Cyanogenesis, herbivory and plant defense in *Turnera ulmifolia* on Jamaica

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Abstract: Field surveys of eight populations of *Turnera ulmifolia* L., a Jamaican weed exhibiting quantitative genetic variation for cyanogenesis, were undertaken to assess the effectiveness of cyanogenesis as a plant defense. Populations known to be characteristically acyanogenic, cyanogenic or to exhibit within-population variation were surveyed for cyanogenesis, plant size, and the presence and identity of invertebrate plant visitors. A developmental series of 10 leaves from a shoot of each surveyed plant was analyzed, using image analysis techniques, for the type and extent of damage present. We also surveyed two additional plant populations for the presence of plants with eggs or larvae of *Euptoieta hegesia* Cramer, a Nymphalid butterfly that is potentially the most damaging herbivore of *T. ulmifolia*, and a paired comparison analysis of cyanogenesis in plants with the herbivore versus plants without the herbivore was conducted. We found that *T. ulmifolia* are attacked by a reasonably diverse insect fauna, but a relatively small suite of specialist herbivores that are seemingly undeterred by cyanogenesis.

Keywords: natural selection, variation, cost, herbivore damage, chemical defense, colonization, specialization, *Euptoieta hegesia*.

Résumé: Nous avons étudié huit populations de *Turnera ulmifolia*, une mauvaise herbe de la Jamaïque démontrant une variation quantitative génétique de la production de cyanure, afin d’évaluer l’efficacité du cyanure comme mécanisme de défense chez les plantes. Nous avons examiné la taille des plantes dans des populations connues, ne produisant aucun cyanure, étant cyanurique ou démontrant une variation dans cette production dans une même population aussi bien que la présence et l’identification des invertébrés visitant ces plantes. Une série développements de dix feuilles de pousse de chaque plante ont été examinées à l’aide des techniques d’analyse d’images pour évaluer le type et le niveau des dommages. Deux autres populations de plantes ont été étudiées afin de déceler la présence d’œufs et de larves de *Euptoieta hegesia* Cramer (Nymphalidae), un papillon qui est un des herbivores éventuellement les plus destructifs de *T. ulmifolia*. Une analyse du cyanure des plantes avec ou sans herbivores a aussi été effectuée. Nous avons trouvé que *T. ulmifolia* est attaquée par un assez grand nombre d’insectes, mais que seulement un petit nombre d’herbivores spécialisés, causant le plus de dommages, semblent n’être d’aucune façon affectés par le cyanure. Le cyanure semble jouer un rôle dans la quantité, le type et la présence des herbivores généralisés dans une même population et parmi les populations de plantes. La perte de tissu végétal dans une même population et entre les populations de plantes varie, mais la proportion de cette perte demeure faible, variant en moyenne de 1 % à 9 %; cette dernière ne semble pas être corrélée de façon constante avec le cyanure. Une analyse de covariance a par contre démontré que la production de cyanure a un effet significatif sur la hauteur et le nombre de pousses que chaque plante produit. Ces résultats suggèrent que la production de cyanure peut fournir une certaine protection contre l’herbivorie par les herbivores généralistes.

Mots-clés : sélection naturelle, variation, coût, dommages causés par les herbivores, défense chimique, colonisation, spécialisation, *Euptoieta hegesia*.

Introduction

Plants use a variety of methods to reduce the extent of herbivore damage including the elaboration of chemical defenses which act as deterrents to colonization or herbivory by generalist herbivores (Ellis, Keymer & Jones, 1977; Woodhead & Bernays, 1978; Bernays & Chapman, 1987). However, chemical defenses may also act as oviposition or gustatory acceptability cues, or be co-opted for other purposes (e.g., the defense of the herbivore against its own predators or parasites). Specialist herbivores are often capable of recognizing, detoxifying, or sequestering the chemical, or are otherwise immune to the defense (Ehrlich & Raven, 1964; Scriber, 1978; Brattsten et al., 1983; Compton & Jones, 1985; Nahrstedt, 1985; 1988; but see Dritschilo et al., 1979). Plant species exhibiting variation for defensive traits are especially useful because they allow a direct comparison of the efficacy of such defenses against any or all of the suite of herbivores that may prey upon the plants (Jones, 1971; 1988; Burgess & Ennos, 1987).

Cyanogenesis, the ability of plants to liberate hydrogen cyanide (HCN) when damaged, is known to occur in more than 3000 species including ferns, gymnosperms, and angiosperms (Poulton, 1990). In the vast majority of these species, cyanogenesis is due to the hydrolysis of cyanogenic glycosides by β-glycosidases. Due to the constitutive nature of these compounds it has been suggested that cyanogenesis does not provide overt cues that allow insects or other
herbivores to locate and recognize plants over distances because it is necessary for herbivores to damage plant tissues to perceive and act upon the defense (Jones, 1988). Cyanogenesis is a passive defense, rarely autotoxic, yet acts extremely quickly when tissue disruption occurs (Jones, 1988; Seigler, 1991).

*Turnera ulmifolia* L. (Turneraceae), a neotropical weed, is known to exhibit quantitative genetic variation for cyanogenesis within and between populations on Jamaica (Schappert & Shore, 1995). Damage to plant tissues liberates cyclopentenoid cyanogenic glycosides, which, when they come in contact with β-glycosidases, are hydrolyzed to release HCN and ketones (Tobler & Conn, 1985; Seigler, 1991). Both of these end products may be toxic and/or act as deterrents to herbivory (Compton & Jones, 1985; Jones, 1988; Nahrstedt, 1988; Spencer, 1988; Seigler, 1991; but see Scriber, 1978 and Woodhead & Bernays, 1978). All *Turnera spp.* investigated by Shore & Obrist (1992) appeared to contain the hydrolyzing β-glycosidases whether the species were cyanogenic or not. Variation in the rate of release and the total amount of cyanide liberated in *T. ulmifolia* appears to depend upon the amount of cyanogenic glycoside in tissues (Schappert & Shore, 1995).

Our studies of cyanogenesis have focused on the *T. ulmifolia-E. hegesia* hostplant-herbivore system. Euptoieta hegesia Cramer (Lepidoptera: Nymphalidae) uses *T. ulmifolia* as its primary hostplant, and cyanogenic *Passiflora foetida* L. and *P. suberosa* L. to a lesser extent, on the island of Jamaica (Schappert & Shore, 1998). *Euptoieta hegesia* is potentially the most damaging (in terms of tissue loss per unit time) of the herbivores commonly found on *T. ulmifolia* on Jamaica. We are examining variation in the ability of this plant to liberate hydrogen cyanide, and the interaction of this variation with *E. hegesia*, to explore the potential coevolutionary interplay between these species.

In this paper we examine the potential of cyanogenesis to operate as a plant defense in *T. ulmifolia* and we search for possible selective agents. We (i) document the type and presence of invertebrate herbivores and other visitors found on *T. ulmifolia* plants on Jamaica, (ii) compare cyanogenesis levels of plants that are used versus those that are not used by the specialist herbivore *E. hegesia*, (iii) assess the role that cyanogenesis plays in the presence and types of invertebrates found on the plants, and on the types and amounts of tissue damage they inflict, (iv) examine plant size (height and number of shoots) to assess the possible role of plant apparency and potential correlations of size and cyanogenesis in influencing herbivore loads, and (v) assess whether cyanogenesis in *T. ulmifolia* reduces the extent of herbivory.

### Material and methods

*Turnera ulmifolia* L. is a shrub common to roadsides, coastal scrub, and marginal habitats throughout the Neotropics and islands of the Caribbean (Barrett, 1978; Barrett & Shore, 1987). It is a weedy perennial with discrete, generally small, and widely separated populations, with potentially little gene flow among populations, possibly due to limited seed dispersal distances by ants and/or restricted flights of pollinators (Barrett, 1978; Baker & Shore, 1995; Belaoussoff & Shore, 1995). *T. ulmifolia* exhibits a wide range of morphological and reproductive variation on Jamaica and usually highly inbreeding (duQuesnay, 1971; Barrett & Shore, 1987; Baker & Shore, 1995, Belaoussoff & Shore, 1995). Flowers of *T. ulmifolia* are self-compatible, short-lived (open for four to eight hours in the morning) and are borne singly on the petiole of a subtending leaf directly above a pair of extrafloral nectaries (Elías, Rozich & Newcombe, 1975; Belaoussoff & Shore, 1995). Shore & Obrist (1992) documented the presence of variation in cyanogenesis across a number of species and taxonomic varieties of *Turnera*, and we have subsequently documented the existence of a wide range of cyanogenesis among populations of *T. ulmifolia* on Jamaica (Schappert & Shore, 1995).

Cyanogenesis was measured using the quantitative method of Lambert, Ramsamy & Paukstelis (1975), as detailed in Brinker & Seigler (1989), but modified so that HCN extractions were completed in the field, frozen, then assayed in our laboratory in Toronto, Canada. Briefly, an entire leaf subtending a flower or the largest flower bud (to control for developmental variation), was crushed in a 1.5-mL microcentrifuge tube and the tube was placed open into a 20 mL scintillation vial containing a trap of 0.5 mL of 1M NaOH. The vials were sealed and left to incubate for 24 hours at ambient temperatures (25-30°C). The NaOH was then transferred from the vial to a fresh 1.5-mL microcentrifuge tube, which was sealed and frozen at approximately -20°C. We had previously determined that there is no significant loss of HCN from tubes for up to 10 days. After transporting the trap samples back to our laboratory they were frozen at -80°C until the amount of cyanide could be quantified spectrophotometrically (Brinker & Seigler, 1989). We prepared a standard curve following the methods of Brinker & Seigler (1989). The microcentrifuge tubes containing the original crushed leaf tissue were left open and the tissue was allowed to dry at ambient temperatures for three days before the tubes were sealed. Upon our return to the laboratory, the tissue samples were dried to constant weight in a drying oven at 65°C so that we could determine the μg HCN liberated per gram dry weight of leaf tissue. Cyanogenesis is a quantitative trait in this species and for presentation purposes we have ranked the plants over all populations and divided them into quartiles (e.g., Figure 1 and see Schappert & Shore, 1995).

### Field surveys of plant populations

In June of 1995, we surveyed eight Jamaican populations of *T. ulmifolia* for cyanogenesis, plant size, the presence and type of invertebrates, and tissue damage. Populations were chosen to provide a range of variation in cyanogenesis based upon our previous work (Schappert & Shore, 1995) and to provide good geographic coverage of the island (see Table I, Figure 1 for locations, mean cyanogenesis levels, and profiles). Two additional populations, KN and MB (see Table I, Figure 1 for location), were surveyed for plants with eggs and/or larvae of *E. hegesia*. We recorded the number of eggs and/or larvae present on each plant, paired each herbivore-containing plant with the nearest neighbor plant that did not have eggs or larvae present, and tested both plants for cyanogenesis.

Surveys of the eight plant populations, 185 plants in total, were conducted using the following protocol. A
A preliminary survey of each population was made to provide an estimate of the numbers of plants present. Most populations were large (> 75 plants) so we selected plants at random. For one small population (IT) we surveyed every plant. We restricted our sampling to plants that had visible buds, flowers or fruits (i.e., mature plants). Each plant sampled was first examined for the presence and type of invertebrate visitors or, in the case of fruit borers, stem borers and leaf miners, the types of damage present on the plant. Plant visitors were identified and later collated for subsequent analysis into the categories of herbivores, non-herbivores, and predators. The size of each plant was recorded by measuring the length of the longest shoot and counting the total number of shoots. A single flowering shoot, or a shoot with a recent flower or large bud, approximately 15-20 cm in length was removed from each surveyed plant, placed in a plastic bag, and kept cool until they could be processed later that day. If there were fewer than a dozen leaves on the shoot, or if the number of leaves was more than 100, the shoot was not collected.

The locations of populations Kenilworth (KN) and Mammee Bay (MB), which were surveyed to examine the relationship between cyanogenesis and host-plant use by E. hegesia, are denoted by their two-digit ID codes.

Table I. Population identification, survey sample sizes, population locations, elevation, mean annual precipitation, and cyanogenesis levels of T. ulmifolia populations surveyed

<table>
<thead>
<tr>
<th>Population</th>
<th>ID</th>
<th>N</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Elevation (m)</th>
<th>Mean annual precipitation (mm)</th>
<th>µg HCN/g dry mass mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falmouth</td>
<td>FM</td>
<td>25</td>
<td>77° 40' w</td>
<td>18° 29' N</td>
<td>8</td>
<td>894</td>
<td>5.4 (1.3)^a</td>
</tr>
<tr>
<td>Oracabessa</td>
<td>OB</td>
<td>25</td>
<td>76° 56' w</td>
<td>18° 24' N</td>
<td>8</td>
<td>1968</td>
<td>6.4 (5.4)^a</td>
</tr>
<tr>
<td>Kinloss</td>
<td>KL</td>
<td>25</td>
<td>77° 33' w</td>
<td>18° 23' N</td>
<td>343</td>
<td>1527</td>
<td>20.5 (25.6)^b</td>
</tr>
<tr>
<td>Sandy Bay</td>
<td>SB</td>
<td>25</td>
<td>78° 05' w</td>
<td>18° 27' N</td>
<td>23</td>
<td>1449</td>
<td>52.9 (66.7)^c</td>
</tr>
<tr>
<td>Quickstep</td>
<td>QS</td>
<td>24</td>
<td>77° 43' w</td>
<td>18° 15' N</td>
<td>419</td>
<td>2677</td>
<td>183.3 (124.6)^d</td>
</tr>
<tr>
<td>Portland Point</td>
<td>PP</td>
<td>22</td>
<td>77° 10' w</td>
<td>17° 45' N</td>
<td>8</td>
<td>1014</td>
<td>451.7 (144.5)^e</td>
</tr>
<tr>
<td>Mandeville</td>
<td>MN</td>
<td>25</td>
<td>77° 34' w</td>
<td>18° 00' N</td>
<td>800</td>
<td>2075</td>
<td>632.4 (336.4)^d</td>
</tr>
<tr>
<td>Irish Town</td>
<td>IT</td>
<td>14</td>
<td>76° 44' w</td>
<td>18° 03' N</td>
<td>648</td>
<td>1700</td>
<td>640.4 (167.4)^e</td>
</tr>
</tbody>
</table>

Means with different letters are significantly different (P < 0.05, SNK a posteriori test).

One-way ANOVA among populations, $F_{7,178} = 131.6, P < 0.001$.

FIGURE 1. Map of Jamaica showing phenotypic distributions of cyanogenesis, proportion of plants with herbivores (Herb), non-herbivores (NHrb), and insect predators (Pred), and the distribution of foliar damage at eight populations of *T. ulmifolia*. Cyanogenesis intensity increases with shading density, clockwise from 12 o’clock. 0: µg HCN/g < 6.3; 1: 6.3 < µg HCN/g < 48.5; 2: 48.5 < µg HCN/g < 398.4; 3: µg HCN/g > 398.4. The proportion of leaf damage (total area damaged/total leaf area) increases similarly. 0: Damage < 0.4%; 1: 0.4% < Damage < 1.6%; 2: 1.6% < Damage < 4.0%; 3: Damage > 4%. The locations of populations Kenilworth (KN) and Mammee Bay (MB), which were surveyed to examine the relationship between cyanogenesis and host-plant use by *E. hegesia*, are denoted by their two-digit ID codes.
shoot was less than 20 cm long, a second shoot was also removed. Cyanogenesis tests were performed on the leaf of the shoot that contained the largest bud, flower, or youngest fruit where no flowers or buds were present. Very large leaves were halved longitudinally to allow thorough grinding of the tissue. The next ten leaves (from the apex to the base) of each shoot were removed, affixed with tape by the leaf petiole to pages in a notebook, pressed and dried at 65°C upon our return to the laboratory. Where there were fewer than ten leaves on single shoots (24% of plants), apical leaves were selected from the second shoot, beginning at the leaf below the leaf containing the largest bud, flower, or fruit. The developmental order of the leaves was maintained and the leaf and shoot orders were recorded.

**IMAGE ANALYSIS OF LEAF DAMAGE**

After the pressed leaves were dry, each leaf was digitized using a flatbed scanner connected to a desktop computer. Each image was stored in 8 bit format (256 shades of gray) with a resolution of 200 dpi (dots per inch). A pretest of 10 leaves digitized at 150, 200, 250, and 300 dpi showed that scanning and storing the images at more than 200 dpi did not yield a significant increase in the precision of subsequent measurements. A total of 1848 leaf scans were made and each leaf image was analyzed using NIH Image, version 1.60. Image analysis was conducted on a desktop PC computer running NIH Image under Executor version 2.0, a Macintosh System 6.0.8 emulator from Abacus Research and Development Inc., Albuquerque, New Mexico.

Each leaf image was analyzed as follows. Each image was thresholded and the default threshold level (the mean pixel value of the entire image frame, e.g., leaf and background) was recorded. Thresholding is a process by which the software reduces the image from 8 bit, 256 shades of gray to 1 bit, black and white using the gray level information contained in the image: all gray levels below the threshold are “objects of interest”, in this case the area of a leaf mine, as the measurable area. Figure 2 provides examples of foliage damage types and their potential sources.

The mean proportion of leaf tissue damaged or missing (i.e., total damage/total leaf area for each leaf) per plant was used in the analyses to control for differences in leaf size among plants and populations. The proportion of damaged or missing tissue is a quantitative measure and we have ranked and divided the distribution of damage into quartiles for presentation purposes (Figure 1).

**DATA ANALYSIS**

Statistical analyses were conducted using Minitab release 11 (Minitab, 1996) except for linear regressions, a split-plot ANOVA using PROC GLM and type III sums of squares, and an analysis of covariance (ANCOVA) using SAS vers. 6.12 (SAS, 1996). Analyses involving HCN were conducted using log transformation to stabilize variances. A small positive constant (0.011) was added to each HCN data point prior to log transformation, to offset the infrequent occurrence of small negative spectrophotometer readings.

**Results**

The sample size, location, elevation, mean annual precipitation (based on 91-year means, see Schappert & Shore, 1995) and mean cyanogenesis levels of the T. ulmifolia populations surveyed are presented in Table I and Figure 1. Most populations, except for QS which was in a wet forest clearing, were roughly linear with plants strung out along roadsides. FM was an open sandy site, whereas plants at OB were along a path through second-growth vegetation. The KL population consisted of three sub-populations of plants in second growth vegetation along the roadside over a 7 km distance. SB was at the edge of a pasture along the roadside, plants at PP were in a dry forest savannah adjacent to mangroves on the other side of the road, and plants at MN and IT were both in elevated banks along the roadside. The plants at KN were in a sandy beach beside the road, whereas those at MB were in a glade along an abandoned road.

**CYANOGENESIS IN PLANTS USED BY E. HEGESIA**

The paired comparison of cyanogenesis levels in plants with eggs and larvae vers vs those without eggs and larvae of the specialist herbivore, E. hegesia, revealed that plants with these herbivores had lower mean cyanogenesis levels than plants lacking these herbivores (Table II). The results are not statistically significant when analyzed over both populations using a split-plot ANOVA (Table III), although a paired t-test for the MB population alone was marginally significant (t13 = 2.33, P < 0.05).
INVERTEBRATE VISITORS

We have observed a variety of invertebrates (Table IV) on plants of T. ulmifolia during the course of our studies (1990 through 1995) that span a number of different guilds (herbivore, predator, flower visitor, and extrafloral nectary visitor). The herbivores include members of leaf mining (Agromyzidae, Gelechiidae), fruit/stem boring (Noctuidae), sap feeding (Fulgoroidea, Aphidoidea, Hemiptera, Homoptera), pollen/flower feeding (Thysanoptera, Aleyrodoidea, Chrysomelidae, Lepidoptera), leaf chewing (Lepidoptera, Chrysomelidae, Orthoptera), and rasping (Mollusca) guilds (Table IV). From 1 to 13 different types of herbivores were recorded on up to 88% of the plants in the eight populations surveyed for damage (Table V and Figure 1). Interestingly, the number of herbivore taxa found in populations declines significantly with increasing mean cyanogenesis level of the populations (Figure 3, linear regression $F_{1, 6} = 7.76, P < 0.05$).

Non-herbivores that were present included a total of six species of ants and a variety of predators (Table V). Two to five species of ants were found on 50% to 96% of all of the plants in each population, and predators were present on at least a few plants in seven of the eight populations (Table V and Figure 1). A minimum of three and a maximum of 12 of...
A possible total of 20 invertebrate taxa (all herbivores and non-herbivores) were recorded with the combined presence of all visitors ranging from 64% to 100% of all plants surveyed in the eight populations. The number of taxa of all plant visitors (herbivores and non-herbivores combined) was not related to plant cyanogenesis; however, the proportion of plants in populations without any invertebrates was linearly related to population mean cyanogenesis levels (Figure 4, linear regression \( F_{1, 6} = 104.2, P < 0.001 \)).

**HERBIVORY**

The extent of damage to leaf tissue in *T. ulmifolia* varied both within and between plant populations (Figure 1, Table VI). The proportion of leaves damaged varied from 26% to almost 98% depending on the population. The proportion of plants damaged per population was considerably higher, however, ranging from over 80% to 100% (Table VI). At SB, for example, only 26% of leaves were found to have sustained damage, but these leaves were distributed among 80% of the plants.

The type of damage sustained was often diagnostic and easily attributed to source. The diagnostic features are a combination of hole size (*Disonycha* spp. produce characteristically larger holes than do *Parchicola*; see Figure 2) and clarity (for example, first instar larvae of *E. hegesia*).
feed primarily on the undersides of leaves, chewing incomplete holes that create distinctive “fenêtres” or windows in the leaves). Similarly, a combination of arc length and the angularity of the missing leaf edges can be attributed to large caterpillars such as *E. hegesia* larvae (long arcs and wide angles) or small beetles such as *Disonycha* (short arcs and narrow angles). Aphid damage and leaf miner damage are also easily recognized. Leaf damage from holes and perimeter edge damage was ubiquitous, whereas aphid and leaf miner damage was less common (Table VI). Interestingly, aphids were found more commonly in the more cyanogenic plant populations, whereas leaf miners were only found in relatively acyanogenic populations (Tables I and VI).

**Relationship between cyanogenesis, damage, and plant size**

An ANCOVA using the proportion of leaf area removed by herbivores per plant as the response variable, with population and log cyanogenesis as explanatory variables, revealed significant differences between populations (\(F_{1,169} = 5.43, P < 0.001\)), but there were no cyanogenesis (\(F_{1,169} = 1.32, P = 0.25\)) or cyanogenesis-population interaction effects (\(F_{7,169} = 0.43, P = 0.88\)). An ANCOVA, using numbers of shoots per plant as the response variable, gave a significant cyanogenesis effect (Table VII) with a positive slope, indicating that plants with more shoots are more highly cyanogenic. Plant height showed both significant cyanogenesis and population effects, again with taller plants showing a positive relationship with cyanogenesis level.

**Discussion**

*Turnera ulmifolia* on the island of Jamaica varies for cyanogenesis, a heritable chemical trait (Schappert & Shore, 1995). The plants are visited by a reasonably diverse herbivore and non-herbivore fauna, and the occurrence, proportion, and intensity of foliage damage varies within and between populations (Table I, Figure 1). The diversity of herbivores attacking plants is negatively correlated with the mean cyanogenesis level of the population, supporting the hypothesis that cyanogenesis provides a defense against some herbivores (Figure 3). The correlation of the proportion of plants lacking any invertebrate visitors with mean cyanogenesis level (Figure 4) provides further support for this hypothesis. Our survey results suggest that cyanogenesis plays a role in the defense of the plants against some herbivores. Marquis & Braker (1994) note, however, that herbivore diversity does not often, or necessarily, correspond in an obvious way to damage levels sustained by plants. It is clear, however, that other herbivores do not discriminate among plants based on their cyanogenesis levels. This appears to be the case for larvae of *Euptoieta hegesia* (Tables II and III, Schappert & Shore, 1999).

In contrast to our herbivore diversity data, an analysis of covariance did not reveal any effect of cyanogenesis levels on the proportion of leaf area removed per plant, although populations differ for this parameter (Table VII). However, two fitness correlates, i.e., number of shoots per plant and plant height, are significantly related to cyanogenesis level (Table VII). The reproductive output of these perennial plants is likely to be highly correlated with the number of shoots since each shoot produces flowers and fruits regularly throughout the year. The number of shoots per plant and plant height likely integrate the effects of herbivory over the life of each plant up to the time we assayed them. Therefore, number of shoots and plant height might provide increased power to detect the effects of herbivory. Experimental plots consisting of seeds of known frequencies of cyanogenesis sown into natural populations would be necessary to clearly test the hypothesis that cyanogenesis provides protection against some herbivores, following the approach used by Ennos (1981) for *Trifolium repens*. Because *T. ulmifolia* is a perennial, it would be necessary to follow cohorts for a considerable length of time to discover if and when selection might be acting on cyanogenesis.

Almost all of the damage found during the image analysis of leaves can be attributed to five types of herbivores: (i) the larvae of *E. hegesia*, (ii) the large holes of the flea beetles *Disonycha* (*Chrysomelidae: Alticinae*, likely a new species; C. N. Duckett, pers. comm.), (iii) the small holes of two species of *Parchicola* (*Chrysomelidae: Alticinae*, at least one of which is also an undescribed species; C. N. Duckett, pers. comm.), (iv) aphids, and (v) leaf miners. Figure 2 shows that the larvae of *E. hegesia* and the larvae and adults of *Disonycha* flea beetles are able to inflict severe damage to leaves. *Euptoieta hegesia* is a host specialist that only feeds on *T. ulmifolia* and, less commonly, *Passiflora spp.* (Schappert & Shore 1998; 1999). Preliminary laboratory studies of the *Disonycha* flea beetles reveal that these insects will only accept *T. ulmifolia* and will not accept the two most common species of *Passiflora* used by *E. hegesia* on Jamaica (unpubl. data). We have reason to expect that both *Parchicola sp.* flea beetles will also turn out to be host specialists (C. N. Duckett, pers. comm.). Aphids that were found feeding on cyanogenic plants may be capable of effectively avoiding cyanogenesis, since they may not lyse many cells (a necessity for the cyanogenic response), and so are able to feed on highly cyanogenic plants with some impunity. Leaf miners (Diptera: Agromyzidae and Lepidoptera: Gelechiidae) which feed on *T. ulmifolia* seem to avoid cyanogenesis by avoiding cyanogenic plants (Table VI). Since these insects are tightly enclosed in leaf tissue, they might find themselves in a toxic environment having a high HCN concentration. Experimental feeding trials with leaf miners would be necessary to test this hypothesis.

Mean levels of tissue loss recorded in this study (1- 9%; Table VI) appear to be unusually low when compared to studies of other tropical species or other tropical habitats. Marquis (1992) and Marquis & Braker (1994), summarizing a number of studies from Costa Rica and other tropical and

<p>| Table VII. Influence of population source and cyanogenesis level on proportion of leaf area removed by herbivores, number of shoots per plant and plant height using analysis of covariance |</p>
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Proportion damage</th>
<th>Number of shoots</th>
<th>Plant height</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>MS</td>
<td>MS</td>
</tr>
<tr>
<td>logHCN</td>
<td>1</td>
<td>0.003</td>
<td>1.32</td>
<td>606</td>
</tr>
<tr>
<td>Population</td>
<td>7</td>
<td>0.014</td>
<td>5.43***</td>
<td>256</td>
</tr>
<tr>
<td>Interaction</td>
<td>7</td>
<td>0.001</td>
<td>0.43</td>
<td>156</td>
</tr>
<tr>
<td>Residual</td>
<td>169</td>
<td>0.002</td>
<td>142</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^{*} P < 0.05, ^{***} P < 0.001.\)
temperate sites, report that mean tissue loss levels average between 5-15% (7-23% at tropical sites and 1.8-12.3% at temperate sites). Marquis (1990) recorded tissue loss levels as high as 50% for *Piper aricideum* (Piperaceae), but found that leaf area loss in one- to three-month census intervals averaged 1–3% in this species. It is likely that the temporal interval represented by the 10 leaves we measured for damage in *T. ulmifolia* represents a time span similar to the census intervals of Marquis (1990). The levels recorded in this study may not be as low as the means suggest. Confounding factors may also include differences in plant and herbivore communities/habitats between studies, and the potential for unusual perturbations in small samples (e.g., dispersion of damage by Lepidopteran herbivores versus herbivores which do not disperse their damage; Mauricio & Bowers, 1990).

This study adds to the evidence provided by a large number of studies of cyanogenesis polymorphisms in temperate species such as *Lotus corniculatus* (Compton & Jones, 1985), *Trifolium repens* (Burgess & Ennos, 1987), and bracken fern, *Pteridium aquilinum* (Cooper-Driver & Swain, 1976), that “cyanogenic glycosides act as functional defenses at an intraspecific level” (Pollard, 1992; but see Hruska, 1988). Importantly, this study adds evidence for cyanogenesis as a functional defense in a tropical plant that exhibits quantitative genetic variation. These findings do not preclude the influence of other factors that may aid in the defense of the plants. For example, there is a positive correspondence between measures of plant size and the number of types of invertebrate plant visitors found, suggesting that plant apparency may play a role in the perception of plants by potential herbivores and their predators alike (Table VII; Feeny, 1976; Chew & Courtney, 1991; but see Thomas, 1990; Parmesan, 1991). There are several other factors that may play a role in the defense of *T. ulmifolia* plants. Many of these factors are properties of particular herbivores or plants, and include herbivore type, movement and colonization patterns (Denno & Donnelly, 1981; Bach, 1990; Bowers & Stamp, 1993), insect life history strategies (contrast between Orthoptera and Lepidoptera; Berenbaum & Isman, 1989), co-occurrence of facilitating herbivores (Pilson, 1992) or predators (Bernays & Graham, 1988), resource availability and variation in plant allocation of resources (Coley, Bryant & Chapin, 1985; Simms, 1992; Zangerl & Bazzaz, 1992), variation in plant tolerance (e.g., the ability to grow and reproduce following damage; Rosenthal & Kotanen, 1994), combinations of local plant resistance and local herbivore preference (Singer & Parmesan, 1993), and variation in impact on different tissues by different herbivores (Zangerl & Bazzaz, 1992).

The variable associations between non-herbivores (e.g., ants and predators) and herbivores found on the plants hint at the possibility that triUTURE interactions may also be important components of plant defense. Certainly, ants are ubiquitous in *T. ulmifolia* populations both in this study and in other reports (Barrett, 1978; Schubart & Anderson, 1978). Ants are often suggested as playing an important role in defending plants like *T. ulmifolia*, which possess extrafloral nectararies (Rehr et al., 1973 [but see Seigler, 1991]; Douglas, 1983; McLain, 1983; Tempel, 1983; Beattie, 1985; Smiley, 1985; 1986; Barton, 1986). Predators and parasitoids, which may also be associated with extrafloral nectararies, may also play a significant role in plant defense (Pemberton & Lee, 1996). Hallman (1979) has even suggested that plants of *T. ulmifolia* (likely *T. subulata* Smith) should be planted around commercial crops in Colombia to attract *E. hegesia* as a means of increasing the abundance of generalizing egg parasitoids of Lepidoptera for crop defenses.

Acknowledgements

We wish to thank J. Lewin and P. Schappert for technical assistance in the laboratory and field, C. Duckett for identifying the flea beetles, and J. D. Woodley, M. Haley, the staff at the Discovery Bay Marine Laboratory, and R. and A. Sutton for logistical aid in the field. P. Schappert kindly translated the abstract. The study was funded by a Natural Sciences and Engineering Research Council operating grant to J. S. Shore.

Literature cited


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