<b>24.07</b>
1, 79.0	2.06	1, 81	0.65	1, 77.3	0.61	1, 72.5	1.07	1, 78.7	0.12	1, 86.4	0.73

Minor herbivorous pooled		Proportional undamaged leaf area		Absolute undamaged leaf area		Viable seed production	
df	F	df	F	df	F	df	F
1, 83.3	<b>155.65</b>	1, 81.7	<b>73.39</b>	1, 79.0	<b>409.71</b>	1, 93.0	<b>292.44</b>
1, 83.3	<b>68.44</b>	1, 81.7	0.90	1, 79.0	0.56	1, 93.0	0.04
1, 83.2	<b>9.37</b>	1, 81.9	0.22	1, 79.2	3.21	1, 92.7	<b>10.66</b>
1, 83.2	1.91	1, 81.9	0.07	1, 79.2	0.09	1, 92.7	2.06

duction (Table 3). The slope of the relationship between viable seed production and plant canopy size was greater for velvety plants than for sticky plants in 1999 ( $F = 6.27$ ,  $df = 1, 81$ ,  $P = 0.014$ ) but not in 1998 ( $F = 1.59$ ,  $df = 1, 98$ ,  $P = 0.2$ ).

Perhaps surprisingly, feeding by *T. notatus* did not significantly reduce total viable seed production of sticky plants. In 1998, damage by *T. notatus* and undamaged remaining leaf area were significantly and positively correlated ( $r = 0.94$ ,  $df = 43$ ,  $P < 0.0001$ ). Larger plants had larger absolute quantities of undamaged leaf area, but larger plants also had larger quantities of absolute leaf area damaged by *T. notatus*. The

high positive correlation between undamaged leaf area and *T. notatus* damage resulted in high collinearity of the data such that the effect of remaining leaf area and *T. notatus* damage on total viable seed production could not be separated. Total viable seed production increased with increasing quantities of either remaining leaf area or *T. notatus* feeding damage. To eliminate the problem of collinearity, we performed our regression analyses after removing one of the two highly correlated variables. Because it is more reasonable biologically to expect seed production to increase with increasing size and duration of the leaf canopy than to expect seed production to increase with *T. notatus* feeding injury,

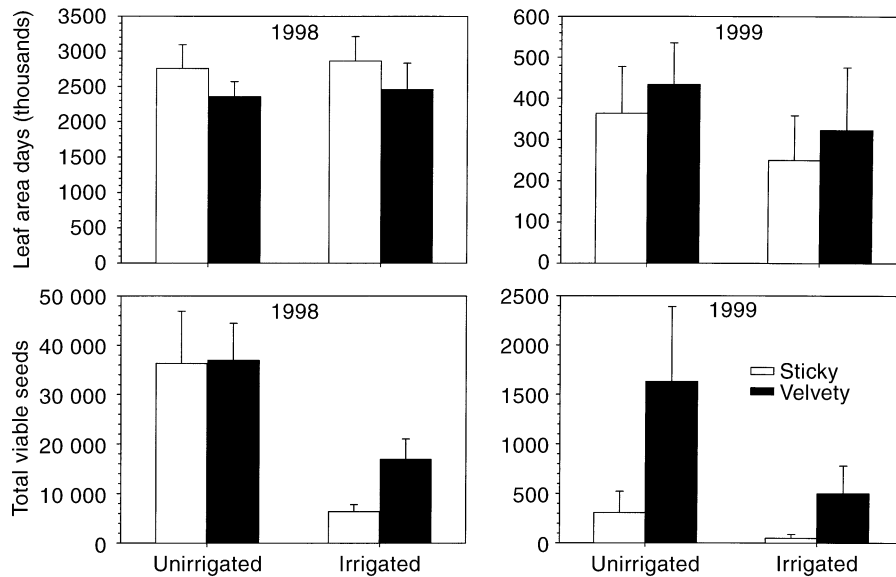


FIG. 2. Mean (+1 SE) remaining leaf area days (cm<sup>2</sup>-d) and total viable seed production of unirrigated and irrigated sticky and velvety plants in 1998 and 1999.



TABLE 3. Results of multiple regression analyses for variation in total viable seeds as a function of variation in undamaged plant leaf area integrated over time and leaf area losses from individual herbivores in 1998 and 1999.

Variable	1998				1999			
	Sticky plants		Velvety plants		Sticky plants		Velvety plants	
	Slope	$r^2$	Slope	$r^2$	Slope	$r^2$	Slope	$r^2$
Undamaged leaf area	0.271***	0.37	0.479****	0.53	0.159**	0.65	0.322****	0.74
Losses due to <i>L. t. daturaphila</i>	-0.194 NS		0.049 NS		-0.075 NS		-0.242****	0.07
Losses due to <i>T. notatus</i>	NA		0.073 NS		0.027 NS		0.107 NS	
Losses due to all other herbivores	0.067 NS		-0.464*	0.04	-0.099 NS		-0.147 NS	

Note: NA = test not applicable

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ ; NS, not significant.

we deleted *T. notatus* feeding injury from the regression analyses for sticky plants in 1998. In 1999, the collinearity problem did not occur, and *T. notatus* damage was not significantly related to total viable seed production of sticky plants (Table 3).

Fitness declined significantly due to damage by *L. t. daturaphila* only in velvety plants in 1999, and fitness also declined significantly with increasing combined damage by the minor herbivores on velvety plants in 1998 (Table 3). These leaf-area losses only accounted for 4% and 7% of the variation in total viable seed production (Table 3).

#### DISCUSSION

Glandular trichomes provide *D. wrightii* varying levels of defense against different members of the local herbivore community. These trichomes either eliminated or significantly reduced damage by *Empoasca* sp., whiteflies, *Epirix* spp., and *T. compacta*, but they did not reduce damage by the most abundant chewing insect, *L. t. daturaphila*. Plants with glandular trichomes were consistently more susceptible to damage from *T. notatus*. Because the latter two herbivores caused most of the damage by far, plants with glandular trichomes suffered greater damage overall than plants with nonglandular trichomes. The relative damage of all herbivores varied between years, however, and relative losses from *L. t. daturaphila* increased substantially from 1998 to 1999.

Based upon differences in overall damage levels, we judged the sticky plants to be more resistant than velvety plants in 1997 (Elle et al. 1999). Using the same criterion, then the apparent resistance changed in the next two years as *T. notatus* and *L. t. daturaphila* increased the overall damage of sticky plants from 1997 through 1999. The differences in herbivore densities and consequent losses in 1999 compared to 1998 could have been the result of several factors, including the gradual growth of the *L. t. daturaphila* population over time, year-to-year variation in overwintering mortality within and among species, and other factors beyond the scope of the present study.

The utilization of the two plant types by different herbivore species suggested that an ecological trade-off due to glandular trichome production might exist

(van Dam and Hare 1998b). Resistance to whiteflies and leafhoppers, and reduced susceptibility to flea beetles and weevils, occurs at the expense of increased susceptibility to *T. notatus*. Indeed, because the overall leaf area damage on sticky plants was substantially greater than the damage to velvety plants (Fig. 1), it would seem that the ecological costs are unacceptably high. However, analyses of data from natural populations suggested that damage by herbivores that remove leaf area reduced seed production more than damage by herbivores that did not cause leaf area removal (Elle and Hare 2000). Results described here are in general agreement with that interpretation. First, although total viable seed production increased with increasing size and persistence of leaf canopies, it was not significantly reduced by *T. notatus* damage. Preliminary data show that leaves damaged by *T. notatus* retain at least 40% of their photosynthetic activity, which suggests that feeding damage by *T. notatus* may be less debilitating than removal of an equivalent level of leaf area by chewing insects (J. D. Hare, unpublished data). The apparent ecological cost of trichome production is less than might be predicted from overall damage levels alone.

The observations from natural populations also suggested that plant seed production was strongly tied to the amount and persistence of leaf canopy area rather than to the losses imposed by any specific herbivore species, and that relationship was stronger for velvety plants than for sticky plants (Elle and Hare 2000). These conclusions also are generally supported by the current study. The primary factor influencing total viable seed production was the size and persistence of the undamaged leaf canopy in all analyses, although seed production was significantly but weakly reduced by increasing damage from minor herbivores in 1998 and *L. t. daturaphila* in 1999.

We had initially hypothesized that additional water would allow plants to better recover from herbivore damage (Elle et al. 1999). In 1997, irrigation benefited seed production of both sticky and velvety plants, both when exposed to and protected from herbivores (Elle et al. 1999). In the next two years of the study, total viable seed production was higher on unirrigated plants than irrigated plants. The relative increase in seed pro-

duction of the unirrigated plants is probably the result of their relatively lower leaf area losses from most herbivores except *T. notatus* (Fig. 1). Responses of other plant–insect associations to drought stress are known to vary with the form of the stress (e.g., short-term vs. chronic), the feeding guild (e.g., chewing vs. sucking insects), and plant type (e.g., woody vs. herbaceous, wild vs. cultivated; Waring and Cobb 1992, Koricheva et al. 1998). The reduced feeding damage on unirrigated *D. wrightii* is consistent with the general trend that herbivores of herbaceous plants are commonly negatively affected by drought stress (Waring and Cobb 1992). We attribute the greater feeding damage on irrigated plants to the fact that they were more succulent, more nutritionally rich, and less defended (Forkner and Hare 2000). The greater feeding damage by *T. notatus* on unirrigated plants is an exception to the general trend but is also likely the result of the greater quantities of acyl glucose esters produced by unirrigated plants. These esters, which confer resistance to some herbivores, stimulate feeding and oviposition by *T. notatus* (van Dam and Hare 1998b). Because the irrigated plants were so much more heavily damaged than unirrigated plants, we were unable to determine if irrigated plants better compensate for herbivore damage than unirrigated plants.

Variation in the herbivore community attacking genetically variable plants provides a major challenge to our ability to predict how plants may respond evolutionarily to attack by herbivores. For example, *Piper arieianum* is attacked by 95 different herbivore species in Costa Rica but different plant genotypes differed in their resistance to individual herbivore species (Marquis 1990). Moreover, resistance to one herbivore species did not confer resistance to the others. Similarly, Fritz and Price (1988) showed that the herbivore community attacking willows varied with plant genotype because of differences in responses among four herbivorous sawfly species to plant variation. Because of the variation in responses of different herbivores to particular genetically determined plant traits, the evolutionary trajectories of different plant genotypes may diverge as a result of variation among herbivore communities attacking them (Fritz and Price 1988). The trajectory of trichome evolution in *D. wrightii* also may be difficult to predict, not only because of the spatial variation in the structure of herbivore communities (Elle and Hare 2000), but also due to the temporal variation in the structure of such a community at one geographic site (shown here).

Compounding these relationships is the previously documented high genetic cost of glandular trichome production manifest by a nearly 45% reduction in the total viable seed production of sticky plants in the absence of herbivores. On balance, residual costs remained for sticky plants in 1997 (Elle et al. 1999), but the seed production of sticky plants was equal to that of velvety plants in 1998 and 1999 (Table 2). One may

argue that the benefits of glandular trichomes eventually equaled their costs in these latter two years, but such equivalence required a substantial level of plant damage, and nearly a 57% reduction in seed production in unirrigated plants and more than a 90% reduction in seed production in irrigated plants. Even at these high herbivory levels, the seed production of sticky plants never significantly exceeded that of velvety plants, so the trait seems to be deleterious at low levels of herbivory and no more than neutral at high levels. Thus, the question of why the sticky type persists in natural populations remains unanswered (Elle et al. 1999, Elle and Hare 2000).

Although the impression may be that costs of resistance have been rarely demonstrated, recent reviews of the topic showed that significant costs of resistance are commonly found (Bergelson and Purrington 1996, Purrington 2000, Koricheva 2002). Rather than continue to search for costs, we suggest that the mechanisms underlying such costs, the impact of environmental variation on the expression of such costs, and the implications of the existence of those costs in the maintenance of resistance polymorphisms represent more fruitful avenues of research. Trichome morphology plays a direct role in shaping the herbivore community that feeds on *D. wrightii* phenotypes, however, the maintenance of the trichome dimorphism may be due as much to the expression of the correlated differences in plant growth and size associated with glandular trichome production in different environments (Elle et al. 1999) as to the herbivore resistance that those trichomes provide.

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