

Fungal infection and soybean competition induce plastic responses in velvetleaf (*Abutilon theophrasti*) growth and reproductive output

Jacob N. Barney
Department of Horticulture, Cornell University,
Ithaca, NY 14853

Antonio DiTommaso
Corresponding author. Department of Crop and
Soil Sciences, Cornell University, Ithaca, NY 14853;
ad97@cornell.edu

Hameed A. Baloch
Alan K. Watson
Department of Plant Science, Macdonald Campus
of McGill University, Ste-Anne-de-Bellevue,
PQ H9X 3V9 Canada

The historic maternal environment; the identity of competing, neighboring plants; and biotic stresses, such as fungal disease and herbivory, interact to influence the competitive ability, reproductive output, and plasticity of weed populations. A weed capable of altering its phenotype in response to environmental factors is better able to compete for resources in agroecosystems, thereby reducing crop yields and contributing more seeds to the seed bank. Velvetleaf is a highly competitive annual weed in many North American cropping systems, exhibiting a high degree of phenotypic plasticity in response to biotic and abiotic factors. We examined the effects of seed size (small and large), competitive environment (with and without soybean), and stress level (none = no treatment, moderate = pathogen inoculation, high = pathogen plus herbicide) on velvetleaf allometry and reproductive output during three field seasons. Only under the high-stress treatment was velvetleaf biomass, height, and reproductive output compromised (> 15% each), and these traits were further reduced with soybean competition. Soybean competition alone reduced velvetleaf biomass (> 50%), height (25%), and seed output (50%), but effects of competition varied with stress level. Velvetleaf plants that originated from large seeds (≥ 10 mg) generally outperformed plants originating from small seeds (< 10 mg) except under the high-stress treatment. These findings suggest that velvetleaf life-history traits and reproductive output in the current generation are a function of both present and historic factors. Management strategies for this species should involve tactics that not only reduce its competitive ability (e.g., biomass, height) but also decrease its seed production via synergistic stressors such as increased crop competitiveness, reduced-rate herbicide applications, and pathogen infection.

Nomenclature: Velvetleaf, *Abutilon theophrasti* Medic. ABUTH; soybean, *Glycine max* (L.) Merr. 'Bayfield'.

Key words: Resource allocation, phenotypic plasticity, biocontrol, pathogen, maternal effects, seed size.

Plant community composition and competitive interactions in annual cropping systems are largely governed by stochastic processes (Radosevich et al. 1997). Unlike most crops, weeds are typically able to alter their phenotype to maximize resource capture via changes in height, leaf area, leaf architecture, and resource allocation (Agrawal 2001; Sugiyama and Bazzaz 1997). Phenotypic plasticity in weeds can be induced by both inter- and intraspecific competition, pathogen infection, herbivory, abiotic factors, and the predictability of these environmental variables (Lee and Bazzaz 1981; Lindquist et al. 1995; Wymore and Watson 1989). Continuous cultivation of a single crop ensures a relatively predictable environment, thereby selecting against plastic traits in weedy species (Weinig 2000a). However, practices such as crop rotation and biotic and abiotic factors (i.e., pathogens or herbicides) create an unpredictable environment, selecting for more plastic individuals (Rendon and Nunez-Farfan 2000; Weinig 2000a). Phenotypic plasticity can impact cropping systems for many generations (Ernande et al. 2004; Yeh and Price 2004), especially if increased seed dormancy or seed production are affected when weed individuals are subject to stress or intense competition.

Velvetleaf is a problematic weed in many cropping systems in the United States and Canada. Crop losses attri-

buted to velvetleaf competition are substantial, especially in soybean production (Akey et al. 1990; Begonia et al. 1991; Dekker and Meggitt 1983). Velvetleaf is difficult to control because of its rapid growth rate, asymmetric competitive dominance against interspecific competitors, high seed production, and long-lived seeds with significant physical dormancy (Warwick and Black 1988). Velvetleaf adaptation, plasticity, and seed dormancy have been studied extensively (Sugiyama and Bazzaz 1997, 1998; Weinig 2000a, 2000b, 2000c, 2005; Weinig and Delph 2000). Velvetleaf can increase the number and vigor of seeds produced in response to increased soil nutrient levels (Garbutt and Bazzaz 1987; Sugiyama and Bazzaz 1997), resulting in future generations of more competitive individuals. Additionally, crop rotation determines the direction of genetic selection, creating either stable or plastic velvetleaf individuals (Weinig 2000a, 2005). Therefore, velvetleaf competitive ability and reproductive output are a function of the seed genetics, maternal environment, current neighborhood composition and structure, and biotic and abiotic stresses. However, the interaction of these factors is not well studied.

This study was designed to elucidate both the main and interactive effects of seed size, neighborhood composition, and stress load on velvetleaf growth and reproductive out-

put. Thus, we asked the following questions: (1) Does velvetleaf alter its phenotype in response to neighborhood composition and stress load? (2) Does resource allocation to reproductive organs change under the tested range of stress and competitive environments? (3) Do maternal effects on seed size influence the growth and reproductive potential of velvetleaf? We hypothesized that velvetleaf individuals originating from larger seeds, under no stress, and in monoculture will be larger than individuals originating from smaller seeds in mixture under all stress conditions. Additionally, we theorized that reduced-rate herbicide in combination with pathogen infection would synergistically reduce velvetleaf fitness, more so in a soybean mixture than in a velvetleaf monoculture.

Materials and Methods

Study Site and Experimental Design

Field trials were conducted during the 1997, 1998, and 1999 growing seasons at the Emile A. Lods Agronomy Research Centre, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, PQ, Canada (45°25'N, 73°56'W). Plots were located on a St. Bernard fine sandy loam (Orthic Melanic Brunisol) with an organic matter content of 3.0% and pH of 6.8. In all years, the experimental site was moldboard-plowed during the previous fall and disked and cultivated in the spring. Fertilizer (0–30–45, N–P–K) was applied each year at 250 kg ha⁻¹. Because of the presence of quackgrass [*Elytrigia repens* (L.) Nevski] and yellow nutsedge (*Cyperus esculentus* L.) at the field site in 1999, the area was treated with glyphosate (0.9 kg ai ha⁻¹) before seedbed preparation.

The experimental design consisted of a full factorial arranged in a randomized complete-block design with four replicates. The three factors evaluated were velvetleaf seed size (small: < 10 mg and large: ≥ 10 mg), competitive environment (with and without soybean), and stress level (low = no treatment, medium = inoculation with *Colletotrichum coccodes* (Wallr.) S.J. Hughes, and high = early POST reduced-rate herbicide application plus *C. coccodes* inoculation) established in 1-m by 2-m plots. *Colletotrichum coccodes* has been identified as a potential biocontrol agent for velvetleaf management (Wymore et al. 1988). Typically, this pathogen causes grey-brown foliar lesions on infected velvetleaf plants. Initially, lesions appear as small flecks but later enlarge to become necrotic (Wymore et al. 1988). The efficacy of velvetleaf control with *C. coccodes* is variable in the field, with mortality generally observed with application at the cotyledon stage only (Gotlieb et al. 1986; Wymore and Watson 1989). When the pathogen is applied at later velvetleaf growth stages, it causes a severe anthracnose on inoculated leaves, but although infected plants are stunted and development is delayed, the plant continues to grow once it sheds the diseased leaves. Therefore, application of *C. coccodes* at later life stages (e.g., two- to three-leaf stages), as in this study, serves as a moderate-stress treatment, whereas the combination of a reduced rate of the herbicide bentazon plus *C. coccodes* serves as a high-stress treatment. The use of reduced rates of bentazon and *C. coccodes* in combination has been investigated for the control of velvetleaf (Ahn et al. 2005; Watson et al. 1986). Bentazon is an inhibitor of electron flow at the D1 polypeptide in photosystem II

(Cobb 1992). The inhibition of electron transport generates reactive oxygen species that deleteriously affect unsaturated membrane lipids, ultimately leading to wilting and desiccation of affected plants (Devine et al. 1993).

Velvetleaf seeds for this study were collected annually from at least 20 randomly selected plants from an adjacent soybean field at the study site and were separated into the chosen size fractions. Two seed-size classes were chosen because they provided an adequate number of seeds in each class for the experiment and because they maximized seed-size differences. Each year between May 22 and May 29, soybeans ('Bayfield') were seeded at 350,000 plants ha⁻¹ at a row spacing of 40 cm, using a five-row plot seeder. To break physical dormancy, velvetleaf seeds were soaked in boiling water for 10 s and then placed on filter paper moistened with distilled water in petri dishes at room temperature (~ 21 C) for 24 h. A total of 200 imbibed velvetleaf seeds were hand-scattered throughout each plot on June 3, 1997; May 27, 1998; and May 29, 1999. No velvetleaf plants were observed growing at the field site for at least 4 yr before the start of the study; thus, the number of velvetleaf seeds initially present in the seed bank was likely negligible. Moreover, care was taken during the study to harvest all mature velvetleaf capsules at the site to reduce seed rain. Soybeans were hand-weeded from velvetleaf monoculture plots, and all other weeds, except seeded velvetleaf, were hand-weeded from all plots in all years. Near the center of each 1-m by 2-m plot, two 0.5-m by 0.5-m (0.25 m²) subplots were established and used for data collection.

Inoculum Production

A stock culture of *C. coccodes* was maintained on potato dextrose agar (PDA) slants under mineral oil at 3 C. Mycelia from the stock culture were then transferred to PDA plates and incubated for 10 d at 24 C. Mycelial plugs were removed and mixed in 100-ml modified Richards solution (10 g sucrose, 10 g KNO₃, 2.5 g MgSO₄, 5 g KH₂PO₄, and 0.02 g FeCl₃ · 6H₂O, 150 ml V-8 juice,¹ and distilled water to a final volume of 1 L) (DiTommaso and Watson 1997). Cultures were incubated for 7 d on a rotary shaker (250 rpm) at room temperature (~ 21 C), then conidia were harvested and washed by filtering through four layers of cheesecloth and centrifuging (6,500 × g for 20 min). The spore pellet recovered was subsequently resuspended in distilled water, and the inoculum density was adjusted using a hemocytometer (DiTommaso and Watson 1997).

Pathogen and Herbicide Application

Plots in the pathogen-plus-herbicide treatment received bentazon at 25% of labeled rate (0.24 kg ai ha⁻¹) 17 d after seeding (DAS) with velvetleaf, when velvetleaf was at the two- to three-leaf stage, and soybean was at the first trifoliolate stage. Pathogen inoculum (*C. coccodes*) in both pathogen-only and pathogen-plus-herbicide plots was applied 22 DAS at the two- to three-leaf stage of velvetleaf, at a rate of 10⁸ conidia m⁻² in an oil-in-water formulation (5% corn oil plus 4% oil surfactant [15% of Tween 80 {polyoxyethylene-sorbitan monooleate}] and 85% v/v of Span 80 [Sorbitan monooleate]) using a pressurized hand-held sprayer at 200 kPa delivering 450 L ha⁻¹. The rationale in the pathogen-plus-herbicide treatment for applying bentazon 5 d before

inoculating plants with *C. coccodes* was to weaken velvetleaf plants and make them more susceptible to fungal infection.

Environmental Data

Daily relative humidity and precipitation were obtained for the period beginning 1 wk before, and ending 3 wk after, *C. coccodes* inoculation in all years from Environment Canada for the Ste-Anne-de-Bellevue Station located ~ 1 km from the field site.

Data Collection

A visual disease rating was determined by estimating the percentage of velvetleaf leaves infected with *C. coccodes* (to the nearest 10%) at 31 and 47 d after pathogen application (DAP) in 1997, 7 and 18 DAP in 1998, and 15 and 30 DAP in 1999. Dates when disease ratings were carried out varied for the 3 yr because of differences in disease development due to weather.

To estimate maximum velvetleaf aboveground biomass, one 0.25-m² subplot was harvested for all aboveground biomass at 85 DAS. The mean number of velvetleaf plants per subplot at 14 DAS was 12.5 ± 0.3 (or 50 m⁻²), which was reduced to 7.9 ± 0.09 (or 32 m⁻²) by 85 DAS (first harvest). There were no differences in mortality between treatments (data not shown). At harvest, stem height was recorded from soil level to apical meristem for all plants within the subplot. Plant material was then dried at 65 C for 72 h, and mass was recorded for each velvetleaf individual. One velvetleaf plant from a second 0.25-m² subplot in each main plot was randomly chosen and tagged at the start of the experiment, and mature capsules were collected and stored at 20 C (± 1) throughout production (August to September) until destructive harvest 115 DAS. For each tagged velvetleaf plant, the number of capsules and seeds per capsule were recorded, and 100 seeds were randomly sampled and weighed. For logistical reasons, seed weights were not determined for 1999.

Statistical Analysis

All of the following analyses were conducted for each year individually (1997, 1998, and 1999) and with years pooled to assess year variation and overall trends, respectively. A mixed model (PROC MIXED)² was used to test the effects of seed size, soybean competition, and stress level on velvetleaf height and aboveground dry biomass (natural log transformed) collected at 85 DAS and on individual seed mass and total seeds per plant (square root transformed) collected at 115 DAS. Transformations were performed to homogenize variances. All mixed-model analyses had the block as a random effect and included all interactions among the main effects. Because the number of velvetleaf plants within a subplot was unbalanced, we nested these values within the block as a random effect in the mixed model for data from 85 DAS.

Results and Discussion

Fungal Pathogenicity and Allometric Growth Response

Pathogenicity of *C. coccodes* differed among years and ranged from no visible infection to a maximum 68% infec-

tion of velvetleaf leaves (data not shown). Variability among years was likely due to variation in precipitation, relative humidity, and leaf wetness directly following inoculation (data not shown) (DiTommaso et al. 1996). The percentage of infected leaves was generally greater in the velvetleaf monoculture than in the mixture with soybean ($P < 0.01$ in 1997 and $P < 0.001$ in 1999). This result is surprising given that previous work using this pathogen–weed system has shown that foliar infection levels are substantially greater in velvetleaf grown in mixture with soybean than in velvetleaf grown in monoculture (DiTommaso et al. 1996). It is possible, however, that *C. coccodes* pathogenicity was underestimated in velvetleaf plants grown in mixture with soybean because highly diseased leaves were shed prematurely and thus infection levels for these senesced leaves were not recorded (DiTommaso and Watson 1995). The high-stress treatment (herbicide and *C. coccodes*) did not affect the proportion of infected velvetleaf leaves. We had expected the severity of infection to be greatest for velvetleaf plants subjected to this treatment, and it is unclear why that was not observed in this study. Ahn et al. (2005) reported that when bentazon (0.25 kg ai ha⁻¹) was applied in a tank mixture with *C. coccodes* (10⁹ conidia m⁻²) at the three-leaf stage of velvetleaf, it significantly reduced aboveground biomass of velvetleaf by substantially reducing the activity of the plant defense enzyme, phenylalanine ammonia lyase (PAL) and preventing the activation of peroxidase, thus enhancing the susceptibility of velvetleaf to *C. coccodes* infection.

Velvetleaf stem height varied among years with all main effects and interactions being highly significant, except for the year-by-competition-by-stress-level and seed-size-by-competition-by-stress-level interactions (Table 1). In individual years, main effects and most high-order interactions were significant, demonstrating the plasticity of this life history trait (Table 1). Velvetleaf plants grown in monoculture were, on average, 30% taller than velvetleaf grown with soybean, whereas velvetleaf plants receiving no stress treatment were, on average, 10% and 16% taller than plants receiving the medium- and high-stress treatments, respectively (Tables 2 and 3). These findings are consistent with expectations because the typical response of velvetleaf plants to increased intraspecific competition is increased height to capture a greater proportion of light, and plant stresses, such as pathogens and herbicides, decrease growth (DiTommaso et al. 1996). Surprisingly, velvetleaf plants originating from small seeds were often taller (–5 to 18%) than plants originating from large seeds (Tables 2 and 3).

Aboveground dry biomass was similarly explained by complex three-way and lower interactions when all years were pooled and either three-way (1998 only) or two-way interactions among main effects in individual years—all demonstrating a highly responsive life history trait (Table 1). Velvetleaf plants had greater aboveground biomass (1) in monoculture (110% larger) vs. in soybean mixture, (2) when originating from small seeds (16% larger) vs. large seeds, and (3) under no stress (16% larger) vs. either stress treatment (Tables 2 and 3). Consistent with our results, Paul and Ayres (1987) found greater aboveground common groundsel (*Senecio vulgaris* L.) biomass in monoculture compared with a mixture with lettuce (*Lactuca sativa* L.) and in a control compared with inoculation with the selective rust pathogen *Puccinia lagenophorae* Cooke. Interestingly, velvet-

TABLE 1. Mixed-model ANOVA for velvetleaf height and aboveground biomass at 85 d after seeding. Factors with $P \leq 0.05$ are in bold font.^a

Source	All years		1997		1998		1999	
	Height	Biomass	Height	Biomass	Height	Biomass	Height	Biomass
	P value							
Year (Y)	< 0.0001	< 0.0001	—	—	—	—	—	—
Seed Size (S)	0.0079	0.2494	< 0.0001	< 0.0001	0.0273	0.1117	< 0.0001	< 0.0001
Competition (C)	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Stress Treatment (T)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Y × S	< 0.0001	0.0006	—	—	—	—	—	—
Y × C	< 0.0001	< 0.0001	—	—	—	—	—	—
Y × T	< 0.0001	0.0468	—	—	—	—	—	—
S × C	0.0065	0.4085	< 0.0001	0.0090	0.0001	< 0.0001	0.0078	0.0012
S × T	< 0.0001	< 0.0001	0.4012	0.1191	< 0.0001	< 0.0001	0.1230	0.1413
C × T	< 0.0001	0.0013	< 0.0001	< 0.0001	0.0566	0.0359	0.2451	0.1366
Y × S × C	< 0.0001	< 0.0001	—	—	—	—	—	—
Y × S × T	< 0.0001	< 0.0001	—	—	—	—	—	—
Y × C × T	0.0583	0.1820	—	—	—	—	—	—
S × C × T	0.7313	0.0181	0.0032	0.0692	0.1110	0.0070	0.0073	0.1727
Y × S × C × T	0.0006	0.0224	—	—	—	—	—	—

^a Dashes indicate effect is not applicable.

leaf that originated from the small seed-size class developed into taller and larger plants when compared with velvetleaf that originated from the large seed-size class. It is generally accepted that the greater resources in larger seeds lead to both increased survival and larger seedlings, especially under competitive conditions, than seedlings originating from smaller seeds (Sugiyama and Bazzaz 1997; Susko and Lovett-Doust 2000; Westoby et al. 1996). However, plants originating from small seeds tend to have higher potential growth rates and, thus, may outgrow seedlings originating from larger seeds, especially when resources are not limiting (Gross 1984; Maranon and Grubb 1993; Swanborough and Westoby 1996).

A clear relationship between plant height and biomass existed, regardless of stress treatment: plants with greater aboveground biomass were taller (Tables 2 and 3). This pattern was most evident when comparing velvetleaf in monoculture vs. in mixed plantings (Table 2). Competition from

soybean resulted in a dramatic decrease in velvetleaf size—decreasing both plant height and total aboveground biomass. In this study, soybean plants with their large trifoliate leaves were able to quickly establish and overtop velvetleaf plants, substantially reducing velvetleaf growth. Higgins et al. (1984) found a ninefold decrease in velvetleaf biomass when grown in soybean mixture at very low density (< 1 velvetleaf m^{-2}). The increased stress from pathogen infection (with or without herbicide) resulted in smaller, shorter plants compared with control plants. DiTommaso et al. (1996) also reported reductions in height and biomass of velvetleaf plants inoculated with *C. coccodes* at the two-leaf stage and when grown in mixture with soybean.

The biomass per unit of stem height differed slightly between the small ($0.089 \pm 0.003 \text{ g cm}^{-1}$) and large seed class ($0.080 \pm 0.002 \text{ g cm}^{-1}$). However, soybean competition elicited a large difference in the biomass-to-height ratio, with velvetleaf in monoculture ($0.108 \pm 0.003 \text{ g cm}^{-1}$)

TABLE 2. Effect of seed size and stress treatment under monoculture + soybean competition on velvetleaf height and aboveground dry biomass (SE) at 85 d after seeding for all 3 yr pooled and individually.

Seed size and stress treatment	All years		1997		1998		1999	
	Height	Biomass	Height	Biomass	Height	Biomass	Height	Biomass
	cm	g	cm	g	cm	g	cm	g
Small								
None	123 (2)	17.3 (1.3)	127 (4)	23.8 (2.7)	111 (4)	12.7 (1.9)	117 (5)	13.7 (2.0)
	99 (3)	8.8 (0.7)	119 (4)	15.5 (1.5)	94 (5)	7.3 (0.6)	107 (3)	8.6 (1.0)
Pathogen	107 (3)	14.0 (1.2)	128 (4)	24.3 (2.9)	80 (4)	6.8 (1.5)	97 (4)	9.5 (1.4)
	82 (3)	6.3 (0.6)	116 (4)	14.0 (1.6)	68 (4)	4.1 (0.4)	91 (4)	5.4 (0.6)
Path/herb ^a	114 (3)	15.5 (1.3)	114 (5)	23.3 (3.2)	102 (4)	8.8 (1.0)	113 (5)	12.4 (1.2)
	79 (4)	5.6 (0.7)	97 (5)	9.1 (1.3)	80 (6)	6.4 (0.5)	83 (5)	5.2 (1.0)
Large								
None	118 (2)	14.7 (1.0)	103 (6)	21.7 (3.2)	109 (3)	9.7 (1.1)	123 (3)	13.1 (0.9)
	93 (3)	7.5 (0.8)	124 (5)	20.1 (2.7)	91 (5)	6.3 (0.8)	88 (3)	4.4 (0.4)
Pathogen	110 (2)	12.2 (1.0)	111 (5)	19.6 (3.3)	120 (4)	12.0 (1.2)	99 (3)	7.1 (0.8)
	85 (3)	6.7 (0.7)	130 (4)	20.5 (1.9)	81 (5)	4.9 (0.6)	72 (4)	3.4 (0.5)
Path/herb	97 (3)	11.7 (1.1)	111 (5)	20.7 (3.3)	74 (6)	7.1 (1.6)	89 (4)	8.6 (1.1)
	68 (4)	4.6 (0.7)	96 (7)	8.3 (1.6)	88 (6)	9.7 (1.1)	70 (6)	3.5 (0.7)

^a Abbreviation: Path/herb, pathogen + herbicide.

TABLE 3. Main effect means (SE) for both velvetleaf reproductive and life history characters. Data pooled across years (1997–1999).

Main effect by factor	Dependent variables			
	Seed number	Seed mass	Biomass	Height
	seed plant ⁻¹	mg	g	cm
Seed size				
Small	654 (59)	9.52 (0.02)	11.4 (0.4)	102 (1)
Large	698 (73)	9.62 (0.02)	9.8 (0.4)	97 (1)
Competition				
Monoculture	886 (70)	9.47 (0.02)	14.1 (0.5)	112 (1)
+ soybean	466 (52)	9.67 (0.02)	6.7 (0.3)	86 (1)
Stress treatment				
None	734 (87)	9.55 (0.02)	11.6 (0.5)	107 (1)
Pathogen	686 (80)	9.60 (0.02)	10.0 (0.5)	98 (2)
Path/herb	607 (75)	9.56 (0.03)	10.0 (0.6)	92 (2)

being nearly twofold greater than velvetleaf in soybean mixture ($0.058 \pm 0.002 \text{ g cm}^{-1}$). As mentioned, soybean competition severely reduced velvetleaf growth relative to plants grown in monoculture, with plants in mixture plots being shorter (86 cm) and having relatively fewer and smaller leaves compared with velvetleaf plants grown in monoculture (112 cm). The imposed stress treatments also induced a response in the velvetleaf biomass-to-height ratio (no stress: $0.091 \pm 0.003 \text{ g cm}^{-1}$, medium stress: $0.080 \pm 0.003 \text{ g cm}^{-1}$, and high stress: $0.082 \pm 0.004 \text{ g cm}^{-1}$). Plasticity in this allometric ratio may allow velvetleaf to respond to the identity of neighbors and biotic stressors—maintaining resource capture and seed production in stressful or unpredictable environments.

Reproductive Response

The number of seeds produced per velvetleaf plant and the mass of individual seeds varied among years (Table 4).

Total seed production per plant was strongly influenced by competition in all years and by a three-way interaction among year, seed size, and competition. Averaged across all years, velvetleaf plants in monoculture yielded 90% more seeds per plant than velvetleaf in mixture, plants originating from large seeds yielded 7% more seeds than plants originating from small seeds, and velvetleaf receiving no stress yielded 7 to 21% more seeds per plant than in either pathogen treatment (Table 3). Averaged across all years, control plants (monoculture and no stress) produced nearly fourfold (large-seed cohort) and twofold (small-seed cohort) more seeds per plant compared with plants grown in mixture and subjected to the high-stress treatment (Table 5). DiTommaso et al. (1996) found that *C. coccoodes* inoculation did not affect velvetleaf seed production in monoculture, but when velvetleaf was grown with soybean, *C. coccoodes* inoculation reduced velvetleaf fecundity. Moreover, velvetleaf seed output was reduced by soybean competition and was affected by total plant density both in monoculture and in mixture with soybean (DiTommaso et al. 1996). Similarly, Nurse and DiTommaso (2005) reported that the number of capsules produced per velvetleaf plant was reduced at least 10-fold when grown with corn (16 capsules plant⁻¹) at densities of ~ 50 velvetleaf plants m⁻² relative to velvetleaf grown in monoculture (176 capsules plant⁻¹).

Seed mass varied among years, and most interactions among main effects were significant (Table 4). Seed size was affected by maternal seed size and competitive environment, but was unaffected by stress. However, the effects of maternal seed-size class and competition on velvetleaf seed size differed among stress treatments. Overall, plants originating from large seeds produced slightly heavier seeds (9.62 mg) than plants grown from smaller seeds (9.52 mg), which is evidence of current traits reflecting historic growing conditions (Table 3). Velvetleaf grown in mixture yielded slightly heavier (9.67 mg) seeds than seeds produced by velvetleaf plants in monoculture (9.47 mg) (Table 3). These differences may reflect a source–sink relationship, whereby velvetleaf plants in mixture may allocate a greater proportion

TABLE 4. Mixed-model ANOVA for the number of velvetleaf seeds per plant and seed mass at 115 d after seeding.^a Factors with $P \leq 0.05$ are in bold font.

Source	All years ^b		1997		1998		1999	
	Seeds plant ⁻¹	Seed mass	Seeds plant ⁻¹	Seed mass	Seeds plant ⁻¹	Seed mass	Seeds plant ⁻¹	Seed mass
	P value							
Year (Y)	< 0.0001	< 0.0001	—	—	—	—	—	—
Seed Size (S)	0.7391	< 0.0001	0.4007	0.0007	0.7123	0.0128	0.8578	—
Competition (C)	< 0.0001	< 0.0001	0.0005	0.0886	< 0.0001	< 0.0001	0.0027	—
Stress Treatment (T)	0.1679	0.2022	0.3245	< 0.0001	0.8367	0.0001	0.2485	—
Y × S	0.5867	0.4425	—	—	—	—	—	—
Y × C	0.1161	< 0.0001	—	—	—	—	—	—
Y × T	0.7455	< 0.0001	—	—	—	—	—	—
S × C	0.3992	< 0.0001	0.0435	0.0281	0.3124	< 0.0001	0.7781	—
S × T	0.4454	< 0.0001	0.6556	0.0146	0.7055	< 0.0001	0.7254	—
C × T	0.1465	< 0.0001	0.6072	< 0.0001	0.1838	0.3406	0.4751	—
Y × S × C	0.0417	0.1078	—	—	—	—	—	—
Y × S × T	0.9693	< 0.0001	—	—	—	—	—	—
Y × C × T	0.9046	< 0.0001	—	—	—	—	—	—
S × C × T	0.8238	0.5223	0.5920	0.8385	0.8762	0.2159	0.6073	—
Y × S × C × T	0.7312	0.2779	—	—	—	—	—	—

^a Dashes indicate that effect is not applicable.

^b Only 1997 and 1998 seed-mass data were used in pooled-year (All years) analysis.

TABLE 5. Effect of seed size and stress treatment under monoculture + soybean competition on mean velvetleaf seed production and mean seed mass (SE) for all 3 yr pooled and individually.

Seed size and stress treatment	All years		1997		1998		1999	
	Seed production	Seed mass	Seed production	Seed mass	Seed production	Seed mass	Seed production	Seed mass ^a
	seeds plant ⁻¹	mg	seeds plant ⁻¹	mg	seeds plant ⁻¹	mg	seeds plant ⁻¹	mg
Small								
None	855 (87)	9.3 (0.1)	1,133 (51)	9.2 (0.1)	888 (89)	9.4 (0.1)	545 (87)	—
	516 (90)	9.9 (0.1)	954 (91)	9.8 (0.1)	259 (38)	10.0 (0.1)	334 (61)	—
Pathogen	757 (114)	9.5 (0.1)	1,207 (154)	9.8 (0.1)	646 (68)	9.1 (0.1)	418 (27)	—
	507 (101)	9.7 (0.1)	991 (127)	9.7 (0.1)	312 (28)	9.8 (0.1)	217 (16)	—
Path/herb ^b	853 (92)	9.3 (0.1)	1,154 (68)	9.7 (0.1)	951 (107)	8.9 (0.1)	456 (28)	—
	437 (110)	9.4 (0.1)	871 (162)	9.3 (0.1)	180 (20)	9.6 (0.1)	261 (42)	—
Large								
None	1,116 (180)	9.3 (0.1)	2,137 (153)	9.3 (0.1)	643 (36)	9.2 (0.1)	568 (84)	—
	451 (58)	9.7 (0.1)	647 (52)	9.8 (0.1)	318 (61)	9.6 (0.1)	388 (52)	—
Pathogen	893 (128)	9.6 (0.1)	1,645 (57)	9.9 (0.1)	700 (76)	9.4 (0.1)	333 (12)	—
	587 (107)	9.6 (0.1)	1,074 (141)	9.7 (0.1)	392 (45)	9.5 (0.1)	296 (15)	—
Path/herb	840 (112)	9.9 (0.1)	1,367 (131)	10.1 (0.1)	673 (14)	9.6 (0.1)	481 (65)	—
	300 (68)	9.7 (0.1)	565 (97)	9.5 (0.1)	209 (36)	9.9 (0.1)	128 (12)	—

^a Dash indicates that data were not available.
^b Abbreviation: Path/herb, pathogen + herbicide.

of available resources to the few seeds produced relative to the allocation of resources to the greater number of seeds produced by plants in monoculture (Alkio et al. 2003). Contrary to our findings, Nurse and DiTommaso (2005) found that velvetleaf seeds produced in monoculture were 25% heavier than seeds produced from plants grown in competition with corn (*Zea mays* L.).

Resource Allocation

One mechanism by which plants respond to biotic stress (e.g., inter- or intraspecific competition, pathogen infection, herbivory) is to reallocate resources to maximize competitive ability or reproductive output, i.e., phenotypic plasticity (Agrawal 2001; Schlichting 1986). The stresses imposed on velvetleaf in this study, soybean competition and pathogen

infection, induced both allometric and reproductive responses. Velvetleaf has been shown to sacrifice aboveground biomass for seed production in soybean cropping systems (Akey et al. 1991), demonstrating its plasticity in response to interspecific competition (i.e., to neighbor identity). In our study, we focused on the trade-off between aboveground biomass and reproduction and seed number for seed mass as a function of the interaction between neighbor identity and stress level.

Soybean competition induced a dramatic decrease in velvetleaf aboveground biomass and total seed production (Figure 1) while increasing mean seed mass (Figure 2). Similarly, Nurse and DiTommaso (2005) found that velvetleaf grown in monoculture produced ~ 27 seeds g⁻¹ aboveground dry mass, whereas under corn competition, the relationship was reduced to ~ 16 seeds g⁻¹ aboveground dry mass. Mean

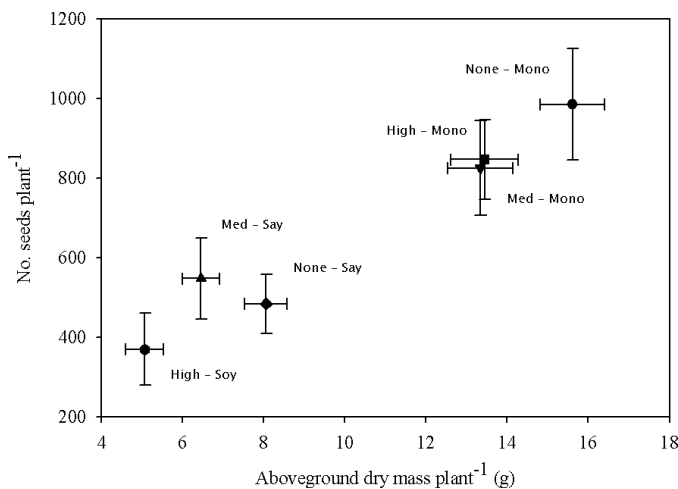


FIGURE 1. Velvetleaf seed production and aboveground biomass means (\pm SE) for each competition–stress treatment combination. Data pooled across years (1997 to 1999) and initial seed size classes.

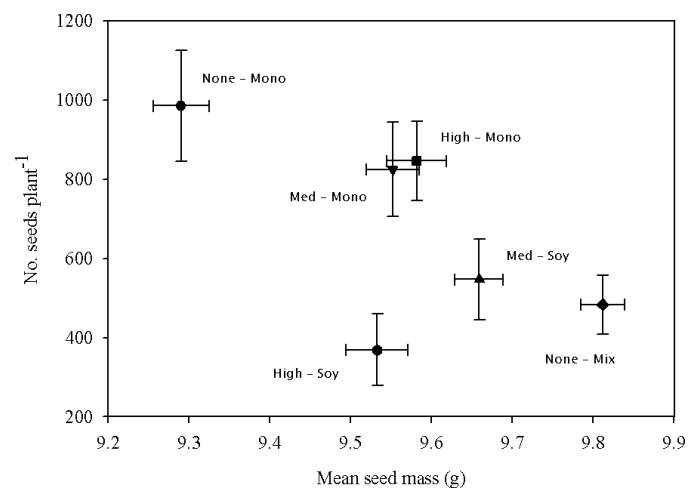


FIGURE 2. Velvetleaf seed production and harvested seed mass means (\pm SE) for each competition–stress treatment combination. Data pooled across years (1997 to 1999) and initial seed-size classes.

velvetleaf seed mass was ~ 200 to 400 µg greater in soybean mixture than in monoculture—a small but ecologically important difference. Studies of velvetleaf (Baloch et al. 2001; Garbutt and Bazzaz 1987; Nurse and DiTommaso 2005) and other weedy species (Susko and Lovett-Doust 2000) have shown that very small differences in seed mass can result in differences in seed dormancy, seedling emergence, and vigor, although other physiologically related factors may also be involved. Therefore, the more unpredictable, soybean-mixture environment may have induced velvetleaf plants to produce fewer but heavier seeds at the expense of aboveground biomass. However, this effect can also be due to differences in source–sink ratios between velvetleaf plants grown in monoculture and in mixture. The larger seeds produced in mixture may be more dormant and produce more vigorous seedlings—favorable traits in competitive (shade) and unpredictable environments (Gross 1984; Rees 1996). Work by Nurse and DiTommaso (2005), showing that velvetleaf seeds have lower weights and dormancy when produced from plants grown in competition with corn compared with plants grown in monoculture, suggests that this relationship may not be consistent for different competing species. Within each competitive environment, velvetleaf resource-allocation patterns were influenced by the stress level imposed. The no-stress treatment resulted in the tallest, heaviest individuals, regardless of competitive environment (Figure 1). In monoculture, the medium- and high-stress treatments resulted in velvetleaf individuals of comparable size (biomass), whereas soybean competition caused the high-stress individuals to be ~ 30% smaller than the medium-stress plants (Figure 1). No differences in total seed production within a competitive environment (Figure 1) were observed, suggesting no trade-off of aboveground biomass for reproductive output. However, stress levels did alter mean seed mass without affecting total seed production within a competitive environment. Velvetleaf individuals under no stress in monoculture produced the lightest seeds, whereas in soybean-mixture velvetleaf, individuals under no stress yielded the heaviest seeds (Figure 2). In monoculture, there were no differences between the medium- and high-stress treatments in velvetleaf seed mass, whereas in mixture, the high-stress treatment yielded the lightest seeds.

Stress levels have different effects on the velvetleaf phenotype depending on the competitive environment. In monoculture, there is no difference between the medium- and high-stress treatments with respect to aboveground biomass and reproductive output (seed mass and number). However, soybean competition acted synergistically with pathogen infection to reduce both velvetleaf aboveground biomass and reproductive output. This synergistic interaction resulted in the smallest individuals producing the fewest, lightest seeds. However, velvetleaf in mixture and under high-stress produced heavier seeds, compared with velvetleaf in monoculture (Figure 2). These numerous, light seeds produced under no competition or stress might reflect the predictability of the environment, whereas the stressful, more unpredictable environment yielded fewer, heavier seeds (Weinig 2005). These results demonstrate the ability of velvetleaf to adapt locally to its habitat, whether agroecosystems, with relatively predictable environment with low microsite variation, or weedy fields, with relatively unpredict-

able environment with high microsite variation (Weinig 2005).

Results from this study demonstrate that velvetleaf is a highly plastic weed, capable of altering its phenotype in response to neighborhood composition and stress level. Moreover, this plasticity is influenced by maternal seed effects (i.e., historic growing conditions). Plants grown in the absence of stress were larger and produced greater numbers of seed, whereas plants experiencing medium and high stress were able to maintain high reproductive output at the expense of vegetative growth. Soybean competition acted synergistically with pathogen infection, dramatically reducing size (competitive ability) and reproductive output of velvetleaf. Weed managers aim to reduce weed competitive ability to increase crop performance and yield while often ignoring weed seed production. However, our results demonstrate that seed production is maintained in seemingly compromised velvetleaf individuals. Effective long-term weed management should incorporate strategies that induce enough stress on target weeds to compromise both their competitive ability and reproductive output.

Sources of Materials

¹ Campbell Soup Co., Ltd., 60 Birmingham Street, Toronto, ON M8V 2B8, Canada.

² Statistical Analysis Systems (version 9.1), SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513.

Acknowledgments

H.A.B. is grateful to the government of Canada for financial support through a Canadian Commonwealth Scholarship. We thank Anurag Agrawal, Daniel Brainard, Andrew McDonald, Charles Mohler, and two anonymous reviewers for providing valuable comments on an earlier version of this manuscript.

Literature Cited

- Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* 294:321–326.
- Ahn, B., T. Paulitz, S. Jabaji-Hare, and A. Watson. 2005. Enzyme responses of *Abutilon theophrasti* in an enhanced biocontrol system. *BioControl* 50:803–817.
- Akey, W. C., T. W. Jurik, and J. Dekker. 1990. Competition for light between velvetleaf (*Abutilon theophrasti*) and soybean (*Glycine max*). *Weed Res.* 30:403–411.
- Akey, W. C., T. W. Jurik, and J. Dekker. 1991. A replacement series evaluation of competition between velvetleaf (*Abutilon theophrasti*) and soybean (*Glycine max*). *Weed Res.* 31:63–72.
- Alkio, M., A. Schubert, W. Diepenbrock, and E. Grimm. 2003. Effect of source–sink ratio on seed set and filling in sunflower (*Helianthus annuus* L.). *Plant Cell Environ.* 26:1609–1619.
- Baloch, H. A., A. DiTommaso, and A. K. Watson. 2001. Intrapopulation variation in *Abutilon theophrasti* seed mass and its relationship to seed germinability. *Seed Sci. Res.* 11:335–343.
- Begonia, G. B., R. J. Aldrich, and C. D. Salisbury. 1991. Soybean yield and yield components as influenced by canopy heights and duration of competition of velvetleaf (*Abutilon theophrasti* Medik.). *Weed Res.* 31:117–124.
- Cobb, A. 1992. *Herbicides and Plant Physiology*. London: Chapman and Hall. 176 p.
- Dekker, J. and W. F. Meggitt. 1983. Interference between velvetleaf (*Abutilon theophrasti* Medic.) and soybean [*Glycine max* (L.) Merr.], I: growth. *Weed Res.* 23:91–101.
- Devine, M., S. O. Duke, and C. Fedtke. 1993. Herbicidal inhibition of photosynthetic electron transport. Pages 113–140 in M. Devine, S. O. Duke, and C. Fedtke, eds. *Physiology of Herbicide Action*. London: Prentice Hall.

- DiTommaso, A. and A. K. Watson. 1995. Impact of a fungal pathogen, *Colletotrichum coccodes* on growth and competitive ability of *Abutilon theophrasti*. *New Phytol.* 131:51–60.
- DiTommaso, A. and A. K. Watson. 1997. Effect of the fungal pathogen, *Colletotrichum coccodes*, on *Abutilon theophrasti* height hierarchy development. *J. Appl. Ecol.* 34:518–529.
- DiTommaso, A., A. K. Watson, and S. G. Hallett. 1996. Infection by the fungal pathogen *Colletotrichum coccodes* affects velvetleaf (*Abutilon theophrasti*)–soybean competition in the field. *Weed Sci.* 44:924–933.
- Ernande, B., U. Dieckmann, and M. Heino. 2004. Adaptive changes in harvested populations: plasticity and evolution of age and size at maturation. *Proc. R. Soc. Lond. B Biol. Sci.* 271:415–523.
- Garbutt, K. and F. A. Bazzaz. 1987. Population niche structure: differential response of *Abutilon theophrasti* progeny to resource gradients. *Oecologia (Berl.)* 72:291–296.
- Gotlieb, A. R., A. K. Watson, and C. Poirier. 1986. First report of an isolate of *Colletotrichum coccodes* which results in severe anthracnose of velvetleaf. *Plant Dis.* 71:281.
- Gross, K. L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *J. Ecol.* 72:369–387.
- Higgins, R. A., D. W. Stanforth, and L. P. Pedigo. 1984. Effects of weed density and defoliated or undefoliated soybeans (*Glycine max*) on velvetleaf (*Abutilon theophrasti*) development. *Weed Sci.* 32:511–519.
- Lee, T. D. and F. A. Bazzaz. 1981. Effects of defoliation and competition on growth and reproduction in the annual plant *Abutilon theophrasti*. *J. Ecol.* 68:813–821.
- Lindquist, J. L., B. Maxwell, D. D. Buhler, and J. L. Gunsolus. 1995. Velvetleaf (*Abutilon theophrasti*) recruitment, survival, seed production and interference in soybean (*Glycine max*). *Weed Sci.* 43:226–232.
- Maranon, T. and P. J. Grubb. 1993. Physiological basis and ecological significance of the seed size and relative growth rate relationship in Mediterranean annuals. *Funct. Ecol.* 7:591–599.
- Nurse, R. E. and A. DiTommaso. 2005. Corn competition alters the germinability of velvetleaf (*Abutilon theophrasti*) seeds. *Weed Sci.* 53:479–488.
- Paul, N. D. and P. G. Ayres. 1987. Effects of rust infection of *Senecio vulgaris* on competition with lettuce. *Weed Res.* 27:431–441.
- Radosevich, S. R., J. S. Holt, and C. Ghersa. 1997. *Weed Ecology: Implications for Management*. 2nd ed. New York: J Wiley. 608 p.
- Rees, M. 1996. Evolutionary ecology of seed dormancy and seed size. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351:1299–1308.
- Rendon, B. and J. Nunez-Farfan. 2000. Population differentiation and phenotypic plasticity of wild and agrestal populations of the annual *Anoda cristata* (Malvaceae) growing in two contrasting habitats. *Plant Ecol.* 156:1–9.
- Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* 17:667–693.
- Sugiyama, S. and F. A. Bazzaz. 1997. Plasticity of seed output in response to soil nutrients and density in *Abutilon theophrasti*: implications for maintenance of genetic variation. *Oecologia (Berl.)* 112:35–41.
- Sugiyama, S. and F. A. Bazzaz. 1998. Size dependence of reproductive allocation: the influence of resource availability, competition and genetic identity. *Funct. Ecol.* 12:280–288.
- Susko, D. J. and L. Lovett-Doust. 2000. Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). *Am. J. Bot.* 87:56–66.
- Swanborough, P. and M. Westoby. 1996. Seedling relative growth rate and its components in relation to seed size: phylogenetically independent contrasts. *Funct. Ecol.* 10:176–184.
- Warwick, S. I. and L. D. Black. 1988. The biology of Canadian weeds, 90: *Abutilon theophrasti*. *Can. J. Plant Sci.* 68:1068–1085.
- Watson, A. K., A. R. Gotlieb, and L. A. Wymore. 1986. Interactions between a mycoherbicide, *Colletotrichum coccodes*, and herbicides for control of velvetleaf (*Abutilon theophrasti* Medic.). *Weed Sci. Soc. Am. Abstr.* 26:52–53.
- Weinig, C. 2000a. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution* 54:124–136.
- Weinig, C. 2000b. Limits to adaptive plasticity: temperature and photoperiod influence shade-avoidance responses. *Am. J. Bot.* 87:1660–1668.
- Weinig, C. 2000c. Plasticity versus canalization: population differences in the timing of shade-avoidance responses. *Evolution* 54:441–451.
- Weinig, C. 2005. Rapid evolutionary responses to selection in heterogeneous environments among agricultural and nonagricultural weeds. *Int. J. Plant Sci.* 166:641–647.
- Weinig, C. and L. F. Delph. 2000. Phenotypic plasticity early in life constrains developmental responses later. *Evolution* 55:930–936.
- Westoby, M., M. R. Leishman, and J. Lord. 1996. Comparative ecology of seed size and dispersal. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351:1309–1318.
- Wymore, L. A., C. Poirier, A. K. Watson, and A. R. Gotlieb. 1988. *Colletotrichum coccodes*, a potential bioherbicide for control of velvetleaf (*Abutilon theophrasti*). *Plant Dis.* 72:534–538.
- Wymore, L. A. and A. K. Watson. 1989. Interaction between a velvetleaf isolate of *Colletotrichum coccodes* and thidiazuron for velvetleaf (*Abutilon theophrasti*) control in the field. *Weed Sci.* 37:478–483.
- Yeh, P. J. and T. D. Price. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am. Nat.* 164:531–542.

Received December 8, 2005, and approved May 23, 2006.