

Phytotoxicity and Potential Allelopathy in Pale (*Cynanchum rossicum*) and Black swallowwort (*C. nigrum*)

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The introduced exotic vines pale and black swallowwort rapidly have become invasive throughout regions of the northeastern United States and adjoining areas of Canada. Preliminary studies have reported that the species are allelopathic, possibly contributing to their competitive ability and invasiveness. Results from our laboratory assays indicated that swallowwort root exudates caused significant root length reductions (e.g., 40% for butterfly milkweed and 20% for large crabgrass) and reduced germination (e.g., 25% for lettuce) of indicator species. Additional bioassays with dried swallowwort tissues demonstrated that tissue leachates caused varied responses in indicators, with both significant stimulatory and inhibitory effects. In particular, significant congeneric interactions were noted between the two swallowwort species. Evidence from this study of swallowwort tissue phytotoxicity has important implications for developing effective management and habitat restoration strategies for the two invasive species.

Nomenclature: Annual bluegrass, *Poa annua* L.; barnyardgrass, *Echinochloa crus-gallis* L. P. Beauv.; black swallowwort, *Cynanchum nigrum* (L.) Pers., non Cav.; butterfly milkweed, *Asclepias tuberosa* L.; common milkweed, *Asclepias syriaca* L.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.; orchardgrass, *Dactylis glomerata* L.; pale swallowwort, *Cynanchum rossicum* (Kleopow) Borhidi; lettuce, *Lactuca sativa* L.; tomato, *Solanum lycopersicum* L.

Key words: Allelopathy; black swallowwort; interference; invasive species; pale swallowwort; phytotoxicity.

Pale (PSW) [*Cynanchum rossicum* (Kleopow) Borhidi] and black swallowwort (BSW) [*Cynanchum nigrum* (L.) Pers., non Cav.] are invasive plant species that were introduced into North America over a hundred years ago from the Ukraine and the Iberian Peninsula, respectively (DiTommaso et al. 2005b). Although in their native ranges the two vines are relatively rare, in novel habitats they have spread aggressively and typically establish impenetrable, vertical thickets (DiTommaso et al. 2005b; Weston et al. 2005). Dense infestations have displaced and threatened endemic and rare flora, disturbed unique alvar ecosystems in western New York, and also have led to reductions in arthropod and avian populations in old-field sites (Di-

Tommaso et al. 2005b; Douglass et al. 2009; Ernst and Cappuccino 2005; Lawlor 2000). Furthermore, the swallowwort species are becoming increasingly common in perennial and no-till agricultural and horticultural croplands, and have been especially damaging in tree nurseries and orchards (Douglass et al. 2009; Lawlor 2003; Weston et al. 2005).

Possible explanations for the success of invasive swallowworts include reproductive and vegetative characteristics that make the two species highly competitive. Both species produce large quantities of wind-dispersed seeds, many of which, particularly in PSW, are polyembryonic (a condition in which two or more embryos develop from a single egg) (Blanchard et al. 2010; Douglass et al. 2009; Hotchkiss et al. 2008). Although there have been reports that swallowwort plants formed stout rhizome-like roots, recent studies have concluded that these structures do not support vegetative propagation or spread (K. Averill, personal communication; Cappuccino 2004; Lumer and Yost 1995). Additionally, the species have large root-to-shoot biomass ratios, with a ratio of 6.7 for PSW plants in northern New York (Smith 2006). Finally, several authors have proposed that the monocultural nature of swallowwort stands in North America could be due in part to the

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Interpretive Summary

Our laboratory studies provided evidence for the negative impact of pale (PSW) and black swallowwort (BSW) tissue leachates and root exudates upon the growth and germination of indicator species, in particular common and butterfly milkweed. Additionally, we found important congeneric interactions between the two species, with results suggesting a possible competitive advantage for BSW. Our study findings indicate that allelopathic interference might help to facilitate the dominance of swallowworts in cases of interspecific competition. However, field studies are needed to demonstrate whether this interference occurs under natural conditions, and whether it ultimately has an ecological significance in the invasiveness of the swallowworts in natural settings.

With regard to management, these results suggest that there could be unexpected ramifications to what is currently one of the recommended control strategies (in addition to chemical controls) for the two species—mowing. Mowing can be effective at preventing populations from spreading if timed to coincide with seed set and repeated throughout the growing season. Our research suggests that the abundant swallowwort leaf and stem material remaining on the soil surface after mowing potentially could release biologically active compounds into the soil rhizosphere as it decomposes. These leachates have the potential to inhibit the germination and seedling growth of sensitive plant species in the seedbank, or those used to restore treated sites. Consequently, if mowing is used to control invasive swallowwort populations, mowed biomass should be collected and removed from the treated site if possible.

release of allelochemicals into the rhizosphere from either tissue leachates or standing biomass (Cappuccino 2004; DiTommaso et al. 2005b; Weston et al. 2005). This putative allelopathic activity has been hypothesized to alter mycorrhizal fungi associations that significantly benefit the competitive dominance of PSW in particular (DiTommaso et al. 2005b; Greipsson and DiTommaso 2006; Smith 2006; Smith et al. 2008). Mogg et al. (2008) proposed that the presence of antifungal compounds (most notably (-)-antofine) in introduced populations of *C. rossicum* could allow the species to escape Old World pathogenic associations.

Pale and black swallowwort historically have been used for medicinal purposes in their native ranges, and roots of related *Cynanchum* species are known to contain the poisonous glycoside vincetoxin (Weston et al. 2005). Also, Nowak and Kisiel (2000) reported the isolation of the triterpenoid hancokinol from aerial portions of PSW plants, and determined that this structure possessed antitumor activity. Numerous authors also have reported that (-)-antofine, a phenanthroindolizidine alkaloid isolated from roots, fruits, and leaves of swallowwort species has pronounced antifungal, antibacterial, and antifeedant properties (Capo and Saa 1989; Mogg et al. 2008). For example, Mogg et al. (2008) found that root extracts containing > 95% pure (-)-antofine resulted in significant

growth reductions in a wide variety of fungal growth forms. However, although the authors found high concentrations of the alkaloid (-)-antofine in PSW root extracts, they concluded that the toxic and antifeedant properties of the crude extracts were actually due to a separate, unidentified polar compound in the acidic fraction of the root tissue. Cappuccino (2004) was the first to study directly whether such compounds had allelopathic effects, and found that aqueous PSW root extracts inhibited germination of radish (*Raphanus sativus* L.) seeds. However, studies on the phytochemical constituents of the swallowwort species have focused specifically on their cytotoxic and antibiotic properties, rather than allelopathic interactions of the chemical compounds with neighboring plant species.

Therefore, the objectives of this study were to determine whether swallowwort root exudates or tissue leachates exhibited significant phytotoxic or allelopathic activity. Specifically, we wished to determine the impact of: (1) root exudates of both swallowwort species on germination and growth of selected juvenile indicator species; and (2) leachates from dried swallowwort tissues on germination and growth of selected indicator species.

Materials and Methods

Swallowwort (SW) Seed Stratification Protocol. PSW seeds were collected at a patchy forest understory site at the Mulholland Wildflower Preserve in Ithaca, NY (42°25'97"N, 76°29'05"W) in September 2006. BSW seeds were collected at an understory site at Mashomack Nature Preserve in Shelter Island, NY (41°03'58"N, 72°17'49"W) and in an open field besides the Dutchess County Cornell Cooperative Extension office in Millbrook, NY (41°46'76"N, 73°44'40"W), in September 2006. The two BSW seed lots were mixed before being used for subsequent assays. All seeds were sorted to cull individuals with visibly abnormal or enlarged endosperms in order to improve germination probabilities (DiTommaso et al. 2005a; Smith 2006). Seeds were placed on three layers of filter paper¹ in a vacuum filtration flask, and rinsed for 30 s in 100 ml (3.38 oz) deionized water. Seeds were sterilized for 30 s in 100 ml of 50% ethanol, and rinsed three times (each for 30 s) in 100 ml of deionized water.

Germination paper² disks were placed in the bottom of round 100 by 20 mm (3.94 by 0.79 in) glass Petri dishes. Three milliliters of deionized water were added to moisten the paper disk, and up to 65 PSW seeds or 45 BSW seeds placed in each Petri dish. A second disk of germination paper was placed on top of the seeds and moistened with an additional 3 ml of deionized water. Petri dishes were sealed with Parafilm and placed in a refrigerator at 1 C (33.8 F) and 20% relative humidity (RH) for 6 wk of cold stratification (Blanchard et al. 2010). Petri dishes were

checked every several days for adequate moisture, and deionized water was added in 500 µl increments when necessary to ensure that germination paper disks remained moist. Simultaneously, seeds were checked for fungal growth, and those seeds with prolific fungal contamination were removed.

Preparation of Agar. A 0.5% (v/v) solution of Sonneveld nutrient solution (Sonneveld and Straver 1994) was prepared in deionized water. A 10 g L⁻¹ (0.35 oz gal⁻¹) solution was prepared by mixing agar powder³ into the Sonneveld mixture and heating the solution until the liquid boiled and was clear. One hundred and fifty ml of Sonneveld-enriched agar gel was poured into a Magenta box⁴ and autoclaved (Bertin et al. 2003).

Growth and Germination Responses to SW Root Exudates. Five seeds of each swallowwort species were randomly selected from stratified seeds. Seeds were placed in a 100 ml glass beaker with 1 ml of 50% (v/v) ethanol and the solution agitated for 30 s. Seeds were drained on a metal mesh sieve, rinsed in deionized water for 30 s, and blotted dry with paper towels. Five surface-sterilized seeds were placed atop the agar gel in a Magenta box in an “X” pattern. Magenta boxes were subsequently placed 5 cm (1.97 in) below fluorescent lights (Sylvania 34 W Cool White Supersaver Ecologic T12 bulbs⁵) on stainless steel shelves in the laboratory. Ambient conditions were 25 ± 2 C and 37 ± 3% RH for the duration. After 5 d of growth (a period which corresponded to at least 75% germination of SW seeds) indicator species were seeded into the Magenta boxes, and permitted to grow for 15 d. Germination of indicator species was monitored daily, and at the conclusion of the experiment root length and shoot length for each germinated seed was measured.

Indicator plants were chosen to evaluate in these studies because they were known to germinate reliably—i.e., lettuce (*Lactuca sativa* L. ‘Black seeded Simpson’⁶) and large crabgrass, [*Digitaria sanguinalis* (L.) Scop.⁷—or because these species naturally cohabit with swallowworts in New York State—i.e., butterfly milkweed (*Asclepias tuberosa* L.⁸), common milkweed (*Asclepias syriaca* L.⁹), and orchardgrass (*Dactylis glomerata* L.¹⁰). Seeds of these species were sterilized using the surface-sterilization protocol described previously and were placed on agar in Magenta boxes around germinated SW seeds so that no indicator seed was more than 5 mm away from any one SW seed. Twenty-five seeds of the two monocot (grass) indicators and lettuce were added to each Magenta box, but only five seeds of the two milkweed species were used for the experiment due to their large size. Experiments were arranged as a Randomized Complete Block Design (RCBD) with trials using lettuce and large crabgrass having three replicates each and repeated twice over time, and trials with the remaining indicator species run only once

with six replicates per treatment. Variation in number of replicates in experiments was due to spatial constraints. Treatments in each block were rerandomized every 7 d for the duration of the experiment.

Growth and Germination Responses to SW Tissue Leachates. A modification of the Parker (1966) soil-plant residue assay similar to that used by Weston et al. (1989) was utilized to determine the phytotoxicity of swallowwort plant tissues leachates. Whole PSW plants were collected at the Mulholland Wildflower Preserve in Ithaca, NY on June 19, 2006. BSW plants were collected in Millbrook, NY on June 21, 2007. Roots, stems, and leaves were separated, then dried at 65 C for 2 h, and 35 C for 5 d. Dried leaves were placed into a Ziploc plastic bag and hand-crushed until a mixture of evenly sized pieces (0.2 to 0.3 cm²) was obtained. Roots were cut away from the root crown, then placed into a Ziploc plastic bag and hand-crushed until pieces were 10 to 15 cm in length. Dried stems were cut into 2-cm segments and then ground further using a small electric coffee grinder¹¹ pulsed for 15 to 20 s.

Williamson very fine sandy loam soil (mixed, active, mesic Fragiudept) was collected from the Bluegrass Lane Turf and Landscape Research Center (42°27'36"N, 76°27'40"W) in Ithaca, NY and oven dried for 12 h at 80 to 90 C. Soil was sifted using a 2-mm mesh metal sieve and combined with dried fine quartz sand 1:1 v/v. For each experimental unit, 100 g of soil mixture was placed in the bottom half of a 100 by 15 mm square plastic Petri dish. Either 0.5 or 1 g (0.02 or 0.04 oz) of one of three types of prepared SW tissue was weighed and spread evenly across the surface of the soil media. An additional 50 g of the soil mixture was then placed on top of the plant tissue and 30 ml of deionized water was carefully placed onto the soil surface to moisten the dish. A single piece of filter paper was placed on top of the moistened soil and an additional 5 ml of deionized water added to evenly wet the filter paper.

Seeds of 10 indicator species—Annual bluegrass (*Poa annua* L.),⁷ Barnyardgrass [*Echinochloa crus-gallis* (L.) P. Beauv.],¹² BSW, butterfly and common milkweed, large crabgrass, lettuce, PSW, orchardgrass, and tomato (*Solanum lycopersicum* L. ‘Red Pear’¹³)—were placed in two evenly spaced rows across the moistened filter paper. Pairs of monocot and dicot indicator species (monocots: barnyardgrass and large crabgrass; annual bluegrass and orchardgrass; dicots: lettuce and tomato; butterfly and common milkweed; BSW and PSW) were seeded at the same time in arranged pairings in the same Petri dishes. Ten seeds per row were used for all monocots, lettuce, and tomato. Five to six seeds per row were placed in dishes containing the other species due to larger seed size. To better simulate field conditions, seeds of indicator species for this experiment were not presterilized. SW and

milkweed seeds were cold stratified for a period of 6 wk before seeding, as previously described (Blanchard et al. 2010). Once seeds were organized in each dish, lids of the Petri dishes were firmly secured with several 3 to 4 cm pieces of masking tape, and dishes were placed vertically to encourage downward root growth. Germination of indicator species was monitored daily, and at the conclusion of the experiment root length and shoot length for each germinated seed was measured.

Treatments consisted of combinations of the two SW tissue weights (0.5 and 1.0 g), three tissue types (root, shoot, and leaf), and the two SW species. Control treatments consisted of Petri dishes containing soil and filter paper, but with plant tissue replaced by 0.75 g of shredded (1.5 by 1.5 cm) unbleached paper toweling (Weston et al. 1989). The experiment was repeated twice for barnyardgrass, large crabgrass, lettuce, and tomato, with each trial containing three replicates. For the remaining species, the experiment was conducted once with each treatment replicated four times. Each trial of the experiment contained a number of blocks equal to the number of treatment replicates, with the order of the Petri dishes containing the indicator seed pairs randomized. All dishes and blocks were rerandomized every other day during the 7-d experiment. After seeding, dishes were maintained vertically in the dark at room temperature (24 C).

Statistical Analyses. Prior to analysis of variance (ANOVA), all data were tested for homogeneity of population variances using Levene's test, and equality of variances using plots of residuals and Shapiro-Wilks tests. When proper assumptions were met, data were run using RCBD ANOVA models. Indicator species populations did not share equivalent standard deviations, and so for model terms including this factor Welch test statistics were calculated, given the ability for this test to account for the presence of heterogeneous variances (Clinch and Keselman 1982; Zimmerman and Zumbo 1993). Student's *t* tests or Tukey Honestly Significant Differences (HSD) tests were used for treatment comparisons in experiments when appropriate. Significance of treatment means were compared with the control (i.e., 100% or no change) using Dunnett's Method. All statistical analyses were conducted using JMP software (version 7).¹⁴

Results

Elongation and Germination Responses to SW Root Exudates. PSW root exudates reduced butterfly milkweed (39%, $P < 0.0001$) and large crabgrass (16%, $P < 0.025$) root elongation, and shoot elongation in orchardgrass (17%, $P < 0.025$; Figure 1). BSW root exudates had a negative effect on shoot growth of common milkweed

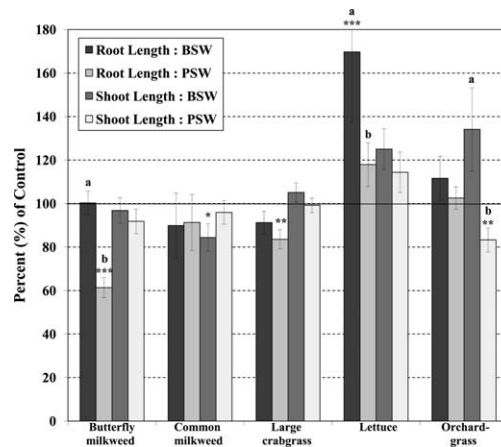


Figure 1. Root and shoot length (as percentage of control) of indicator species (butterfly and common milkweed, large crabgrass, lettuce, and orchardgrass) when grown in the presence of black swallowwort (BSW) and pale swallowwort (PSW) seedlings for 15 d. Each value is the mean from two repetitions, with three replicates per repetition. Vertical bars represent \pm standard error (SE). Significance of difference from control (100%, solid line) for individual treatments means is indicated by *, **, and *** for $P < 0.05$, 0.025 and 0.001 , respectively. Lower-case letters indicate significantly ($P < 0.05$) different effects of swallowwort (SW) species origin (BSW or PSW) on root or shoot elongation, from Student's *t* test.

(16%, $P < 0.05$) only (Figure 1). In contrast, BSW root exudates significantly stimulated root growth of lettuce (70%, $P < 0.0001$; Figure 1). PSW exudates had no significant influence on germination of indicator species, while BSW root exudates caused a 25% ($P < 0.025$) reduction in lettuce germination (Table 1).

Elongation and Germination Responses to SW Tissue Leachates. Indicator species identity was a highly significant factor in the analysis of variance (ANOVA) for all three bioassay response variables ($P < 0.0001$ for root and shoot length, and final germination (Table 2). Swallowwort tissue weight (0.5 or 1 g) also significantly affected growth and germination responses for indicator species, but to a lesser extent than indicator species choice ($P < 0.0001$, $P = 0.0051$ and $P = 0.0001$, respectively; Table 2). Tissue origin (BSW or PSW) significantly influenced indicator species root length and germination, $P = 0.0056$ and $P = 0.0258$, respectively (Table 2). Finally, swallowwort tissue type (leaf, root, or shoot) was only a significant factor ($P < 0.0001$) for root length of indicator species (Table 2).

Without exception, higher-level interactions were highly significant ($P < 0.0001$) for all three response variables when they included indicator species as a term (Table 2). There was a great deal of variety in indicator species responses to treatments, both in effect direction (i.e.,

Table 1. Final germination of indicator species (butterfly and common milkweed, large crabgrass, lettuce and orchardgrass) as affected by the presence of pale swallowwort (PSW) and black swallowwort (BSW) seedlings, from laboratory light bench trials in agar-filled Magenta boxes in which indicators species were grown with swallowworts (SWs) for 15 d. Each value is the mean from two repetitions and three replicates per repetition. Absolute values for control (percentage of seeds germinated with no SWs present) are shown at the left of the table, and mean values for the treatments exposed to SWs (expressed as percentage of the appropriate control) are to the right.

Treatment	Final germination		
	None	BSW	PSW
Indicator species	% of seeds	—————% of control —————	
Butterfly milkweed	85.0	105.9	101.0
Common milkweed	76.7	113.0	100.0
Large crabgrass	94.7	99.2	102.1
Lettuce	94.7	75.1**	89.5
Orchardgrass	75.8	91.2	118.7

** Significant at $P < 0.025$.

stimulation vs. inhibition) and magnitude (Table 3, Supplemental Materials). For example, overall there were 15 cases of significant reductions in indicator species growth or germination, and six cases of significant stimulation (Table 3). Although several indicators had negative responses to specific treatments, black swallowwort alone accounted for four of six stimulatory responses (Table 3). Other indicators that were sensitive to treatments were annual bluegrass, butterfly milkweed, and large crabgrass. In direct contrast, lettuce and orchardgrass were insensitive to all treatments.

Root length responses were significant for every model effect except for tissue origin by weight (Table 2). Interactions including tissue type as a term (origin by type, type by weight, and origin by type by weight) also were highly significant ($P < 0.0001$) for this response variable.

Discussion

Both black and pale swallowwort demonstrated an ability to interfere with the establishment (seed germina-

Table 2. Root and shoot length, and final germination of indicator species (annual bluegrass, barnyardgrass, black swallowwort (BSW), butterfly milkweed, common milkweed, large crabgrass, lettuce, orchardgrass, pale swallowwort (PSW), and tomato) as affected by leachates from BSW and PSW tissues. Results from modified Parker bioassays conducted under laboratory conditions in Petri dishes. ANOVA results giving probability (P) values for main effects (Indicator Species; Tissue Origin [BSW or PSW]; Tissue Type [leaf, root, or shoot]; and Tissue Weight [0.5 or 1 g dry weight]) and all interactions are shown.

	ANOVA (P) values			
	df	Root length	Shoot length	Final germination
Indicator species (I)	9	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$
Tissue origin (O)	1	0.0056*	0.5785	0.0258*
Tissue type (T)	2	$< 0.0001^{*\dagger}$	0.1001	0.5748
Tissue weight (W)	1	$< 0.0001^*$	0.0051*	0.0001*
I * O	9	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	0.0001 *†
I * T	18	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$
I * W	9	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$
O * T	2	$< 0.0001^{*\dagger}$	0.0072*	0.3766
O * W	1	0.6673	0.7834	0.7155
T * W	2	$< 0.0001^{*\dagger}$	0.0674	0.7647
I * O * T	18	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$
I * O * W	9	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$
I * T * W	18	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$
O * T * W	2	$< 0.0001^{*\dagger}$	0.1856	0.0509
I * O * T * W	18	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$	$< 0.0001^{*\dagger}$

^aThe symbol \dagger indicates populations used in the ANOVA comparison that did not satisfy the assumption of homogeneity of variances; thus, the value given in the table is that of the Welch's ANOVA, which corrects for the presence of heterogeneous population variances (Clinch and Keselman 1982; Zimmerman and Zumbo 1993).

* Significant at $P < 0.05$.

Table 3. Summary of the effects of black swallowwort (BSW) and pale swallowwort (PSW) tissue leachates on the root (R) and shoot (S) elongation and final germination (G) of indicator species (annual bluegrass [POANN], barnyardgrass [ECHCG], black swallowwort [CYKNI], butterfly milkweed [ASCTU], common milkweed [ASCSY], large crabgrass [DIGSA], lettuce [LACSA], orchardgrass [DACGL], pale swallowwort [VNCRO], and tomato [LYPES]). Significant ($P > 0.05$) stimulatory effects are indicated by '+', significant ($P > 0.05$) reductions by '-', and non-significant effects are not indicated. See supplementary materials for more complete data set on indicator species responses.

Leaf tissues												
Tissue origin	BSW						PSW					
Tissue weight	0.5 g			1 g			0.5 g			1 g		
Effect	R	S	G	R	S	G	R	S	G	R	S	G
POAAN												
ECHCG												
CYKNI										+		
ASCTU						-		-				
ASCSY												-
DIGSA												-
LACSA												
DACGL												
VNCRO						-						
LYPES				-								
Root tissues												
Tissue origin	BSW						PSW					
Tissue weight	0.5 g			1 g			0.5 g			1 g		
Effect	R	S	G	R	S	G	R	S	G	R	S	G
POAAN						-						-
ECHCG												
CYKNI				+					+			+
ASCTU	+											
ASCSY												
DIGSA			-		-							
LACSA												
DACGL												
VNCRO												
LYPES												
Shoot tissues												
Tissue origin	BSW						PSW					
Tissue weight	0.5 g			1 g			0.5 g			1 g		
Effect	R	S	G	R	S	G	R	S	G	R	S	G
POAAN											+	-
ECHCG						-						
CYKNI			-									
ASCTU												
ASCSY						-						
DIGSA												
LACSA												
DACGL												

Table 3. Continued.

Tissue origin	Shoot tissues											
	BSW						PSW					
	0.5 g			1 g			0.5 g			1 g		
Effect	R	S	G	R	S	G	R	S	G	R	S	G
VNCRO												
LYPES												–

+ Significant for stimulatory effects at $P > 0.05$.

– Significant for reductions at $P > 0.05$. Nonsignificant effects are not indicated.

tion and seedling growth) of neighboring plant species in controlled laboratory studies. The presence of swallowwort root exudates and tissue leachates caused significant responses in eight of 10 indicator species tested (Table 3). Indicator species identity was the most significant factor in determining treatment responses for both exudate and leachate trials. On the whole, the two milkweed species (*A. syriaca* and *A. tuberosa*) were significantly inhibited by either swallowwort root exudates or tissue leachates (Figure 1; Table 3). Although lettuce growth was stimulated by swallowwort root exudates, seed germination was reduced; tissue leachates did not affect either growth or germination in the species (Figure 1; Tables 1 and 3). Finally, black swallowwort seedling growth and germination was stimulated by the presence of tissues of either swallowwort species (Table 3). Variability in the responses of indicator species to suspected allelopathic exudates or tissue leachates is species- and cultivar-dependent and commonly is observed in the literature (Bertin et al. 2007; Newman and Rovira 1975; Orr et al. 2005; Stowe 1979). Schulz and Friebe (1999) observed that differential responses by indicator species to allelochemicals often are due to differential tolerance to or metabolism of the biologically active compounds by specific indicator plants.

Tissue weight also significantly influenced indicator species response (Table 2), with 1 g of swallowwort tissue resulting in more cases of significant inhibition or stimulation than 0.5 g (Table 3). Thresholds often exist to induce biological activity of chemical compounds, which could explain the occurrence of positive and negative indicator species responses at higher rates of tissue amendment (Nilsson 1994).

Indicator species root length and germination responses also were significantly affected by which swallowwort species was tested. Tissues of both swallowwort species had negative effects on several indicator species (in particular the two milkweed species, barnyardgrass, and large crabgrass), but congeneric species responses were more complicated (Table 3). Whereas BSW tissues had negative effects on germination of PSW indicators, PSW tissues

stimulated both root growth and seed germination of BSW plants, while also reducing germination of PSW seeds (Table 3). Although few studies have directly compared allelopathic interference in congeneric plant species, autotoxicity as exhibited by other species is thought to act as a means of limiting the effects of intraspecific competition (Fitter and Hay 1987; Newman and Rovira 1975). Considering that the introduced ranges of the two swallowwort species are beginning to overlap, any potential advantage for BSW from congeneric allelopathic interference could be ecologically important.

Interestingly, tissue type was a significant factor only in determining root length responses of indicators (Table 2). In this case, leaf tissues of either swallowwort species resulted in significant inhibition of root elongation in butterfly milkweed and tomato seedlings (Table 3). Leaf tissues of plants often contain higher concentrations of biologically active secondary metabolites, possibly due to the vulnerability of these structures to herbivory (Herms and Mattson 1992; Orr et al. 2005; Wardle et al. 1996). The aerial portions of swallowwort species recently have been found to contain a number of biologically active constituents that might impact herbivory (Staerk et al. 2000, 2002).

Stimulation in Indicator Species. There was evidence for stimulation of growth or germination in lettuce, BSW, annual bluegrass, and butterfly milkweed seedlings in response to interference from swallowwort species. Stimulatory growth and germination responses in indicator species are common within the literature, and have been found for species as varied as downy brome (*Bromus tectorum* L.), radish, and eastern cottonwood (*Populus deltoides* Bartram ex Marsh.) (Orr et al. 2005; Rice 1986). Stimulation of seed germination might be due to the release of nutrients or hormones by decaying plant tissues, thereby enhancing survival of the affected seedling (Orr et al. 2005). Increases in shoot growth could be due to: nutrient reallocation away from root tissues affected by phytotoxic allelochemicals (Fitter and Hay 1987; Nilsson

1994); or hormesis, a phenomenon in which low concentrations of allelochemicals result in growth stimulations, but higher concentrations cause growth inhibition (An 2005; An et al. 1993).

Invasiveness and Interference by SW Species. Results from our laboratory experiments indicated that compounds released from swallowwort plants have biological activity. However, the ultimate ecological role for potential allelopathic interference explained by these compounds remains unclear, and is species- and density-dependent. Under natural conditions, swallowwort plants produce very large amounts of biomass, particularly below ground (Blanchard et al. 2010; McKague and Cappuccino 2005). Given that we found a strong rate effect in our study, we hypothesize that phytotoxic effects could be more pronounced under natural growing conditions with greater plant biomass (Weidenhamer 1996).

The sensitivity of native Asclepiadaceae species that co-occur with the introduced swallowworts is of particular interest. Growth and germination in both common and butterfly milkweed was very significantly reduced by swallowwort exudates and leachates. Previous work (Blanchard et al. 2010; Cappuccino 2004) found that polyembryony contributed only to swallowwort establishment at disturbed, depauperate sites, and swallowwort individuals generally performed better in the absence of interspecific competition. However, both swallowwort species are able to establish successfully in both disturbed and stable, undisturbed habitats, (DiTommaso et al. 2005b; Weston et al. 2005). Allelopathic interference might provide a partial explanation for the invasiveness of the swallowwort species at such sites and under conditions of interspecific competition.

Alternatively, in addition to direct allelopathic interference with neighbors, invasive SW species could gain a competitive advantage over neighbors from the interaction of released biologically-active compounds with soil microbes or fungal species (Kruse et al. 2000; Radosovich et al. 1997; Wardle et al. 1998; Wolfe and Klironomos 2005). PSW invasions in particular are known to alter the species composition of soil microbial communities, in favor of fungal species that promote the growth of PSW over native plants (DiTommaso et al. 2005b; Greipsson and DiTommaso 2006; Smith et al. 2008). A possible mechanism for this alteration was proposed by Mogg et al. (2008), who reported that pale swallowwort root extracts contained levels of (-)-antofine with potent antifungal activity.

In summary, these studies provided preliminary evidence for phytotoxicity due to root exudates and tissue leachates from pale and black swallowwort plants. Our results suggested that alkaloids or other unknown inhibitors present in the decomposing leaf litter or root exudates of swallowwort plants might contribute to reduced germina-

tion and seedling growth of affected seeds. This direct interference, along with the antifungal nature of root compounds, could help explain the dominant invasiveness demonstrated by pale and black swallowwort in North America. Field investigations are necessary to clarify the role that allelopathic interference plays in establishing and maintaining such populations.

Sources of Materials

- ¹ Filter paper, No. 1, Whatman Inc., Florham Park, NJ.
- ² Germination paper, No. 76, Anchor Paper, St. Paul, MN.
- ³ Agar powder, Fisher Bioreagents, Fair Lawn, NJ.
- ⁴ Magenta culture boxes, Krackeler Scientific Inc., Albany, NY.
- ⁵ 34 W fluorescent light bulbs, OSRAM Sylvania, Danvers, MA.
- ⁶ Lettuce seed, Johnny's Selected Seeds, Winslow, ME.
- ⁷ Large crabgrass and annual bluegrass seed, Herbiseed, Twyford, England, UK.
- ⁸ Butterfly milkweed seed, Everwilde Farms, Bloomer, WI.
- ⁹ Common milkweed seeds, collected in October 2006 along Freese Rd., Ithaca, NY.
- ¹⁰ Orchardgrass seed, Stock Seed Farms, Murdock, NE.
- ¹¹ Grind Central™ Coffee Grinder, Model DCG-12BC, Cuisinart, East Windsor, NJ.
- ¹² Barnyardgrass seed, Valley Seed Service, Fresno, CA.
- ¹³ Tomato seed, Franchi Sementi S.p.A., Grassobbio (Bergamo), Italy.
- ¹⁴ JMP statistical analysis software, ver. 7, SAS Institute Inc., Cary, NC.

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