Journal of Chemical Ecology, Vol. 31, No. 2, February 2005 (© 2005)
DOI: 10.1007/s10886-005-1339-8

ISOLATION AND CHARACTERIZATION OF ALLELOPATHIC VOLATILES FROM MUGWORT (*Artemisia vulgaris*)

JACOB N. BARNEY,[1,*] ANTHONY G. HAY,[2] and LESLIE A. WESTON[1]

[1]*Department of Horticulture*
[2]*Department of Microbiology, Cornell University, Ithaca, New York 14853, USA*

(Received June 11, 2004; accepted October 14, 2004)

Abstract—Several volatile allelochemicals were identified and characterized from fresh leaf tissue of three distinct populations of the invasive perennial weed, mugwort (*Artemisia vulgaris*). A unique bioassay was used to demonstrate the release of volatile allelochemicals from leaf tissues. Leaf volatiles were trapped and analyzed via gas chromatography coupled with mass spectrometry. Some of the components identified were terpenes, including camphor, eucalyptol, α-pinene, and β-pinene. Those commercially available were tested individually to determine their phytotoxicity. Concentrations of detectable volatiles differed in both absolute and relative proportions among the mugwort populations. The three mugwort populations consisted of a taller, highly branched population (ITH-1); a shorter, lesser-branched population (ITH-2) (both grown from rhizome fragments from managed landscapes); and a population grown from seed with lobed leaves (VT). Considerable interspecific variation existed in leaf morphology and leaf surface chemistry. Bioassays revealed that none of the individual monoterpenes could account for the observed phytotoxicity imparted by total leaf volatiles, suggesting a synergistic effect or activity of a component not tested. Despite inability to detect a single dominant phytotoxic compound, decreases in total terpene concentration with increase in leaf age correlated with decreases in phytotoxicity. The presence of bioactive terpenoids in leaf surface chemistry of younger mugwort tissue suggests a potential role for terpenoids in mugwort establishment and proliferation in introduced habitats.

Key Words—*Artemisia vulgaris*, mugwort, allelopathy, monoterpenes, volatiles, invasive weed, volatile bioassay, glands.

INTRODUCTION

Mugwort (*Artemisia vulgaris* L.) is a rhizomatous perennial weed that commonly infests roadsides, waste areas, and landscapes. The US nursery industry considers

* To whom correspondence should be addressed. E-mail: jnb22@cornell.edu

mugwort as one of the ten worst weed problems impacting nursery production (Henderson and Weller, 1985; Holm et al., 1997). Mugwort is a Eurasian dicot that spreads quickly upon introduction via an extensive rhizome system, and is difficult, to control chemically or culturally (Bing, 1983; Henderson and Weller, 1985; Foy, 2001; Neal and Adkins, 2001; Barney and DiTommaso, 2003). With few effective control strategies, this aggressive weed has rapidly colonized new areas in the eastern United States. Mugwort is most troublesome, often forming dense monospecific stands, along roadsides, in turfgrass and rights-of-way, and is a lesser threat in agronomic settings (Barney and DiTommaso, 2003). Not surprisingly, diversity of native flora, namely early successional species, in these habitats has declined following mugwort colonization (Holm et al., 1997; Barney and DiTommaso, 2003). However, the exact mechanism(s) of interference (e.g., allelopathy and/or competition) is unknown.

Allelopathy has been reported in an increasing number of plant species in recent years, several of which are classified as invasive (Qasem and Foy, 2001; Bais et al., 2003; Hierro and Callaway, 2003). The invasive ability of certain vigorous and often nonnative plants was thought to be associated with greater competitive ability of the invasive species, or a release from natural enemies (Keene and Crawley, 2002; Mitchell and Power, 2003). Recently, allelopathic activity of invasive species also has been reported as a significant factor that negatively influences species biodiversity and ecosystem succession, while enhancing nonnative species establishment and proliferation (Ridenour and Callaway, 2001; Hierro and Callaway, 2003).

The presence of volatile allelochemicals in aromatic shrubs was first established in the early 1960s in the semiarid chaparral regions of California (Muller et al., 1964; Muller, 1965). Characteristic volatiles or essential oils of species in *Artemisia* and many other taxa have since been explored for their inhibitory effects on plant growth in both field and laboratory assays (Halligan, 1975; del Amo and Anaya, 1978; Kim and Kil, 1989; Abraham et al., 2000). Major inhibitory components of the California chaparral shrubs are terpenes. Several monoterpenes inhibit seedling root and shoot growth (Penuelas et al., 1996), with specific cytotoxic effects that include the reduction of intracellular mitochondrial and Golgi body populations, inhibition of respiration and photosynthesis, decreasing cell wall permeability, as well as accelerating the oxidative destruction of chloroplast pigments (Charlwood and Charlwood, 1991; Loreto et al., 1996; Abraham et al., 2000). The antimalarial drug artemisinin, a sesquiterpenoid from annual wormwood (*A. annua* L.), is inhibitory to several broadleaf weeds and crops, but its mode of action is unknown (Duke et al., 1987; Lydon et al., 1997). Other *Artemisia* species with terpenoid allelochemics include *A. absinthium* L. (Funke, 1943), *A. californica* Less. (Muller et al., 1964; Muller, 1966), *A. princeps* var. *orientalis* (Yun and Kil, 1992), and *A. tridentata* Nutt. ssp. *vaseyana* (McCahon et al., 1973; Weaver and Klarich, 1976; Weaver and Klarich, 1977). Most *Artemisia* species

produce predominately monoterpenes, which suggests that the mugwort might as well. Previous studies have suggested that mugwort has allelopathic potential, but the source of its inhibitory chemicals is largely unknown (Hale, 1982; Melkania et al., 1982; Inderjit and Foy, 1999). Inderjit and Foy (1999) suggested that decomposing mugwort foliage and rhizomes were highly suppressive to red clover (*Trifolium pratense* L.) seedling growth.

More than 80 compounds have been isolated from the foliage of mugwort populations around the world, many of these being terpenes (Misra and Singh, 1986; Banthorpe and Brown, 1989; Milhau et al., 1997; Pino et al., 1999). To date, none of these has been examined for suppressive potential. Therefore, the objectives of this research were to evaluate the allelopathic potential of several populations of mugwort, determine which terpenes are implicated in allelopathic activity, and establish the potential for soil activity of these volatiles.

METHODS AND MATERIALS

Plant Material. Two separate mugwort populations were collected from two geographically isolated areas in Ithaca, NY; specifically from a cemetery site (labeled ITH-1) and the Cornell Test Gardens (labeled ITH-2). Both populations were managed in a turfgrass setting that received regular mowing to a height of approximately 6 cm, and no additional irrigation or fertilization for at least 5 yr previous to this study. Plant material was collected in August of 2000, and subsequently maintained in a greenhouse at 30°C day/24°C night, under high-pressure sodium lighting (Sylvania LV 400/EC) with a 12/12 L/D photoperiod and an average of 300 μmol m^{-2} s^{-1} of overhead irradiance. Both biotypes were transplanted and potted in a Cornell 1:2:1 media mix (soil:peat:perlite) from 10 cm rhizome pieces and watered as needed with no fertilizer applied throughout the experiment. An additional biotype was obtained from seed, and is referred to as VT (Vermont, USA) for the location of the seed company from which it was purchased. On average, the ITH-1 population was taller (>1 m), exhibited densely pubescent stems and light green, and deeply dissected leaves; ITH-2 was shorter (0.75–1 m) with hairless stems and dark green, and deeply dissected leaves; and VT had a prostrate-like growth habit (>1 m in length) with moderate pubescent stems and light green-lobed leaves.

Species evaluated in the bioassays included curly cress (*Lepidium sativum* L.), foxtail millet (*Setaria italica* (L.) Beauv.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), white clover (*T. repens* L.), and mugwort (Le Jardin seed company, VT).

Chemical Standards. Terpenoids identified from mugwort foliage via gas chromatography coupled with mass spectrometry (GC-MS) that were commercially available were purchased from Sigma-Aldrich, including camphene,

camphor, eucalyptol, D-limonene, β-myrcene, α-pinene, and β-pinene. However, not all of the terpenes identified were available.

Allelopathic Potential of Mugwort Foliage: Volatile Bioassay. Freshly harvested leaves were evaluated for bioactive volatile activity in a manner that allowed only atmospheric contact between the test species and the mugwort foliage. Foliage was harvested from the three populations, ITH-1, ITH-2, and VT, at 60 days after planting (DAP). At harvest, stock plants were nearly 0.75 m tall, and were trimmed to the soil surface. All leaves were stripped from shoots, including small petioles. Sampled foliage was randomly mixed, to obtain leaves of various maturities. Foliage (2, 5, or 10 g f.w.) was placed into a single layer of Grade 50 cheese cloth. Ten seeds of the bioassay indicator species, curly cress, were placed onto a single layer of Whatman #1 filter paper moistened with 2.5 ml Milli-Q water in a 500 ml Erlenmeyer flask. To maximize volatile release, mugwort foliage was hand-crushed in cheese cloth before placement into the flask, which was immediately sealed with a rubber stopper. The negative control contained only cheese cloth and the receptor species with no mugwort foliage, while the positive control contained the receptor species plus 10 g tall fescue shoots (*Festuca arundinacea* Schreb.), in an effort to simulate other commonly released plant volatiles. Sealed flasks were incubated at 27°C for 72 hr in the dark, after which test species root and shoot lengths were recorded. All experiments contained three replicates and were arranged in a completely randomized design, repeated once in time. Data were pooled after homogeneity analysis, and analyzed using a two-sample t-test for comparison to the control.

To examine the effect of foliage age on phytotoxicity, the experiment was repeated with 60 and 120 DAP foliage collected from the VT population, as this mugwort population exhibited the greatest range in test species response in the previous experiment. Flasks were arranged in a completely randomized design, with three replicates of 60 and 120 DAP foliage using 2, 5, or 10 g fresh leaf biomass, in addition to a control (no mugwort biomass). Ten g of fresh mugwort foliage is the equivalent of approximately 15 mature leaves, which represents one plant, 0.3 m in height. Cress radicle elongation was recorded after 72 hr. The experiment was repeated once in time and data were analyzed as above.

Identification of Volatile Compounds. GC-MS was utilized to identify and quantify volatiles collected from the atmosphere surrounding mugwort foliage. One leaf from the top, middle, and bottom of the shoot of each population was selected at 60 and 120 DAP. After being crushed by hand to maximize the release of volatiles, three leaves from each plant were placed together in a 5 ml conical vial with a Teflon septum. The VT population was not available for examination at 60 DAP.

After 30 min of equilibration, a hypodermic needle was used to withdraw 10 μl of headspace from each vial, which were then injected into an Hewlett Packard 6890 GC equipped with a 30 m $\times$ 250 μm $\times$ 0.25 μm HP-5MS (5% phenyl

methyl siloxane) column and an Hewlett Packard 5973 Mass Selective Detector. The oven temperature gradient used was as follows: 35°C (1 min); 200°C at 15°C/min for 10 min; and 300°C at 40°C/min for 5 min. The quadrapole and source settings were 150°C and 230°C, respectively. Two replicates were analyzed for each sample, and peak values were averaged. Individual peaks were identified by comparison of their mass spectrum with published spectra, as well as by comparisons with both the retention times and mass spectra of authentic samples when available.

Response factors for the seven terpenes readily available as standards (camphene, camphor, eucalyptol, D-limonene, β-myrcene, α-pinene, and β-pinene) were determined using standard curves formulated with concentrations of 0.1, 1, 10, 100, and 1,000 mg/l in chloroform. Concentrations of Santolina triene, the only other identified compound in the headspace that was not available as a standard, were estimated using an average of the response factors of the other seven standards.

Phytotoxicity of Identified Terpene Components. Individual terpenes identified above, except Santolina triene, were tested for inhibitory potential, utilizing the volatile bioassay described above. Test tubes (13×100 mm^2) containing pure terpenes dissolved in methanol (30 μl aliquots) were placed inside closed 500 ml Erlenmeyer flasks, which contained the seeds of test species sown on moist filter paper. Activity of each terpene was examined at 0, 1, 10, 50, 100, 250, 500, and 1,000 mg/m^3, plus a methanol control, with a curly cress indicator. In addition, mugwort, white clover, large crabgrass, and foxtail millet indicators were examined at 1,600 mg/m^3 (chosen to exaggerate toxicity effects). Flasks were arranged as a completely randomized design with three replicates, and were incubated for 72 hr at 27°C in the dark, after which root and shoot lengths were recorded. Tests were conducted on multiple days, with two terpenes evaluated per analysis plus a water and methanol control. Control means were significantly different among days as shown by ANOVA analysis. Therefore, data was not pooled. Data for each day were analyzed using ANOVA and means were compared using Dunnetts tests for comparison to the methanol control (SAS ver. 8).

Test for Soil Activity. In order to determine if the mugwort volatiles could impart a residual effect in soils, an assay was developed to examine the germination and establishment of test species in soil that had previously been exposed to mugwort volatiles. ITH-1, ITH-2, and VT greenhouse grown plant material was harvested at 60 DAP, and the foliage was removed, including petioles. Harvested tissue within each population was mixed for uniformity, and 20 g of the foliage was used for each treatment, after hand-crushing. A fine sandy loam soil was dried for 96 hr at 90°C, and subsequently sifted through a 2-mm soil sieve. In a sealed 11.4-l plastic box, 75 g of the soil were placed into a separate 0.71-l plastic dish, adjacent to, but not touching 20 g of the hand-crushed mugwort foliage. An aquarium pump was included inside the box to aid in circulation of the volatiles. The chamber

equilibrated for 24 hr at room temperature before the soil was removed. The soil box was removed and subsequently wetted with 35 ml Milli-Q water, and sown with 25 curly cress seeds. The control consisted of the soil inside the sealed chamber in the absence of mugwort foliage. The soil box was then sealed and incubated for 72 hr at 27°C in the dark, after which time test species root and shoot lengths were recorded. Boxes were arranged in a completely randomized design with three replicates. The experiment was repeated once in time and data were pooled, with means analyzed using a two-sample t-test for comparison to the control.

RESULTS

Phytotoxicity of Mugwort Foliage via Volatile Bioassay. Curly cress radicle elongation is an excellent indicator of plant growth inhibition due to its uniformity of growth and overall sensitivity, and was used as the assay of choice for bioassay-directed identification of unknown volatiles. Of the three mugwort populations evaluated, ITH-1 and ITH-2 significantly inhibited cress radicle elongation at 2 g f.w. whereas the VT population showed minor cress stimulation (Figure 1). Inhibition ($P < 0.05$) of both curly cress root and shoot elongation was observed with 10 g fresh foliage of ITH-1, ITH-2, and VT populations. Only VT, the seed

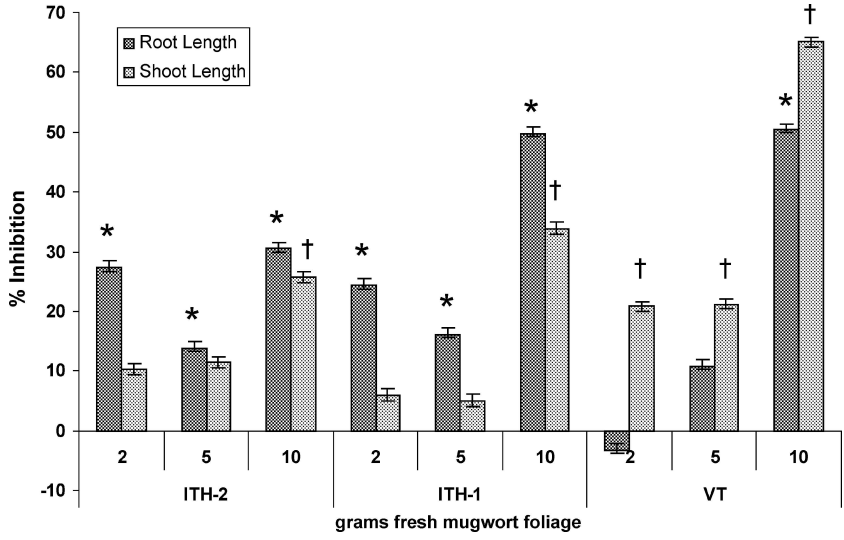

FIG. 1. Percent inhibition of root and shoot elongation of curly cress (*L. sativum*) compared with controls following 72-hr exposure to different amounts of fresh mugwort foliage. *Indicates significant radicle length inhibition ($P < 0.05$) and † indicates significant shoot inhibition compared to the control.

grown population, produced significant shoot growth inhibition of cress at 2, 5, and 10 g f.w. of foliage. Greater than 50% root growth inhibition and 65% shoot growth inhibition were observed with 10 g VT foliage, the most suppressive of all populations evaluated at this tissue concentration. Our findings show that population-level differences exist with respect to mugwort's potential phytotoxicity. Interestingly, percent inhibition of curly cress radicle elongation did not appear to follow a typical dose response relationship. Greatest tissue weight (10 g), however, did result in the greatest inhibition of both radicle and shoot length (Figure 1).

Harvest date, or plant age, also had an impact on phytotoxicity of foliar tissue (Figure 2). Foliar tissue (VT) was evaluated at 60 or 120 d after planting (DAP). The 60 DAP foliage significantly inhibited root elongation at 5 and 10 g, and shoot elongation at 2, 5, and 10 g. Conversely, the 120 DAP foliage significantly reduced cress shoot elongation at 10 g only, while cress radicle length was unaffected.

Test for Soil Activity. To determine if residual phytotoxicity was present in soils previously exposed to mugwort leaf volatiles, a soil bioassay was performed in an enclosed environment. Even after removal of the mugwort leaves, seeds that were germinated in soil that had 24 hr of prior exposure to leaf volatiles were inhibited with respect to root and shoot elongation (Figure 6). Root growth was inhibited up to 27%, whereas shoot growth was inhibited up to 50%. Differences

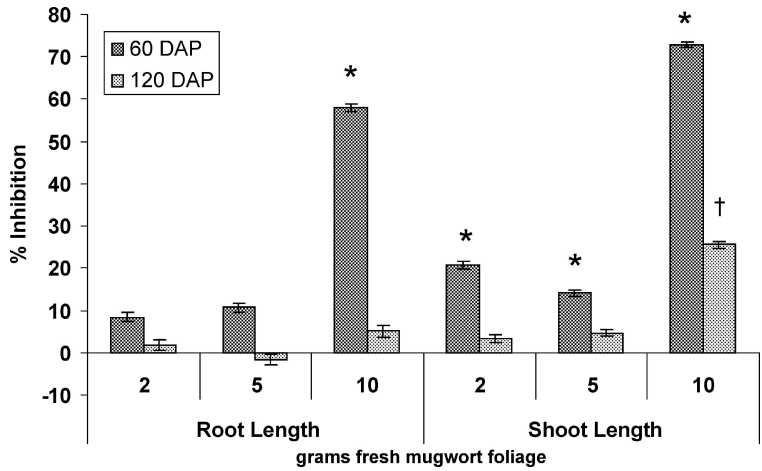

FIG. 2. Percent inhibition of root and shoot elongation of curly cress (*L. sativum*) compared with controls following 72-hr exposure to different ages (60 and 120 d after planting) and weights of fresh mugwort foliage from the VT population. *Indicates significant radicle length inhibition ($P < 0.05$) and † indicates significant shoot inhibition as compared to the control.

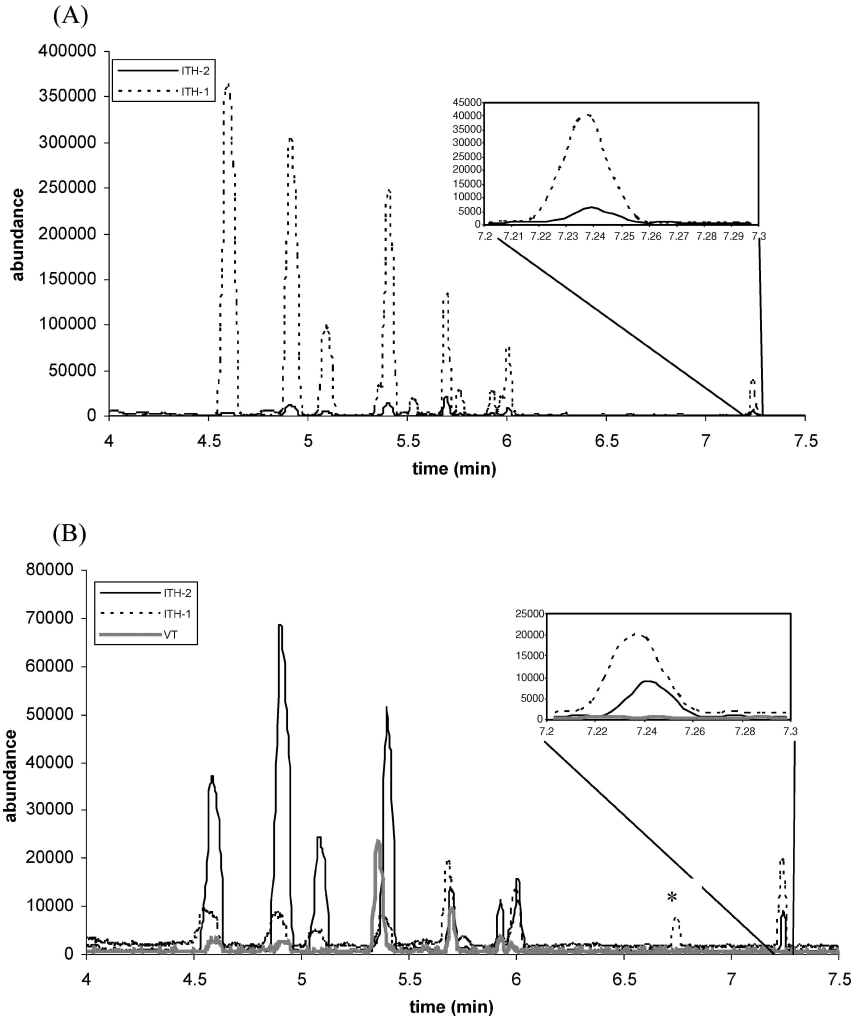

FIG. 3. GC-MS analysis of (A) 60-d-old and (B) 120-d-old plant material. Peaks labeled with "*" did not match any known compounds in the database.

among populations were observed with respect to shoot growth inhibition, with ITH-2 foliage causing greater phytotoxicity than ITH-1 and VT foliage.

Identification of Volatile Compounds. The components of the volatile atmosphere were collected and analyzed via GC-MS. Of the 10 identified peaks (Figure 3), 8 were monoterpenes, including Santolina triene, α-pinene, camphene, β-pinene, β-myrcene, limonene, eucalyptol (1,8-cineole), and camphor (Figure 4).

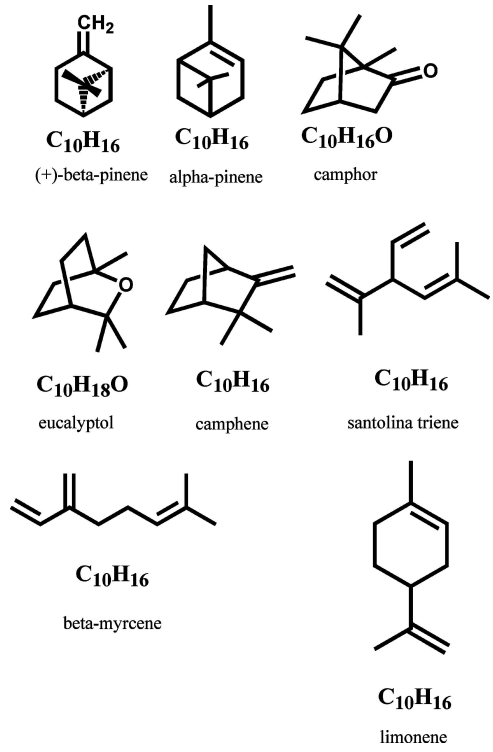

FIG. 4. Terpene structures identified in mugwort foliage.

The 60-d-old ITH-1 foliage contained between 1- and 28-fold higher concentrations of each of the compounds in the leaf headspace compared to older 120-d-old foliage (Table 1). ITH-2 60 DAP foliage contained between 1- and 12-fold greater concentrations of the identified compounds than did the 120 DAP foliage. The major component of ITH-1 foliage at 60 DAP was α-pinene (119.5 mg/m^3), with the second most abundant component being Santolina triene (86.9 mg/m^3). At 120 DAP, the most abundant volatiles were α-pinene and camphene (18.7 and 17.2 mg/m^3, respectively), followed by camphor (13.5 mg/m^3). Volatiles produced by the ITH-2 population included α-pinene (43.5 mg/m^3), followed by camphene (22.1 mg/m^3) at 60 DAP. At 120 DAP, the ITH-2 population contained α-pinene and camphene (19.8 and 17.0 mg/m^3, respectively) followed by camphor (12.0 mg/m^3). At 120 DAP, the VT foliage exhibited slightly different concentrations of volatiles than did ITH-1 and ITH-2 (Table 1). VT foliage at 120 DAP contained α-pinene (16.3 mg/m^3), followed by camphene (15.9 mg/m^3), while camphor and β-pinene were found at concentrations near 10 mg/m^3.

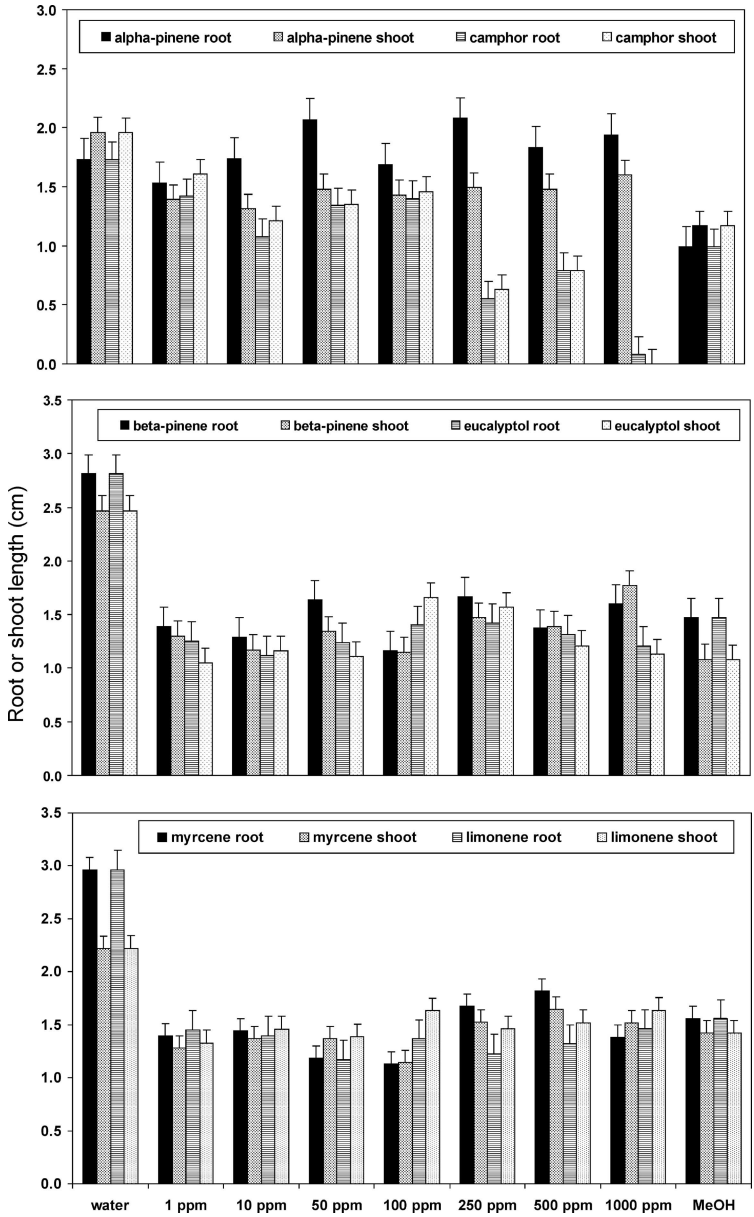

FIG. 5. Response of curly cress (*L. sativum*), to various terpenes identified from mugwort foliage as compared to a methanol and water control, as measured by radicle or shoot elongation. See Table 2 for significance values.

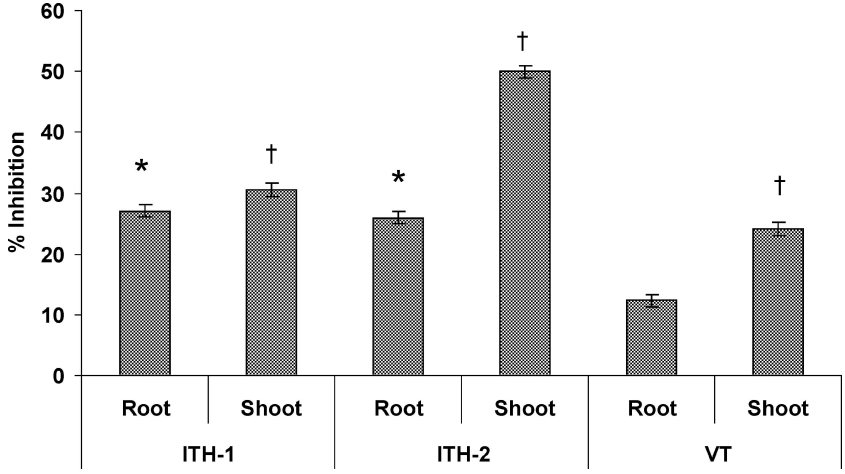

FIG. 6. Percent inhibition of curly cress (*L. sativum*) root and shoot elongation compared with controls after indirect exposure of soil to fresh mugwort foliage of different populations. *Indicates significant radicle length inhibition ($P < 0.05$) and † indicates significant shoot inhibition as compared to the control.

TABLE 1. INFLUENCE OF MUGWORT POPULATION AND TISSUE AGE, 60 AND 120 DAP, ON RELATIVE AMOUNTS OF TERPENES (MG/M^3) AS MEASURED BY GC/MS, AND ESTIMATED LOG Pk_{oc} AND WATER SOLUBILITIES OF COMPONENTS

	Retention times (min)	Log pk_{oc}^a	Water solubility[a] (mg/l)	60 DAP[b]		120 DAP		
				ITH-1	ITH-2	ITH-1	ITH-2	VT
Santolina triene	4.59	3.06	1.99	86.9[c]	10.3[c]	3.1[c]	0.82[c]	0.63[c]
α-Pinene	4.91	3.08	1.89	119.5	43.5	18.7	19.8	16.3
Camphene	5.08	3.08	4.89	37.2	22.1	17.2	17.0	15.9
β-Pinene	5.40	3.08	4.89	74.0	19.4	5.9	7.0	9.7
β-Myrcene	5.53	3.10	6.92	2.8[c]	0	0	0	0
3-Hexen-1-ol, acetate (Z)	5.69							
Hexyl ester acetic acid	5.76							
Limonene	5.93	3.12	3.15	2.9[c]	0.3[c]	0	1.0[c]	0.6[c]
Eucalyptol	6.01	2.03	532.4	5.9	1.4	1.3	0.6	0.1
Camphor	7.24	2.02	186.3	15.0	12.2	13.5	12.0	11.4

[a]Log pk_{oc} values and water solubilities estimated using Epi Suite v 3.10.
[b]Terpene concentrations calculated from response factor equations as determined by standard curves.
[c]Terpene concentrations estimated by average response factor of known standards.

TABLE 2. SIGNIFICANCE TABLE FOR INDIVIDUAL MONOTERPENES ASSESSED IN VOLATILE BIOASSAY

	Camphor		α-Pinene		β-Pinene	
[Terpene] (mg/m^3)	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length
1	$*\uparrow$	$*\uparrow$	$*\uparrow$	ns	ns	ns
10	ns	ns	$*\uparrow$	ns	ns	ns
50	ns	ns	$*\uparrow$	ns	ns	ns
100	ns	ns	$*\uparrow$	ns	ns	ns
250	$*\downarrow$	$*\downarrow$	$*\uparrow$	ns	ns	ns
500	ns	$*\downarrow$	$*\uparrow$	ns	ns	ns
1,000	$*\downarrow$	$*\downarrow$	$*\uparrow$	$*\uparrow$	ns	$*\uparrow$

ns = Nonsignificant ($P > 0.05$), $\uparrow$= mean greater than MeOH control (i.e., stimulation), $\downarrow$= mean lower than MeOH control (i.e., inhibition). Terpenes not shown, myrcene and limonene, are all nonsignificant ($P > 0.05$).
$^*P < 0.05$.

Phytotoxicity of Terpene Components. Terpenes were tested at concentrations bracketing those calculated from the unknown injections (0–1,000 mg/m^3). Overall, myrcene, limonene, and β-pinene had no effect on the test species compared to the methanol control (Figure 5). Camphene had a pattern similar to both limonene and myrcene (data not shown). Eucalyptol had no phytotoxicity to radicle elongation, but did cause shoot length stimulation at 50 and 100 mg/m^3, while α-pinene stimulated cress radicle growth at all concentrations (Table 2). Only camphor suppressed radicle and shoot growth, showing a nonlinear trend (Figure 5 and Table 2). Methanol significantly reduced both radicle and shoot length compared to water ($P < 0.001$).

The terpenes were also evaluated on mugwort, crabgrass, and foxtail millet germination at 1,600 mg/m^3 to determine their phytotoxicity toward a variety of test species at high concentration (Table 3). Mugwort root and shoot inhibition was observed with all terpenes except camphene. Fewer inhibited monocotyledonous test species, suggest that broadleaf species may be more sensitive to the phytotoxic effects.

DISCUSSION

Plant species succession, whether in a natural or agroecosystem, is often disrupted by the introduction of a nonnative species that rapidly displaces native flora or interferes with crop production (Turner, 1988; Sax et al., 2002). Most often this phenomenon is attributed to the greater competitive ability of the introduced plant compared to the native flora or crops in a particular setting

TABLE 3. PERCENT GROWTH INHIBITION OF RADICLE ELONGATION BY MUGWORT TERPENES AT 1,600 MG/M^3

	Curly cress				Mugwort				Large crabgrass				Foxtail millet			
	Roots		Shoots		Roots		Shoots		Roots		Shoots		Roots		Shoots	
α-Pinene	21.0	ns	21.3	*	32.7	**	29.9	**	17.4	ns	−13.7	ns	−6.8	ns	−19.9	ns
β-Pinene	40.5	**	46.0	**	41.5	**	43.2	**	31.5	**	16.22	ns	−19.7	ns	−13.6	ns
Camphor	95.1	**	98.6	**	96.5	**	95.8	**	85.7	**	54.7	**	95.3	**	59.2	**
Camphene	−3.2	ns	16.0	n/s	29.8	**	11.0	ns	3.6	ns	−13.7	ns	35.1	*	−10.2	ns
Eucalyptol	59.5	**	76.6	**	35.0	**	43.2	**	35.9	**	31.6	**	−6.3	ns	3.1	ns
Caryophyllene oxide	1.7	ns	29.4	**	−1.8	n/s	10.8	ns	10.5	ns	13.7	ns	−10.1	ns	5.4	ns

[a] Percent inhibition based on comparison to the control.
[b] n/s = Nonsignificant ($P > 0.05$).
* $P < 0.05$; ** $P < 0.001$.

(Williamson, 1996; Williamson and Fitter, 1996; Lockwood et al., 2001). However, in the case of mugwort and about 80 other weedy species (Singh et al., 2001), habitat invasion and loss of biodiversity may be partially accomplished through the release of allelochemicals that can reduce seedling establishment and overall plant fitness (Ridenour and Callaway, 2001; Bais et al., 2003). A greater comprehension of the growth and ecology of nonnative species would assist in furthering our understanding of the mechanisms that contribute to invasiveness (Whittaker and Feeny, 1971; Wardle et al., 1998). A recent study by Callaway and Aschehoug (2000), suggested the importance of allelopathic interference in the ability of diffuse knapweed (*Centaurea diffusa* Lam.) to invade crop and rangelands across North America. The identified toxin in a related *Centaurea* species (*C. maculosa*), (–)-catechin, is believed to cause the formation of reactive oxygen species and lead to eventual root necrosis (Bais et al., 2003). This allelopathic response, deemed the "novel weapons hypothesis," states that nonnative species exude/emit compounds foreign to the native plant community, resulting in enhanced invasiveness (Bais et al., 2003). We attempted to test the "novel weapons hypothesis" in controlled laboratory studies using the invasive perennial weed mugwort, which belongs to a family known for their production of volatile aromatics.

We know that mugwort tends to establish in dense monospecific stands, gradually resulting in a decrease of plant biodiversity, while increasing its radius of expansion from the nucleus of introduction (Barney, 2003). Related *Artemisia* spp. are also reported to be invasive, and many are considered to be noxious weeds, and also known to contain volatile bioactive compounds, primarily terpenoids (Funke, 1943; Muller et al., 1964). Literature states that mugwort contains similar chemicals (LeFevre, 1964; Dung et al., 1992), although a thorough analysis of foliar chemistry has not been performed. Based on the hypothesis that many of the allelochemicals present in mugwort foliage would be volatile terpenoids, we developed a specific assay to assess this potential toxicity.

The assay allowed only indirect (atmospheric) contact between fresh mugwort leaves (donor) and the test species (receptor) in an enclosed environment. We observed significant inhibition of curly cress radicle elongation by volatiles released from as few as two mature mugwort leaves. Assays performed with leaves of various ages from identical mugwort populations demonstrated that the inhibitory potential of foliage, or quantity of volatiles produced, was leaf-age dependent. Similarly, monoterpene concentrations in peppermint (*Mentha* × *piperita* L.) leaves exhibited an exponential decrease with increase in leaf age (Rohloff, 1999; McConkey et al., 2000). This finding is consistent with the decrease of limonene concentrations in peppermint as the plant ages (Gershenzon et al., 2000), showing that terpene concentrations are not static throughout a plant's life cycle. GC–MS results obtained with the ITH-1 population indicate that younger foliar tissue contained up to 28-fold greater concentrations

of monoterpenes. This increase in total volatiles correlates with increased phytotoxicity, as younger tissue is significantly more inhibitory to seedling growth.

Volatile assays were conducted in enclosed receptacles using hand-crushed leaves, which may have resulted in greater volatile concentrations compared to actual field settings (Fehsenfeld et al., 1992). While this assay cannot mimic field conditions, our intention was to evaluate the potential impact of maximal terpene concentrations. If no phytotoxicity were observed with all possible monoterpenes (null hypothesis), one could assume that monoterpenes were not likely influencing the surrounding plant community structure.

Several studies have suggested the role of volatile compounds in suppression of neighboring vegetation. These include the chaparral species *Salvia* and *Eucalyptus*, and the rangeland weed *A. tridentata* (Muller, 1965; del Moral and Muller, 1970; Klarich and Weaver, 1973; Kohli and Singh, 1991), all of which create "monocultural islands" with no surrounding vegetation. These present studies suggest that foliar terpenes can influence the germinators of competing species. Interestingly, in field settings mugwort produces large quantities of foliage resulting in noticeable quantities of released aromatics (Barney, personal observation). Foliar tissues likely generate volatiles continuously over the course of the season, with environmental conditions influencing emission quantities and the association of potential allelochemicals with soil particulates, as observed in many forest and shrubby ecosystems (Lerdau et al., 1995; Guenther et al., 1996; Hayward et al., 2001). In addition, if terpene storage structures are ruptured, their contents are immediately released into atmosphere, with some terpenes likely partitioning into soil organic matter. Soils in a Sitka spruce forest (*Picea sitchensis* Bong.) re-emit monoterpenes accumulated throughout the growing season from the general emission of the spruce trees (Hayward et al., 2001).

Despite their volatility, these compounds have relatively high log pk_{oc} values, with lipophyllic monoterpenes having a 10-fold greater affinity for organic matter than the more polar terpenes. This suggests a potential for accumulation in soil organic matter over time. Association of phytotoxic terpenes with soil organic matter presents a possible mechanism for allelopathic interactions in natural settings. In addition, the solubility of terpenoids identified in mugwort foliage varies greatly (see Table 1), and toxicity is dependent upon concentration. Weidenhamer et al. (1993) showed that camphor in aqueous solution significantly inhibited germination of *Lactuca sativa* L. and *Rudbeckia hirta* L. with concentrations as low as 25 mg/l. Therefore, our results suggest that the affinity of various terpenoids for soil organic matter, coupled with their relatively high water solubility, may lead to accumulation in multiple environmental compartments.

It is possible that the terpenoids produced by mugwort foliage may be active in the environment. Mugwort tissue incorporation, or decomposition over time reduces neighboring species germination (Inderjit and Foy, 1999). The action

of rainfall or dew may also result in the transport of these compounds to the soil surface, resulting in reduced seed germination. Studies performed by Muller (1965) showed that *Salvia* spp. produced volatiles that bind to the soil surface underneath *Salvia* infestations, and subsequently reduce the growth of surrounding weedy species.

Our results indicate that volatile compounds are produced and released by the foliage of three separate mugwort populations. However, potential phytotoxicity varied with population. Numerous studies have shown similar variations in the production of allelochemicals with genotype and plant age (Weston et al., 1987; Fajer et al., 1992). In addition, Inderjit et al. (2001) found site variation, and likely concomitant mugwort population variation between sites, with respect to the effect of mugwort-infestation on soil characteristics. Population variation in phytotoxic chemicals likely explains some of the inconsistencies reported in field studies examining allelopathy (Muller, 1966; Fuerst and Putnam, 1983).

We attempted to identify the specific monoterpenes associated with the phytotoxicity observed in the presence of mugwort foliage, or soil associated volatiles. No one terpenoid could account for the phytotoxicity observed in foliar assays. Most of the monoterpenes produced no inhibition of either root or shoot growth, while some stimulated growth at low concentrations. Camphor treatments, however, did result in reductions to root and shoot growth of various indicator species. This finding is consistent with Abraham et al. (2000) who examined the effect of four monoterpenes on maize seed germination and found the following order of activity: camphor > eucalyptol > α-pinene > limonene.

It is possible that the toxicity observed in our initial experiments with mugwort foliage is due to synergistic combinations of phytotoxic terpenes. Due to the difficulty involved in accurately mimicking terpene mixtures in the lab, neither we nor any other researchers have examined terpene mixtures for allelopathic activity. Therefore, activity observed with mugwort foliage might include combinations of those tested in this study, as well as those untested, such as santolina triene, which occurs in substantial quantities in the foliage, but which was unavailable for evaluation. It is possible that Santolina triene, or unidentified components may influence the overall phytotoxic potential of mugwort volatiles emitted over time in an additive fashion.

Of the parameters assessed, shoot growth was generally slightly more sensitive than radicle elongation to the presence of mugwort volatiles. The quantity of the terpenes appears to be inversely proportional to plant tissue age, and is also population dependent. However none of the individual monoterpenes tested was highly suppressive. Further research on the production, release, and activity of volatiles produced by populations under field conditions are needed. In addition, the potential role of rhizome exudates should be

evaluated to elucidate the contribution of other allelochemicals involved in mugwort interference.

REFERENCES

ABRAHAM, D., BRAGUINI, W. L., KELMER-BRACHT, A. M., and ISHII-IWAMOTO, E. L. 2000. Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. *J. Chem. Ecol.* 26:611–624.

BAIS, H. P., VEPACHEDU, R., GILROY, S., CALLAWAY, R. M., and VIVANCO, J. M. 2003. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* 301:1377–1380.

BANTHORPE, D. V. and BROWN, G. D. 1989. Two unexpected coumarin derivatives from tissue cultures of compositae species. *Phytochemistry* 28:3003–3007.

BARNEY, J. N. 2003. Characterization of allelopathic and invasive potential of mugwort (*Artemisia vulgaris* L.). Masters Thesis, Cornell University.

BARNEY, J. N. and DITOMMASO, A. 2003. The biology of Canadian weeds. 118. *Artemisia vulgaris* L. *Can. J. Plant Sci.* 83:205–215.

BING, A. 1983. Problems in mugwort control in lawns. *Proc. Northeast Weed Sci. Soc.* 37:376.

CALLAWAY, R. M. and ASCHEHOUG, E. T. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science* 290:521–523.

CHARLWOOD, B. V. and CHARLWOOD, K. A. 1991. Monoterpenoids, p. 565, *in* B. V. Charlwood and D. V. Banthorpe (eds.). Terpenoids. Academic Press, New York, NY.

DEL AMO, S. and ANAYA, A. L. 1978. Effect of some sesquiterpene lactones on the growth of certain secondary tropical species. *J. Chem. Ecol.* 4:305–313.

DEL MORAL, R. and MULLER, C. H. 1970. The allelopathic effects if *Eucalyptus camaldulensis*. *Am. Midl. Nat.* 83:254–282.

DUKE, S. O., VAUGHN, K. C., CROOM, E. M., and ELSHOHLY, H. N. 1987. Artemisinin, a constituent of annual wormwood (*Artemisia annua*) is a selective phytotoxin. *Weed Sci.* 35:499–505.

DUNG, N. X., NAM, V. V., HUONG, H. T., and LECLERECQ, P. A. 1992. Chemical composition of the essential oil of *Artemisia vulgaris* L. var. *indica* Maxim. from Vietnam. *J. Essent. Oil Res.* 4:433–434.

FAJER, E. D., BOWERS, M. D., and BAZZAZ, F. A. 1992. The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in *Plantago*: A test of the carbon/nutrient balance hypothesis. *Am. Nat.* 140:707–723.

FEHSENFELD, F., CALVERT, J., FALL, R., GOLDAN, P., GUENTHER, A. B., HEWITT, C. N., LAMB, B., LIU, S., TRAINER, M. H. W., and ZIMMERMAN, P. 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochem. Cycles* 6:389–430.

FOY, C. L. 2001. Effect of selected herbicide-adjuvant combinations on mugwort (*Artemisia vulgaris*). *Proc. Northeast Weed Sci. Soc.* 55:109.

FUERST, E. P. and PUTNAM, A. R. 1983. Separating the competitive and allelopathic components of interference: Theoretical principles. *J. Chem. Ecol.* 9:937–944.

FUNKE, G. L. 1943. The influence of *Artemisia absinthium* on neighboring plants. *Blumea* 5:281–293.

GERSHENZON, J., MCCONKEY, M. E., and CROTEAU, R. B. 2000. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Phys.* 122:205–213.

GUENTHER, A., GREENBERG, J., HARLEY, P., HELMIG, D., KLINGER, L., VIERLING, L., ZIMMERMAN, P., and GERON, C. 1996. Leaf, branch, stand and landscape scale measurements of volatile organic compound fluxes from U.S. woodlands. *Tree Physiol.* 16:17–24.

HALE, M. 1982. Allelopathic potential of *Artemisia vulgaris* rhizomes. *Plant Physiol.*69 (Supplement 126).

HALLIGAN, J. P. 1975. Toxic terpenes from *Artemisia californica*. *Ecology* 56:999–1003.

HAYWARD, S., MUNCEY, R. J., JAMES, A. E., HALSALL, C. J., and HEWITT, C. N. 2001. Monoterpene emissions from soil in a Sitka spruce forest. *Atmos. Environ.* 35:4081–4087.

HENDERSON, J. C. and WELLER, S. C. 1985. Biology and control of *Artemisia vulgaris*. *Proc. North Central Weed Control Conf.* 40:100–101.

HIERRO, J. L. and CALLAWAY, R. M. 2003. Allelopathy and exotic plant invasion. *Plant Soil* 256:29–39.

HOLM, L., DOLL, J., HOLM, E., PANCHO, J., and HERBERGER, J. 1997. World Weeds: Natural Histories and Distribution. John Wiley and Sons, New York, NY.

INDERJIT and FOY, C. 1999. Nature of the interference mechanism of mugwort (*Artemisia vulgaris*). *Weed Technol.* 13:176–182.

INDERJIT, KAUER, M., and FOY, C. 2001. On the significance of field studies in allelopathy. *Weed Technol.* 15:702–797.

KEENE, R. M. and CRAWLEY, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* 17:164–170.

KIM, Y. S. and KIL, B. S. 1989. Identification and growth inhibition of phytotoxic substances from tomato plant. *Korean J. Bot.* 32:41–49.

KLARICH, D. and WEAVER, T. 1973. Effects of vapors from *Artemisia tridentata* Nutt. on seed germination. *Proc. Montana Acad. Sci.* 33:31–36.

KOHLI, R. K. and SINGH, D. 1991. Allelopathic impacts of volatile components from *Eucalyptus* on crop plants. *Biol. Plant* 33:475–483.

LEFEVRE, C. W. 1964. A light activated growth inhibitor from *Artemisia vulgaris*. *Plant Physiol.* 39 (Supplement S).

LERDAU, M., MATSON, P., FALL, R., and MONSON, R. 1995. Ecological controls over monoterpene emissions from Douglas-fir (*Pseudotsuga menziesii*). *Ecology* 76:2640–2647.

LOCKWOOD, J. L., SIMBERLOFF, D., MCKINNEY, M. L., and VON HOLLE, B. 2001. How many, and which, plants will invade natural areas? *Biol. Invest.* 3:1–8.

LORETO, F., CICCIOLI, P., CECINATO, A., BRANCALEONI, E., FRATTONI, M., and DTRICOLI, D. 1996. Influence of environmental factors and air composition on the emission of a-pinene from *Quercus ilex* leaves. *Plant Physiol.* 110:267–275.

LYDON, J., TEASDALE, J. R., and CHEN, P. K. 1997. Allelopathic activity of annual wormwood (*Artemisia annua*) and the role of artemisinin. *Weed Sci.* 45:807–811.

MCCAHON, C. B., KELSEY, R. G., SHERIDAN, P. P., and SHAFIZADEH, F. 1973. Physiological effects of compounds extracted from sagebrush. *Bull. Torrey Bot. Club* 100:23–28.

MCCONKEY, M. E., GERSHENZON, J., and CROTEAU, R. B. 2000. Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. *Plant Physiol.* 122:215–223.

MELKANIA, N. P., SINGH, J. S., and BISHT, K. K. S. 1982. Allelopathic potential of *Artemisia vulgaris* L. and *Pinus roxburghii* Sargent: A bioassay study. *Proc. Indian Nat. Sci. Acad. B* 48:685–688.

MILHAU, G., VALENTIN, A., BENOIT, F., MALLIÉ, M., BASTIDE, J., PÉLLISSIER, Y., and BESSIÉRE, J. 1997. *In vitro* antimalarial activity of eight essential oils. *J. Essent. Oil Res.* 9:329–333.

MISRA, L. N. and SINGH, S. P. 1986. α-Thujone, the major component of the essential oil from *Artemisia vulgaris* growing wild in Nilgiri Hills. *J. Nat. Prod.* 49:941.

MITCHELL, C. G. and POWER, A. G. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421:625–627.

MULLER, C. H. 1965. Inhibitory terpenes volatilized from *Salvia* shrubs. *Bull. Torrey Bot. Club* 92:38–45.

MULLER, C. H. 1966. The role of chemical inhibition (allelopathy) in vegetational composition. *Bull. Torrey Bot. Club* 93:332–351.

MULLER, C. H., MULLER, W. H., and HAINES, B. L. 1964. Volatile growth inhibitors produced by aromatic shrubs. *Science* 143:471–473.

NEAL, J. C. and ADKINS, C. R. 2001. Comparison of glyphosate and clopyralid for mugwort (*Artemisia vulgaris*) control in field-grown nursery crops. *Proc. SNA* 46:420–421.

PENUELAS, J., RIBAS-CARBO, M., and GILES, L. 1996. Effects of allelochemicals on plant respiration and oxygen isotope fractionation by the alternative oxidase. *J. Chem. Ecol.* 22:801–805.

PINO, J. A., ROSADO, A., and FUENTES, V. 1999. Composition of the essential oil of *Artemisia vulgaris* L. herb from Cuba. *J. Essent. Oil Res.* 11:477–478.

QASEM, J. R. and FOY, C. L. 2001. Weed allelopathy, its ecological impacts and future prospects: A review. *J. Crop Prod.* 4:43–119.

RIDENOUR, W. M. and CALLAWAY, R. M. 2001. The relative importance of allelopathy in interference: The effects of an invasive weed on a native bunchgrass. *Oecologia* 126:444–450.

ROHLOFF, J. 1999. Monoterpene composition of essential oil from peppermint (*Mentha* × *piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrometry analysis. *J. Agric. Food Chem.* 47:3782–3786.

SAX, D. F., GAINES, S. D., and BROWN, J. H. 2002. Species invasions exceed extinctions on islands worldwide: A comparative study of plants and birds. *Am. Nat.* 160:766–783.

SINGH, H. P., BATISH, D. R., and KOHLI, R. K. 2001. Allelopathy in agroecosystems: An overview. *J. Crop Prod.* 4:1–41.

TURNER, C. E. 1988. Ecology of invasions by weeds, pp. 354, *in* M. Liebman (ed.). Weed Management and Agroecosystems: Ecological Approaches. CRC Press, Inc., Boca Raton, FL.

WARDLE, D. A., NILSSON, M.-C., GALLET, C., and ZACKRISSON, O. 1998. An ecosystem-level perspective of allelopathy. *Biol. Rev.* 73:305–319.

WEAVER, T. and KLARICH, D. 1976. Toxic effects of volatile exudates from *Artemisia tridentata* Nutt. on soil microbes. *Proc. Montana Acad. Sci.* 36:80–85.

WEAVER, T. W. and KLARICH, D. 1977. Allelopathic effects of volatile substances from *Artemisia tridentata* Nutt. *Am. Midl. Nat.* 97:508–512.

WEIDENHAMER, J. D., MACIAS, F. A., FISCHER, N. H., and WILLIAMSON, G. B. 1993. Just how insoluble are monoterpenes? *J. Chem. Ecol.* 19:1799–1807.

WESTON, L. A., BURKE, B. A., and PUTNAM, A. R. 1987. Isolation, characterization and activity of phytotoxic compounds from quackgrass (*Agropyron repens* (L.) Beauv.). *Weed Sci.* 13:403–421.

WHITTAKER, R. H. and FEENY, P. P. 1971. Allelochemicals: Chemical interactions between species. *Science* 171:757–770.

WILLIAMSON, M. 1996. Biological Invasions. Chapman & Hall, London.

WILLIAMSON, M. and FITTER, A. 1996. The characters of successful invaders. *Biol. Cons.* 78:163–170.

YUN, K. W. and KIL, B.-S. 1992. Assesment of allelopathic potential in *Artemisia princeps* var. *orientalis* residues. *J. Chem. Ecol.* 18:1933–1940.