

Cyanogenesis in *Turnera ulmifolia* L. (Turneraceae): II. Developmental expression, heritability and cost of cyanogenesis

Phillip J. Schappert* and Joel S. Shore

Department of Biology, York University, 4700 Keele Street, North York, Ontario M3J 1P3, Canada

ABSTRACT

We examine the cyanogenesis polymorphism in *Turnera ulmifolia* on Jamaica with respect to our hypothesis that seedlings in some populations have significantly higher levels of cyanogenesis than mature plants because of the developmental loss of cyanogenesis. Furthermore, we provide estimates of among-family variance, as estimates of broad sense heritability, for a number of fitness-related traits, and also examine the potential cost of cyanogenesis in this species. Our data reveal that there is a marked developmental loss of cyanogenesis in some populations of *T. ulmifolia*. Seedlings have significantly greater levels of cyanogenesis than mature plants in 'acyanogenic' populations, but this developmental loss is absent in predominantly cyanogenic populations. This is the first study to document extensive developmental loss of chemical defence in a cyanogenic species. We suggest that, in these populations, there might be selection favouring cyanogenesis in seedlings. There is substantial among-family variance in a number of traits in five *T. ulmifolia* populations examined, including plant height, time to first flowering, total flower production and cyanogenesis. Phenotypic and, more importantly, negative genetic correlations between total flower production and cyanogenesis provide evidence for a cost of cyanogenesis in three of five populations.

Keywords: chemical defence, genetic variation, life-history trade-offs, natural selection, plant growth, reproduction.

INTRODUCTION

Cyanogenesis is a common chemical defence that has been reported in over 3000 plant species (Poulton, 1990). When plant tissue is damaged, a cyanogenic glycoside (in most species) is brought into contact with a β -glycosidase, resulting in the liberation of hydrogen cyanide (HCN) and an aldehyde or ketone moiety (Conn, 1979; Poulton, 1990; Seigler, 1991). Both HCN and the carbonyl product might act as defence compounds (Compton and Jones, 1985; Jones, 1988; Spencer, 1988; Seigler, 1991). The occurrence of genetic polymorphism for cyanogenesis in some species (e.g. *Trifolium repens* and *Lotus corniculatus*) has provided useful experimental systems for studies of the evolution of chemical defence in

* Address all correspondence to Phillip J. Schappert, Section of Integrative Biology, School of Biological Science, University of Texas, Austin, TX 78712-1064, USA. e-mail: philjs@mail.utexas.edu
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plants and has received considerable attention (Daday, 1954a,b, 1965; Jones, 1962, 1966, 1971, 1973, 1981, 1988; Bishop and Korn, 1969; Angseesing and Angseesing, 1973; Cooper-Driver and Swain, 1976; Abbott, 1977; Cooper-Driver *et al.*, 1977; Ellis *et al.*, 1977a,b,c; Jones *et al.*, 1978; Scriber, 1978; Dritschilo *et al.*, 1979; Ennos, 1981; Dirzo and Harper, 1982a,b; Schreiner *et al.*, 1984; Compton and Jones, 1985; Horrill and Richards, 1986; Burgess and Ennos, 1987; Blaise *et al.*, 1991; Schappert and Shore, 1995, 1999a). In some species, the polymorphisms are under relatively simple genetic control (Dawson, 1941; Jones, 1977; Hughes *et al.*, 1984), whereas in others the variation is quantitative and appears to be polygenically based (Nass, 1972; Eck *et al.*, 1975; Schappert and Shore, 1995).

One unanswered question in the evolution of plant defence is whether there are costs associated with the defence. Hypotheses that there is a cost of defence may be tested by determining whether there are negative genetic correlations between the defensive and fitness-related traits (Simms and Rausher, 1992), an approach used to examine life-history trade-offs (Reznick, 1985). Quantitative genetic analyses, or selection experiments, provide a means of estimating the magnitude of the heritability of defensive and fitness-related traits as well as of genetic correlations among them. Results of analyses of a variety of different plant species, their chemistry and their herbivores or pathogens, have been equivocal (Bergelson and Purrington, 1996; Agrawal and Karban, 1999), with some showing costs (Berenbaum *et al.*, 1986; Han and Lincoln, 1994; Baldwin, 1998; Agrawal *et al.*, 1999; Strauss *et al.*, 1999) and others providing little or no direct evidence of costs (Simms and Rausher, 1989; Briggs and Schultz, 1990; Agren and Schemske, 1993; Simms and Triplett, 1994).

Turnera ulmifolia L. is a weedy shrub common in disturbed habitats on the island of Jamaica. Plants are self-compatible hexaploids that are commonly highly self-fertilizing (Barrett and Shore, 1987; Belaoussoff and Shore, 1995) and are often distributed in small discreet populations across Jamaica. Populations are genetically differentiated for a number of floral (Barrett and Shore, 1987; Belaoussoff and Shore, 1995) and vegetative traits (duQuesnay, 1971). Although no estimates of gene flow are currently available, it is likely that relatively little gene flow occurs among populations as a result of their patchy distribution, limits on distance of seed dispersal by ants, and high levels of selfing limiting opportunities for outcrossing. Furthermore, Baker and Shore (1995) demonstrated that some populations are partially reproductively isolated through competitive advantages favouring within-population pollen. Flowers are borne singly and are short-lived (4–8 h).

Significantly, populations of *T. ulmifolia* on Jamaica show considerable differentiation for cyanogenesis and the variation is quantitative, possibly controlled by a number of genes (Schappert and Shore, 1995). We previously mapped the phenotypic distribution of cyanogenesis for 39 populations on Jamaica and revealed a number of populations virtually devoid of adult cyanogenic plants, while some populations, particularly on the south coast, were composed solely of plants with high cyanogenesis levels (see figure 3 in Schappert and Shore, 1995). Cyanogenesis appears to be widespread in the Turneraceae and plants of *T. ulmifolia* and other species in the genus *Turnera* possess cyclopentenoid cyanogenic glycosides (Spencer and Seigler, 1980, 1981; Spencer *et al.*, 1985; Tober and Conn, 1985; Olafsdottir *et al.*, 1990; Shore and Obrist, 1992). Acyanogenic plants of *T. ulmifolia* apparently lack the cyanogenic glycosides but do possess β -glycosidases capable of hydrolysing them, as evidenced by the liberation of HCN after the addition of exogenous cyanogenic glycosides to crushed leaves of acyanogenic plants (Schappert and Shore, 1995).

Cyanogenesis in *T. ulmifolia* may act as a plant defence against some invertebrate herbivores, as cyanogenic plant populations possess fewer herbivore guilds and large proportions of plants within these populations are free of insects (Schappert and Shore, 1999a). Some insects, such as the host specialist *Euptoieta hegesia* (Schappert and Shore, 1998), appear to be unimpeded by cyanogenesis and apparently sequester the cyanogenic glycoside, possibly for their own chemical defence (Schappert and Shore, 1999b). We found that, in some field-sampled populations, there appeared to be a difference in the levels of cyanogenesis among seedling versus adult plants, with seedlings showing significantly greater levels of cyanogenesis than adults, while there was no such effect in other populations (Schappert and Shore, 1995). We hypothesized that either there was selection against cyanogenesis in some populations, yielding low levels of cyanogenesis in adults, or that there was a developmental loss of cyanogenic expression (Schappert and Shore, 1995).

Here we report on controlled glasshouse experiments to: (1) demonstrate the developmental loss of cyanogenesis for some plants and populations of *T. ulmifolia*; (2) provide estimates of broad sense heritability of cyanogenesis and reproductive traits for five populations; and (3) determine if there is a cost of cyanogenesis (in the absence of herbivores) by examining phenotypic and approximate genetic correlations among cyanogenesis and reproductive and growth traits, including time to first flower, total flower production and plant height.

METHODS

Seeds from a number of fruits of individual plants (yielding open-pollinated seed families) were collected from 10 populations (range: 4–30 seed families per population; total: 139 seed families; see Table 1) of *T. ulmifolia* on Jamaica in the summer of 1991. Limits on seed number and availability were imposed by some populations that had low seed output (both number and size of fruits; e.g. PORTPT) or by populations that had few seed-bearing plants (e.g. HSHIRE). Seeds from each family were sown in pots and placed in the greenhouse at York University, Toronto, Canada, in the spring of 1992 and 10 seedlings from each seed family were later transplanted to individual 7.6 cm pots. Seedlings were transplanted when they were at the two-leaf (cotyledon) stage and were chosen to match approximately the size of seedlings across families within populations. An additional five seedlings per family, from four of the populations, were frozen at -80°C for the determination of cyanogenesis levels of emergent seedlings bearing only cotyledons (cotyledon stage).

We arbitrarily assigned the day when every pot had greater than 50% seed germination to be day 1 (i.e. day of seedling emergence) for subsequent comparison of time to first flowering and total flower production. A total of 1390 potted seedlings were randomized within population blocks, containing up to 21 individuals each, and the blocks (trays) were randomized on greenhouse benches. Trays, and the seedlings in them, were periodically re-randomized. Time to first flowering was recorded for all plants. All flowers produced over 150 days of growth were recorded and removed from the plants (to avoid confounding effects of differences in autogamous seed production influencing subsequent flowering). We assayed all plants for cyanogenesis (see below) and plant height, 90 days after germination. At this time, the point of incipient flowering, 5% of the plants had begun flowering. At 150 days following germination (when 95% of plants had produced 10 or more flowers), we again assayed all plants for cyanogenesis. A small subsample of the plants from each

Table 1. Controlled greenhouse grow-out of 10 Jamaican *T. ulmifolia* populations to compare cyanogenesis at various life-history stages

Population	No. of seed families	Mean HCN ($\mu\text{g CN} \cdot \text{g}^{-1}$) at cotyledon stage		Mean HCN ($\mu\text{g CN} \cdot \text{g}^{-1}$) 90 day seedlings ^a		Mean HCN ($\mu\text{g CN} \cdot \text{g}^{-1}$) 150 day plants ^b		Mean HCN ($\mu\text{g CN} \cdot \text{g}^{-1}$) 1 year plants	
		<i>n</i>	Mean $\pm$ <i>s</i>	<i>n</i>	Mean $\pm$ <i>s</i>	<i>n</i>	Mean $\pm$ <i>s</i>	<i>n</i>	Mean $\pm$ <i>s</i>
S HILL	7	—	—	64	0.5 (51.5)	64	30.6 (706.7)	15	−30.7 (46.1)
KENILW	24	100	1319.4 (477.1)	237	123.8 (114.0)	230	59.3 (187.7)	144	0.5 (23.4)
EWARTN	18	90	1290.9 (612.8)	179	23.0 (62.6)	178	5.4 (179.9)	32	5.9 (15.7)
FALMTH	30	210	525.5 (279.5)	300	35.0 (62.1)	297	22.6 (109.3)	60	8.1 (28.6)
TRYALL	19	—	—	190	61.8 (63.8)	184	109.6 (324.0)	23	34.6 (113.7)
S CRUZ	7	—	—	70	462.2 (411.8)	69	370.0 (504.1)	11	132.8 (193.6)
IRSHTN	4	—	—	39	714.4 (157.5)	38	723.5 (260.1)	37	1155.7 (625.8)
PORTPT	10	—	—	62	1067.7 (351.3)	62	1913.1 (678.5)	56	1275.3 (369.0)
MANDEV	14	70	994.6 (305.4)	140	847.9 (234.3)	139	1552.0 (637.3)	17	1509.0 (563.8)
HSHIRE	6	—	—	57	2053.3 (614.3)	57	3424.3 (971.4)	55	2020.9 (675.8)

Note: Populations ordered from least cyanogenic (top) to most cyanogenic (bottom) based on 1 year sample.

^a Point of incipient flowering (5% of plants flower for first time). ^b Endpoint of flower recording (95% of plants had put on 10 or more flowers).

population were subsequently retained and sampled for cyanogenesis a third time, 300 days following seedling emergence.

Cyanogenesis assays were conducted by removing a leaf approximately 25 mm in length from the tallest shoot. We ground the entire leaf and followed the cyanogenesis assay below. Emergent seedlings from four populations that were destructively sampled (frozen at -80°C) were assayed for cyanogenesis by grinding the entire seedling (after washing in distilled water) following the protocol for quantitative spectrophotometric determinations that we have used previously (following methods detailed in Schappert and Shore, 1995, 1999a; after Brinker and Seigler, 1989).

In a second experiment, to explore more closely the developmental changes in cyanogenesis, we used seeds derived from two open-pollinated families obtained from a population showing considerable variation in cyanogenesis. The plants were originally collected in 1989 and selfed progeny have been raised and grown in the greenhouse since that time. We have used selfed lines of these plants in other studies (Shore and Obrist, 1992; Schappert and Shore, 1995). Two selfed lines were derived from high cyanide plants of one family and two from low cyanide plants (see figure 2 in Schappert and Shore, 1995). Eight progeny from each of four plants from each line were also sown in the spring of 1992 and individual seedlings were subsequently transferred to individual 7.6 cm pots with an additional five seedlings of each being frozen for cyanogenesis determination (cotyledon stage). In total, 64 seedlings were monitored for 150 days after seedling germination. Cyanogenesis determinations were conducted for the frozen seedlings, and at 30, 90, 125 and 150 days from emergence. We recorded time to first flowering and flower production for all plants, as above.

All statistical analyses were carried out using SAS (1996). We log-transformed cyanogenesis values (after adding a small positive constant to offset the occurrence of small negative spectrophotometer readings) to reduce heterogeneity of variances. We calculated the proportion of among-family variance, only for the five populations for which we had a reasonable sampling of families (14 or more families; see Table 1), as an estimate of broad sense heritability for HCN values at 90 and 150 days, days to first flowering, total flower production and height at 90 days. One-way analyses of variance and associated *F*-tests were carried out to assess whether there was a significant family effect for each character, and the among-family variance components were estimated using the maximum likelihood procedure of VARCOMP (SAS, 1996). We used the sequential Bonferroni (Rice, 1989) for each population to control type I error rate, with the type I error rate held at $\alpha = 0.05$ for the five significance tests for each population. For these populations, we also calculated pairwise phenotypic correlations among the characters, as well as family mean correlations, to provide an estimate of the genetic correlations among characters for each population. We used Pearson's product-moment correlation and the sequential Bonferroni (Rice, 1989) for each population to control the type I error rate. For each population and kind of correlation (i.e. phenotypic vs genotypic), we used $\alpha = 0.05$ collectively for the ten pairwise significance tests. Analyses of variance and analyses of covariance were carried out using PROC GLM (SAS, 1996) and correlations using PROC CORR.

RESULTS

All emergent seedlings assayed, regardless of population source, were highly cyanogenic (Table 1). In some populations, cyanogenesis levels declined quickly to near zero at about the time the plants began flowering for the first time, eventually yielding largely acyanogenic

plant population samples (e.g. population S HILL and EWARTN; see Table 1 and Fig. 1). For other populations, cyanogenesis levels remained high or even increased beyond 150 days following germination (e.g. populations MANDEV, PORTPT). By 150 days, some populations were composed of largely acyanogenic plants or plants showing little cyanogenesis (populations S HILL, KENILW, EWARTN, FALMTH, TRYALL, S CRUZ), whereas others remained cyanogenic (populations IRSHTN, PORTPT, MANDEV, HSHIRE). The plants from Hellshire (HSHIRE) exhibited the greatest cyanogenesis levels (mean $3424 \mu\text{g} \cdot \text{g}^{-1}$ at 150 days), while those from Stoney Hill (S HILL) showed the lowest (mean of approximately 0).

Investigation of the selfed lines derived from population A2 (see Schappert and Shore, 1995) revealed that seedlings derived from lines known to be acyanogenic as adults, were cyanogenic at the cotyledon stage and decreased in cyanogenic status from emergence through 30 days to 90 days and remained acyanogenic beyond that point. Seedlings derived from the cyanogenic selfed lines began at somewhat greater cyanogenesis levels, which then dropped somewhat until 120 days following germination, but increased to day 150 (Fig. 2). By the time plants began flowering (approximately day 90), they had segregated into two groups (cyanogenic vs acyanogenic), which were still apparent more than 300 days after germination (unpublished results).

In addition to cyanogenesis, we compared three reproduction and growth-related traits (days to first flowering, total flower production and plant height at 90 days) for each population (Tables 2 and 3). Time to first flowering varied from 91.1 ± 6.4 days for MANDEV to 115.6 ± 12.2 days for HSHIRE. Total flower production over 150 days varied from 0.4 ± 1.1 flowers per plant for the HSHIRE population to 14.1 ± 4.4 flowers

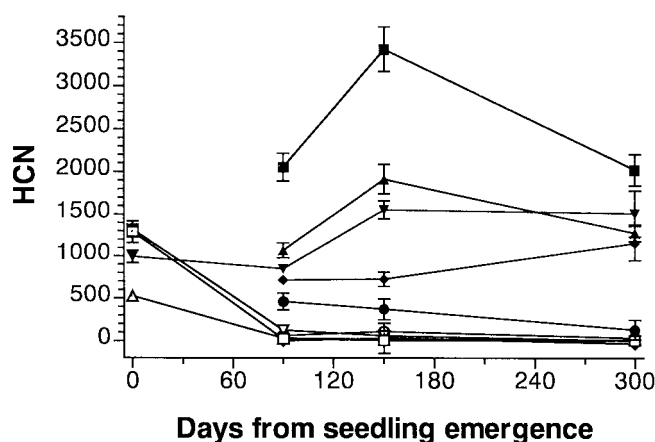


Fig. 1. Mean cyanogenesis (with 95% confidence intervals) for 10 populations of *T. ulmifolia* from Jamaica. Cyanogenesis sampling points represent newly emerged seedlings (day 0; destructively sampled), point of incipient flowering (5% of plants flowered for first time, day 90), end of the experimental flowering period (95% of plants had had 10 or more flowers, day 150) and fully mature adult plants (approximately 1 year old, day 300). Populations are denoted, top to bottom, by symbols as follows: ■, HSHIRE; ▲, PORTPT; ▼, MANDEV; ◆, IRSHTN; ●, S CRUZ; □, EWARTN; △, FALMTH; ▽, KENILW; ◇, S HILL; ○, TRYALL. Cyanogenesis (HCN) is measured in μg HCN per gram dry weight of leaf tissue.

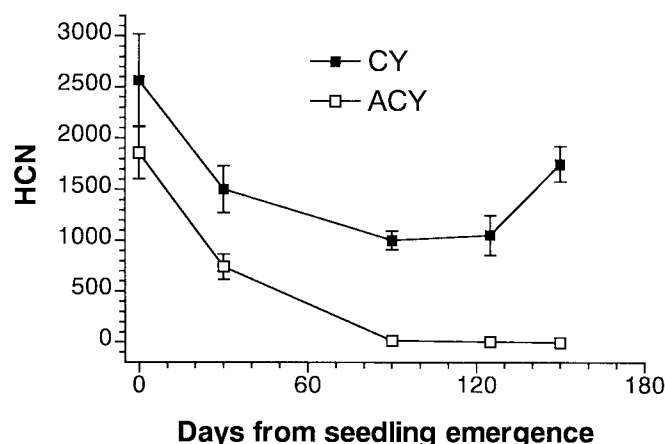


Fig. 2. Mean cyanogenesis (with 95% confidence intervals) for selfed lines of *T. ulmifolia* (population A2 of Shore and Obrist, 1992) from Jamaica which differ markedly in cyanogenic potential. Cyanogenesis sampling points represent newly emerged seedlings (day 0; destructively sampled), pre-flowering seedlings (day 30), point of incipient flowering (5% of plants flowered for first time, day 90), mid-flowering adults (day 125) and end of the experimental flowering period (95% of plants had had 10 or more flowers, day 150). Cyanogenesis (HCN) is measured in μg HCN per gram dry weight of leaf tissue.

per plant for the MANDEV population. Mean plant height at 90 days varied from 21.8 ± 6.3 for HSHIRE to 48.3 ± 9.8 for IRSHTN.

We calculated estimates of among-family variance (expressed as a percent of total variance) for five populations (Table 3) using variance components calculated using the maximum likelihood procedure of PROC VARCOMP (SAS, 1996). Cyanogenesis levels (at 90 and 150 days), days to first flowering, total flower production and plant height at 90 days, showed statistically significant family effects in all populations with only three exceptions: HCN at 150 days showed no significant family effect in the KENILW, EWARTN and MANDEV populations (Table 3). Note that we used the sequential Bonferroni procedure (Rice, 1989) to control the type I error rate for each population. Furthermore, if we apply the correction to the entire set of 25 analyses of variance, we obtain the same set of significant results with only one exception (among-family variance for HCN at 90 days in EWARTN would no longer be statistically significant). These results indicate that there is statistical evidence for among-family variance in virtually all the traits measured in all five populations. The among-family variance components provide estimates of broad sense heritability (although maternal effects cannot be ruled out), which ranges from 0.0% for HCN at day 150 in MANDEV and EWARTN to 57.9% for plant height at 90 days in the FALMTH population. The occurrence of genetic variation for these traits provides the opportunity to investigate whether there are genetic correlations among the traits.

We calculated both phenotypic and family mean correlations among all the traits for each of the five populations to assess whether there is a cost to cyanogenesis when plants are grown in the absence of herbivores (Table 4). The phenotypic correlations are presented in the upper half of each correlation table. In all five populations, plant height, time to first

Table 2. Controlled greenhouse grow-out of 10 Jamaican *T. ulmifolia* populations to compare plant height and reproductive traits

Population	No. of seed families	Days to first flowering		Total flower production		Height (cm) at day 90	
		<i>n</i>	Mean $\pm$ <i>s</i>	<i>n</i>	Mean $\pm$ <i>s</i>	<i>n</i>	Mean $\pm$ <i>s</i>
S HILL	7	64	96.4 (8.21)	64	10.8 (2.88)	64	33.25 (5.70)
KENILW	24	228	98.4 (9.81)	237	11.9 (4.81)	236	23.44 (5.01)
EWARTN	18	176	95.1 (8.13)	179	11.5 (4.04)	179	28.98 (6.64)
FALMTH	30	243	114.4 (14.08)	300	6.4 (4.45)	300	34.20 (9.40)
TRYALL	19	187	99.9 (8.51)	190	11.2 (3.48)	190	25.83 (6.44)
S CRUZ	7	69	95.4 (9.12)	70	10.5 (4.15)	70	32.51 (6.77)
IRSHTN	4	39	95.1 (6.16)	39	10.9 (3.89)	39	48.32 (9.79)
PORTPT	10	53	109.9 (13.35)	62	7.4 (4.98)	62	39.40 (8.17)
MANDEV	14	140	91.1 (6.39)	140	14.1 (4.44)	140	32.27 (6.93)
HSHERE	6	8	115.6 (12.15)	57	0.4 (1.11)	57	21.76 (6.34)

Note: Populations ordered from least cyanogenic (top) to most cyanogenic (bottom) based on 1 year sample.

Table 3. Percent of among-family variance (broad sense heritability) for log-transformed HCN measured at 90 and 150 days, days to first flowering, total flower production and plant height at 90 days, for five populations of *T. ulmifolia*

Population	No. of seed families	HCN (day 90)	HCN (day 150)	Days to first flowering	Total flower production	Height (day 90)
KENILW	24	31.2*	4.6	34.7*	30.0*	15.9*
EWARTN	18	8.0*	0.0	25.0*	23.9*	41.3*
FALMTH	30	57.2*	15.0*	27.1*	33.8*	57.9*
TRYALL	19	56.8*	66.3*	52.3*	38.1*	44.7*
MANDEV	14	28.0*	0.0	24.5*	46.0*	41.1*

Note: Populations ordered from least cyanogenic (top) to most cyanogenic (bottom) based on 1 year sample. For each population, we calculated the *F*-values from one-way analysis of variance and then used the sequential Bonferroni procedure (Rice, 1989) to assess statistical significance holding $\alpha = 0.05$ for each of the five traits. * $P < 0.05$ using sequential Bonferroni for five traits in each population.

Table 4. Family mean correlations (lower half of each matrix) and phenotypic correlations (upper half of each matrix) for log-transformed HCN measured at 90 and 150 days, days to first flowering, total flower production and plant height at 90 days, for five populations of *T. ulmifolia*

	HCN (90 days)	HCN (150 days)	Days to first flowering	Total flower production	Height (90 days)
KENILW (24 seed families, 240 plants)					
HCN (90 days)	—	0.05	0.40*	-0.47*	-0.46*
HCN (150 days)	-0.05	—	-0.01	-0.07	0.01
Days to first flowering	0.63*	-0.21	—	-0.66*	-0.38*
Total flower production	-0.75*	-0.11	-0.79*	—	0.47*
Height (90 days)	-0.42	-0.22	-0.46	0.70*	—
EWARTN (18 seed families, 180 plants)					
HCN (90 days)	—	-0.01	0.13	-0.20*	-0.39*
HCN (150 days)	-0.01	—	0.01	-0.03	0.11
Days to first flowering	0.43	0.21	—	-0.66*	-0.31*
Total flower production	-0.36	0.01	-0.65*	—	-0.45*
Height (90 days)	-0.51	-0.04	-0.14	0.27	—
FALMTH (30 seed families, 300 plants)					
HCN (90 days)	—	0.53*	0.09	-0.48*	-0.70*
HCN (150 days)	0.82*	—	0.01	-0.22*	-0.35*
Days to first flowering	0.32	0.33	—	-0.73*	-0.01
Total flower production	-0.68*	-0.61*	-0.74*	—	0.47*
Height (90 days)	-0.80*	-0.70*	-0.02	0.52*	—
TRYALL (19 seed families, 190 plants)					
HCN (90 days)	—	0.43*	0.45*	-0.59*	-0.71*
HCN (150 days)	0.73*	—	-0.10	-0.53*	-0.51*
Days to first flowering	0.42	-0.08	—	-0.51*	-0.41*
Total flower production	-0.79*	-0.76*	-0.46	—	0.61*
Height (90 days)	-0.90*	-0.82*	-0.43	0.88*	—
MANDEV (14 seed families, 140 plants)					
HCN (90 days)	—	0.16	0.18	-0.16	0.32*
HCN (150 days)	0.49	—	0.05	-0.16	0.07
Days to first flowering	0.10	-0.40	—	-0.63*	-0.40*
Total flower production	-0.06	0.34	-0.76*	—	0.45*
Height (90 days)	0.57	0.50	-0.46	0.69	—

Note: For each half of each matrix we used the sequential Bonferroni procedure (Rice, 1989) to assess statistical significance holding $\alpha = 0.05$ for the 10 correlations in each half matrix.

* $P < 0.05$ using sequential Bonferroni for 10 correlations.

flowering and total flower production are significantly phenotypically correlated, with the exception of days to first flowering and plant height in the FALMTH population. HCN at 90 days and HCN at 150 days are only significantly phenotypically correlated in FALMTH and TRYALL. HCN at 90 days is significantly correlated with days to first flowering in

KENILW and TRYALL, while it is significantly negatively correlated with total flower production in all populations except MANDEV. Plant height is negatively correlated with HCN at 90 days in KENILW, EWARTN, FALMTH and TRYALL, but positively correlated in MANDEV. HCN at 150 days shows significant negative correlations with total flower production and plant height only in FALMTH and TRYALL. Collectively, these results suggest there are trade-offs between cyanogenesis and growth (plant height) and reproductive traits, with more cyanogenic plants within a population taking longer to flower and producing fewer flowers.

Calculation of family mean correlations for each population provides approximate estimates of genetic correlations among traits (Table 4, lower half of each correlation table). Generally, fewer correlations are significant when compared with the phenotypic correlations, in part a function of the smaller number of families and lower degrees of freedom for each test. Total flower production and time to first flowering are significantly negatively correlated for all populations except TRYALL. Plant height is significantly positively correlated with total flower production in three of the five populations. Most interesting are the family mean correlations between HCN and the reproductive traits. HCN at 90 days is significantly positively correlated with days to first flowering in two of the five populations, and HCN at 90 days is significantly negatively correlated with total flower production in three of the five populations. In all populations, the signs of the correlations show a similar pattern with total flower production negatively, and days to first flowering positively, correlated with HCN at 90 days. HCN at 150 days is significantly correlated with HCN at 90 days in two populations. HCN at 150 days is significantly negatively correlated with total flower production in two populations, and is not significantly correlated with days to first flowering in any of the populations. It is significantly negatively correlated with height in two of the populations.

To illustrate the possible trade-off of HCN at 90 days with time to first flowering and total flower production we have plotted family means against HCN at 90 days for each of the populations (Fig. 3). The plots illustrate the positive relationship between days to first flowering and HCN at 90 days for each population, and the negative relationship between HCN at 90 days and total flower production. Using analysis of covariance to test simultaneously for heterogeneity among intercepts and slopes for total flower production, we found significant effects of cyanogenesis ($F_{1,95} = 20.3$, $P < 0.001$) and population ($F_{4,95} = 47.8$, $P < 0.001$) but no interaction effects ($F_{4,95} = 0.6$, $P > 0.6$), indicating that the slopes do not differ significantly among populations. For days to first flowering, there was a significant negative cyanogenesis effect ($F_{1,95} = 32.2$, $P < 0.001$), a population effect ($F_{4,95} = 48.6$, $P < 0.001$) but no interaction ($F_{4,95} = 0.2$, $P > 0.9$), indicating that the slopes do not differ significantly among populations.

To test whether there is a cost to cyanogenesis in population A2, we used nested analyses of variance to compare time to first flowering and total flower production for the cyanogenic versus acyanogenic selfed lines (Fig. 2) derived from population A2. The means for time to first flowering were 112.2 ± 12.0 days for the acyanogenic lines and 114.3 ± 14.7 days for the cyanogenic lines, which were not significantly different ($F_{1,2} = 0.2$, $P > 0.3$). Total flower production did not differ significantly among the lines (acyanogenic = 9.2 ± 3.4 , cyanogenic = 8.1 ± 3.5 ; $F_{1,2} = 1.1$, $P > 0.2$). Both time to flowering and total flower production differed in directions that are consistent with a cost to cyanogenesis but were not significant, possibly due to the small number of lines available for analysis and the small number of degrees of freedom for the nested analysis.

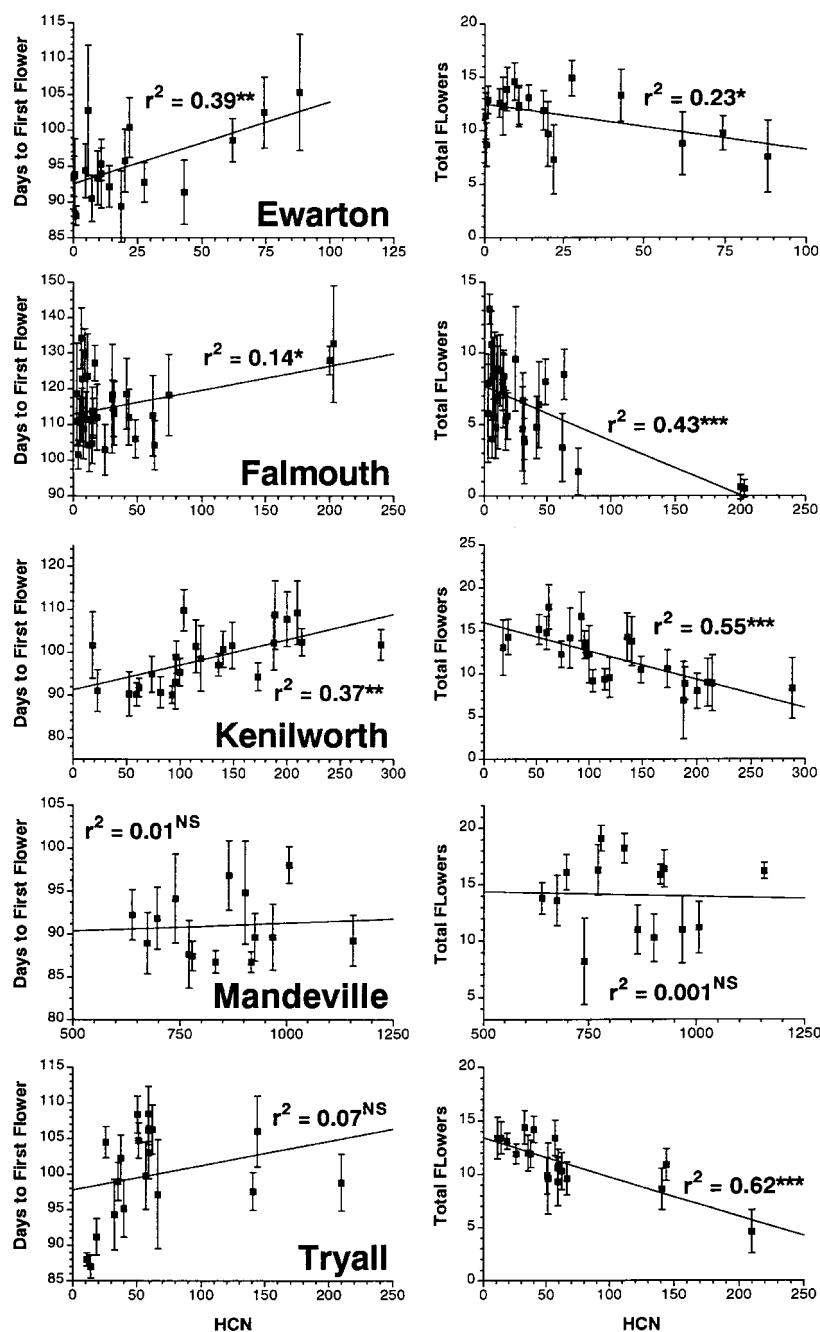


Fig. 3. Relationship, for family means, of time from seedling emergence to first flower (with 95% confidence intervals) and total flower production by day 150 (with 95% confidence intervals) to family mean cyanogenesis in five populations of *T. ulmifolia*. Cyanogenesis (HCN) is measured in μg HCN per gram dry weight of leaf tissue. Linear regression lines and correlation coefficients delineate trends. NS = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

We have previously demonstrated considerable genetically based variation for cyanogenesis among Jamaican populations of *T. ulmifolia* (Schappert and Shore, 1995), but have not clearly identified evolutionary forces responsible for the observed patterns of geographic distribution of cyanogenesis. While some generalizing insect herbivores appear to act as selective agents for cyanogenesis (Schappert and Shore, 1999a), we also require an explanation as to what countervailing forces, if any, might select against cyanogenesis, as a number of Jamaican populations are entirely composed of acyanogenic adult plants (Schappert and Shore, 1995). An assumption of optimality theory is that there should be a cost to plant defence. In the absence of such a cost, the defence should become fixed in populations (Simms and Fritz, 1990). Cyanogenesis appears to have a cost in *Trifolium repens* and *Lotus corniculatus* in response to moisture or cold stress (Daday, 1965; Foulds and Grime, 1972; Abbott, 1977; but see Foulds, 1977; Foulds and Young, 1977) and cyanogenic plants may be poorer competitors than acyanogenic plants in *T. repens* (Dirzo and Harper, 1982b). Briggs and Schultz (1990) reported that cyanogenesis in *L. corniculatus* was lower when the plants were fruiting but that costs of defence change as plants mature.

If there is a cost to cyanogenesis, then, in the absence of herbivores, there should be selection against cyanogenesis. Although populations of *T. ulmifolia* are often small and highly inbreeding (Belaoussoff and Shore, 1995), we have provided evidence for significant broad sense heritability of cyanogenesis and growth and reproductive traits, although we cannot rule out maternal effects contributing to the among-family variance in these open-pollinated families. Note that a single reciprocal cross of two plants from population A2 provided no evidence for maternal effects on cyanogenesis (Schappert and Shore, 1995). Our estimates should be treated as upper limits of heritability for these populations. Phenotypic and, more appropriately, genetic correlations (Simms and Rausher, 1992) provide a means of assessing cost of defence. We have shown that there appears to be a cost to cyanogenesis in three of the five populations, because of the significant negative relationship between total flower production at 150 days and cyanogenesis measured 90 days after germination.

The population with the highest levels of cyanogenesis in adult plants (MANDEV) does not show any evidence of a cost of cyanogenesis and, interestingly, also has the greatest flower production of any of the populations (Table 2). This could indicate that the cost itself has evolved to a low level in this population. Little evidence exists for the evolution of cost in the plant defence literature (Simms and Fritz, 1990). A more mundane explanation, which we cannot rule out, is that the estimate of among-family variance (28%; Table 3) is wholly due to maternal effects and there is no genetic variation for cyanogenesis in this population, leaving no possibility for the occurrence of genetic correlations. Two of the other highly cyanogenic populations (HSHIRE and PORTPT) have about the lowest flower production, which could indicate that cyanogenesis is costly. It is, however, inappropriate to draw conclusions from comparisons among these populations, given that they differ in a number of traits and might be subject to different evolutionary forces.

Remarkably, we have shown that, in controlled glasshouse studies, seedlings from all populations investigated are cyanogenic. This concurs with results from field-sampled plants (Schappert and Shore, 1995). Plants from some populations lose their cyanogenic

status at around the time of first flowering. Although developmental variation for cyanogenesis has been reported in several other species, including *Lotus corniculatus* (Jones, 1962), *Pteridium aquilinum* (Cooper-Driver and Swain, 1976), *Trifolium repens* (Till, 1987) and *Hevea brasiliensis* (Selmar *et al.*, 1988), we believe this to be one of the most marked examples of the loss of expression of cyanogenesis.

Cyanogenesis is widespread in the Turneraceae (Spencer and Seigler, 1980, 1981; Spencer *et al.*, 1985; Olafsdottir *et al.*, 1990; Shore and Obrist, 1992) and in allied families, the Flacourtiaceae (Saupe, 1981; Spencer and Seigler, 1985) and the Passifloraceae (Saupe, 1981; Spencer, 1988; Olafsdottir *et al.*, 1989). Although there is no rigorous phylogeny of the Turneraceae available, this strongly suggests that cyanogenesis is ancestral in the Turneraceae and that acyanogenic populations of *T. ulmifolia* (Schappert and Shore, 1995) arose from cyanogenic populations, perhaps via selection against cyanogenesis. If so, the evolution of acyanogenic populations has proceeded by the developmental loss of cyanogenesis in adult plants, while seedlings remain cyanogenic. Perhaps cyanogenesis is necessary to provide chemical defence against herbivory in seedlings, yet becomes a significant cost and expression is shut down in some habitats on Jamaica. Jones (1971) has suggested that cyanogenesis polymorphism might be of greater significance to seedlings than adult plants. We have not investigated whether selection acts at the seedling level, but appropriate field experiments could be carried out to address this question if sufficient variation in the level of cyanogenesis occurs among seedlings.

A number of workers have described cyanogenic versus acyanogenic species in the Turneraceae (Spencer and Seigler, 1980, 1981; Spencer *et al.*, 1985; Olafsdottir *et al.*, 1990; Shore and Obrist, 1992). Reports of acyanogenic species might, however, be premature given that virtually all the investigations were carried out on adult plants (often from herbarium specimens). A re-investigation of seedlings might be of value in assessing the actual level of cyanogenesis in this family and provide information on the generality of the developmental loss of cyanogenesis in the Turneraceae.

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