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Biogenic volatile organic compounds from an invasive species: impacts on plant–plant interactions

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Abstract Invasive plant species impact both ecosystems and economies worldwide, often by displacing native biota. Many plant species exude/emit compounds into the surrounding environment with minor consequences in their native habitat due to a long coevolutionary history. However, upon introduction to ecosystems naïve to these compounds, unpredictable interactions can manifest. The majority of the putative allelochemicals studied have been root exudates, despite the large number of plant species that emit volatile organic compounds. We quantified the concentrations and ecological consequences of volatile monoterpenes from the North American invasive perennial *Artemisia vulgaris*. Ambient monoterpene-mixing ratios inside an *A. vulgaris* canopy were 0.02–4.15 ppbv in May and 0.01–0.05 ppbv in August, but were negligible (below instrument detection limit of 0.01 ppbv) 10 m away. Foliar disturbance increased total monoterpene concentration to a maximum of 27 ppbv. However, this level remains 1,000-fold lower than that shown to be phytotoxic to sensitive species in laboratory assays. In contrast, soil monoterpene concentrations were >74-fold higher inside [$\leq 35 \pm 11$ ng g^{-1} (SDW)] and 19-fold higher at the edge [9 ± 3 ng g^{-1} (SDW)], compared to outside the *A. vulgaris* stand [0.48 ± 0.05 ng g^{-1} (SDW)]. A common native competitor species, *Solidago canadensis,* grown in pots and resident soil in situ yielded up to 50% less aboveground biomass inside as compared to outside the *A. vulgaris* stand. Activated carbon had no effect on greenhouse-grown *S. canadensis* performance when grown with *A. vulgaris*, suggesting root-derived exudates are not responsible for field observations. Results from this study suggest that *A. vulgaris*-derived monoterpenes have little direct activity in their volatile gaseous state, but are concentrated in the soil matrix within and bordering the *A. vulgaris* stand, thereby reducing interspecific performance and potentially fostering the subsequent local invasion of this species.

Keywords Allelopathy · *Artemisia vulgaris* · Biogenic volatile organic compound · Biological invasion · Monoterpene · Mugwort · *Solidago canadensis*

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Abbreviations

VOC Volatile organic compound
BVOC Biogenic volatile organic compound

Introduction

The mechanisms proposed to explain non-native plant species success in introduced habitats include inherent autecological traits (Williamson and Fitter 1996), evolution of an invasive phenotype (Blossey and Notzold 1995), release from natural enemies (Mitchell and Power 2003), and exudation of novel phytotoxic compounds (Callaway and Aschehoug 2000). The realized success of an introduced plant from rare occurrence to ubiquitous invader is likely the result of many interacting factors that include aspects of each of the above circumstances (Barney and Whitlow 2008). However, it is important to quantify the contribution of each interacting variable if we are to create a holistic picture of the invasion process. One mechanism that requires further empirical investigation in situ is that of chemically mediated plant–plant interactions.

Most plants release biogenic organic compounds (BOCs) into the surrounding environment actively or passively through senescence and cell leakage (Newman 1978). Active release of BOCs into the environment occurs via root exudation or volatile emission from aboveground plant parts, with BOCs typically aiding in nutrient acquisition (Bertin et al. 2003), herbivory defense (Simms and Rausher 1987), mitigating environmental extremes (Lerdau et al. 1997), or microbial and viral defense (Langenheim 1994). Through coevolutionary history, the surrounding biota of the native range "ignore," tolerate, or overcome these BOCs in an evolutionary arms race, thus resulting in predictable ecosystem consequences. However, the same compounds can have unpredictable repercussions in a naïve environment inexperienced with a specific BOC or a particular mixture of BOCs. For example, *Centaurea maculosa* L. exudes a racemic mixture of catechin in both its native and introduced ranges, but in different quantities and with very different ecological consequences depending on the recipient community (Bais et al. 2003). In the introduced range of North America, catechin reduces competitor performance and alters the soil microflora, while having an unknown, yet benign (to neighboring plant species at least), function in the native range (Callaway et al. 2004; Vivanco et al. 2004). Further investigation demonstrated that surrounding biota may evolve tolerance to the previously novel BOCs with increasing exposure (Callaway et al. 2005). Despite the range of chemical compounds (Whittaker and Feeny 1971) and introduced species (Hierro and Callaway 2003) implicated in allelopathic interactions, little research has investigated the potential role of volatile BOCs in invasive species success in recipient communities.

Biogenic volatile organic compounds (BVOCs) are primarily studied for their role in atmospheric chemistry (e.g., Guenther et al. 1995), but also play a role in plant–plant interactions, and include compounds as varied as methanol to the many isoprenoid derivatives (Peñuelas and Llusià 2004). One of the first empirical studies of allelopathy involved BVOCs, and was based on observations of the "spacing and patterning of annual grassland species in and about colonies of *Salvia leucophylla* and *Artemisia californica*" (Muller et al. 1964). Since the groundbreaking work by Muller and colleagues, BVOCs, especially monoterpenes, have been demonstrated to negatively impact recipient plant species (Abrahim et al. 2000; Muller 1965) and alter soil microflora (Weaver and Klarich 1976; Yun et al. 1993). However, these studies have relied exclusively on laboratory assays and often use extraordinarily sensitive test species to demonstrate potential phytotoxicity. Therefore, allelopathy in the gas phase has remained a subject for skepticism, and will continue to be so until demonstrated empirically in the field.

This study was designed to assess the role that BVOCs play in interspecific competition in the herbaceous invasive perennial *Artemisia vulgaris* L. (mugwort). *Artemisia vulgaris* is a robust perennial introduced from Eurasia, and is common along roadsides, urban lots, and abandoned agricultural fields where it is often observed displacing native *Solidago* species (Barney and DiTommaso 2003). In previous studies, we have shown that *A. vulgaris* advances along a distinct invasion front from the locus of introduction, displacing all competing vegetation (Barney et al. 2005a). Similar to Muller's observation in the California chaparral, we observed dead graminaceous and broadleaved species within and surrounding *A. vulgaris* populations, suggestive of a BVOC-mediated interaction. Because the various

terpenoids in the *Artemisia* genus have been well studied, they are good candidates for investigating the role of leaf-derived BVOCs in allelopathy. Previous laboratory volatile assays using *A. vulgaris* foliage reduced the performance of both monocots and dicots, though no single isolated monoterpene was responsible for the observed phytotoxicity (Barney et al. 2005b). Therefore, the aim of this study was to address the following questions relating *A. vulgaris* BVOCs in local competitive dynamics: (1) Are monoterpene concentrations in *A. vulgaris* canopies high enough to elicit phytotoxic responses in competitor species as demonstrated in laboratory assays? (2) Are *A. vulgaris*-derived BVOCs accumulated in the soil matrix? (3) Is *Solidago canadensis* L. (a common *A. vulgaris* competitor) performance differentially affected when grown in pots (aboveground interaction only) or in situ soil (above and belowground interaction) on the inside, outside, or border of an *A. vulgaris* stand? (4) Does activated carbon in the rhizosphere mitigate *A. vulgaris* allelochemicals? Addressing each of these questions will help to elucidate the role and possible mechanism of interference of BVOCs in the invasiveness of *A. vulgaris*.

Materials and methods

In situ BVOC analysis

BVOC concentrations were quantified in two *A. vulgaris* populations at different life stages (May 26, July 10, and August 4, 2005) both pre- and post-disturbance. One *A. vulgaris* population was a 6 × 15 m monoculture located at Cornell's Turfgrass Research Center, and the second population was a 1.5 × 1 m monoculture located in an abandoned urban lot in downtown Ithaca, NY, USA. BVOC samples were collected in the center of each *A. vulgaris* stand at mid-stem height relative to the mean stand height at that date. Initial samples were taken without disturbing the vegetation to quantify ambient BVOC concentrations within the *A. vulgaris* canopy. A second sample was taken at the same location as the first, but after the vegetation had been disturbed by walking through the *A. vulgaris* stand for 1 min to determine the maximum potential concentration within canopy following a physical perturbation. This treatment was introduced as a "best-case" scenario in terms of BVOC load within the *A. vulgaris* canopy, and was not intended to mimic any natural disturbance. To determine ambient atmospheric concentrations, control samples were also collected >10 m from all *A. vulgaris* populations and any known terpenoid-emitting vegetation (e.g., *Pinus* spp.). BVOC concentrations were determined from air samples (approximately 1.5 l) passed through a 2-stage solid absorbent cartridge (Tenax TA/Carbotrap B, Supelco Inc., Bellefonte, PA). BVOCs were subsequently thermally desorbed and analyzed by gas chromatography with mass spectrometric detection (HP5890 gas chromatograph, HP5972 mass spectrometer, Hewlett Packard, Palo Alto, CA) at the National Center for Atmospheric Research (NCAR) in Boulder, Colorado, USA. Details of the BVOC analysis are described previously (Greenberg et al. 1999, 2004). BVOC concentration for each monoterpene and total monoterpene concentration were analyzed using a two-factor ANOVA with date and collection treatments as fixed effects.

Soil BVOC analysis

To quantify *A. vulgaris*-derived BVOC concentrations in the soil environment, we collected soil samples on December 4, 2005, after all aboveground tissue had senesced, and most leaves had abscised. Three replicates of soil samples were taken at three locations along a transect through the *A. vulgaris* stand located on the Cornell campus: (1) in the center of the stand ("inside" hereafter); (2) at the invasion front ("border" hereafter); and (3) 2 m from invasion front in an adjacent oldfield ("outside" hereafter). Snow was removed and a 10 × 10 × 1 cm soil sample, including small stones and plant debris, was collected into 1 l glass jars and stored at 4°C until analysis. Due to cost and logistics, we have analyzed BVOCs at a single date similar to other studies (e.g., Hayward et al. 2001). Measuring soil BVOC emission from a warmed winter soil (see below) likely provides a maximum potential production for a given soil. At this time, the primary source of soil emissions (fragmenting trichomes) is high in abundance and volatilization rates are low due to cool temperatures (van Roon et al. 2005). Therefore, the effective 'pool' of BVOC during heating is high. These values

should be viewed as an index of BVOC pool sizes across treatments, but are not necessarily representative of growing season magnitudes.

For BVOC retrieval, sample jars were outfitted with modified lids with an inlet and outlet port. The inlet was outfitted with 6 cm non-reactive Teflon tubing fastened on the inside of the sample jar ending just above the soil sample, and connected to a pressurized tank of ultrapure air (<1.0 ppmv total hydrocarbon) from the outside of the sample jar. The outlet was connected to a solid-adsorbent cartridge (same as above), followed by a gas flow meter. The sample jar was pre-warmed in a 40°C water bath for 10 min to desorb all BVOCs, followed by 25 min BVOC collection at a flow rate of 200 ml min^{-1}. Soil samples were then dried at 70°C to determine soil dry weight (SDW). BVOC cartridges were analyzed at NCAR similar to within canopy samples (see above for details). Terpene concentrations are expressed as ng g^{-1} (SDW). Individual and total terpenoid concentrations were analyzed using a one-way ANOVA with collection location as a fixed effect.

In situ bioassay

To isolate the effect of *A. vulgaris* BVOCs in the field, we manipulated the location of *S. canadensis* in an *A. vulgaris* stand and whether *S. canadensis* experienced just aboveground or above- and belowground interactions with *A. vulgaris*. *Solidago canadensis* individuals were planted on the inside, border, and outside of the *A. vulgaris* stand in 2005 and 2006. In 2005, *S. canadensis* seeds were sown in soil-less media in early April and maintained in a greenhouse at 26/23°C day/night with natural lighting and watered as needed. *Solidago canadensis* individuals were randomly assigned to one of the following treatments on May 11: (1) a 15.3 cm diameter pot lined with weed fabric and filled with soil-less media (aboveground exposure only) or (2) transplanted to a 10 × 10 × 5 cm hole refilled with resident soil (above- and belowground interaction). *Solidago canadensis* individuals in pots and in resident soil were planted as pairs 0.25 m apart with a total of 10 replicates for each treatment combination. Each *S. canadensis* individual received 6 g of the slow-release fertilizer Osmocote 14-14-14 and 1 l of water on the day of transplanting with no additional watering or fertilization. The same experimental protocol as above was followed in 2006, with *S. canadensis* seeded on April 6 and transplanted to the field on May 25.

In 2005, *S. canadensis* height was recorded three times during the study. In 2006, at 8 weeks after transplanting, final height and number of vegetative ramets were recorded for all *S. canadensis* individuals, and aboveground biomass was harvested and dried at 70°C until constant mass was achieved. A few of the *S. canadensis* individuals were lost to vertebrate herbivory in both years, and were thus excluded from analyses. *Solidago canadensis* variables were analyzed using a two-way ANOVA with exposure (pot or soil), location (control, border, inside), and their interaction as fixed effects. Data from 2005 and 2006 were analyzed separately. Analyses were performed using the JMP v5.1 statistical software (Cary, NC).

Greenhouse activated carbon-competition experiment

To quantify the effects of root/rhizome-derived chemical exudates on competition, we used activated carbon to mitigate carbon-based compounds in a greenhouse experiment. Activated carbon has been demonstrated to reduce the bioavailability of organic compounds from a variety of media, and is a standard treatment to isolate the role of exudates in plant–plant interactions (Callaway and Aschehoug 2000; Kulmatiski and Beard 2006; Qin et al. 2007; Siemens and Blossey 2007). Seeds of six introduced North American *A. vulgaris* populations (Ithaca, Queens, and Port Jefferson, NY; Camden, NJ; Amherst, MA; and Montreal, Quebec) and *S. canadensis* were sown in soil-less media on May 5, 2006, and maintained in a greenhouse at 26/23°C day/night with natural lighting and watered as needed. Seedlings of *A. vulgaris* and *S. canadensis* were transplanted in pairs to 15.3 cm-diameter pots lined with weed fabric and filled with sand (pH 6.5) top-dressed with 6 g of the slow-release fertilizer Osmocote 14-14-14 on June 9. At the time of transplanting, the height of each shoot was recorded. Two treatments were imposed on *A. vulgaris–S. canadensis* pairs: (1) 20 ml finely ground (phosphorus free) activated carbon per 1 l sand (+carbon hereafter) and (2) sand only (−carbon hereafter). Four replications per population per treatment were included. Eight weeks

after transplanting, final height of both *A. vulgaris* and *S. canadensis* were recorded, and aboveground biomass was removed at soil level and roots/rhizomes were washed of sand. Then both were dried at 70°C until constant mass was achieved. Root-to-shoot (R:S) ratios were calculated as well.

To determine whether the presence of activated carbon increased *S. canadensis* growth, a mixed-model ANOVA was performed on each dependent variable with height at transplanting used as a covariate, *A. vulgaris* population as a random effect, the presence or absence of activated carbon as a fixed effect, and the interaction between *A. vulgaris* population and activated carbon as a random effect. Analyses were performed using the JMP v 5.1 statistical software (Cary, NC).

Results

In situ organic volatile analysis

Though not statistically significant, BVOC concentrations were higher early in the season (May) and decreased with time, and a disturbed canopy had higher BVOC concentrations than an undisturbed canopy (Tables 1 and 2). Further, BVOC partial pressures were always higher inside compared to outside the *A. vulgaris* monoculture (Table 1).

Soil BVOC analysis

Soil BVOC concentrations varied between <0.04 and 12.6 ng g $(SDW)^{-1}$ inside the *A. vulgaris* stand, and between 0 and 0.3 ng g $(SDW)^{-1}$ outside the stand (Fig. 1). Total monoterpene content was >70-fold higher on the inside and 18-fold higher at the border of the *A. vulgaris* stand, as compared to the outside (total monoterpenes: $F = 5.39$, $P = 0.05$).

In situ bioassay

In the field experiment conducted during 2005, in pots, *S. canadensis* height did not differ among locations (outside, border, inside) 2 weeks ($F = 1.85$, $P = 0.19$), 6 weeks ($F = 2.15$, $P = 0.15$), or 11 weeks ($F = 1.99$, $P = 0.17$) after transplanting (Fig. 2a). In resident soil, *S. canadensis* height did not differ among locations 2 weeks ($F = 0.75$, $P = 0.54$), 4.5 weeks ($F = 1.31$, $P = 0.31$), or 8 weeks ($F = 1.49$, $P = 0.27$) after transplanting (Fig. 2b). However, *S. canadensis* height inside was lower than the combined borders at 6 weeks ($F = 5.02$, $P = 0.04$) and 11 weeks ($F = 4.42$, $P = 0.05$).

In the field experiment during 2006, in pots, *S. canadensis* inside the *A. vulgaris* stand had ~50% less aboveground biomass than those grown outside, with *S. canadensis* at the invasion front (border) being intermediate ($F = 2.48$, $P = 0.10$,

Table 1 Mean terpenoid* concentrations (standard deviation) from two *Artemisia vulgaris* populations in Ithaca, NY taken early, middle, and late in the growing season

Date	Treatment	α-pinene	β-pinene	Camphene	Sabinene	Myrcene	3-carene	Total
					ppbv			
May 26	Control	0.02 (0.01)	<0.01	0.02 (0.01)	<0.01	–	<0.01	0.05 (0.03)
	Undisturbed	1.61 (2.25)	0.43 (0.52)	1.41 (1.96)	4.15 (5.87)	0.56 (0.76)	0.02 (0.02)	8.18 (11.4)
	Disturbed	3.10 (4.10)	2.00 (0.00)	3.50 (3.54)	17.0 (24.01)	1.20 (1.13)	<0.01	26.82 (32.8)
July 10	Control	0.04 (0.03)	0.01 (0.00)	0.01 (0.00)	<0.01	–	<0.01	0.06 (0.03)
	Undisturbed	0.05 (0.06)	0.02 (0.02)	0.05 (0.07)	<0.01	0.03 (0.04)	<0.01	0.16 (0.21)
	Disturbed	1.51 (2.11)	1.00 (1.41)	1.00 (1.40)	<0.01	<0.01	<0.01	3.53 (4.91)
Aug 4[a]	Control	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Undisturbed	0.05 (0.06)	0.01 (0.00)	0.02 (0.00)	<0.01	0.01 (0.01)	<0.01	0.09 (0.08)
	Disturbed	2.00 (0.00)	1.00 (0.00)	0.40 (0.14)	0.15 (0.07)	0.10 (0.01)	0.01	3.66 (0.20)

Gaseous samples were taken pre-disturbance (Undisturbed) and post-disturbance (Disturbed) of the *Artemisia vulgaris* canopy, and >10 m from the *A. vulgaris* population (Control)

* Dashes indicate terpenoids were not detected

[a] 3.0 l were sampled instead of 1.5 l

Table 2 *F*-statistic and *P*-values (in parentheses) for each identified monoterpene in an *Artemisia vulgaris* monoculture as a function of collection date and collection treatment (with and without disturbance and control)

Source	α-pinene	β-pinene	Camphene	Sabinene	Myrcene	3-carene	Total terpenes
Date	0.8 (0.44)	2.0 (0.18)	2.2 (0.15)	1.4 (0.27)	**3.0 (0.09)**	0.5 (0.61)	1.8 (0.20)
Canopy disturbance	**3.5 (0.06)**	**14.2 (0.0005)**	2.3 (0.14)	0.8 (0.47)	1.3 (0.29)	0.5 (0.61)	1.7 (0.22)

P-values ≤0.10 are in bold

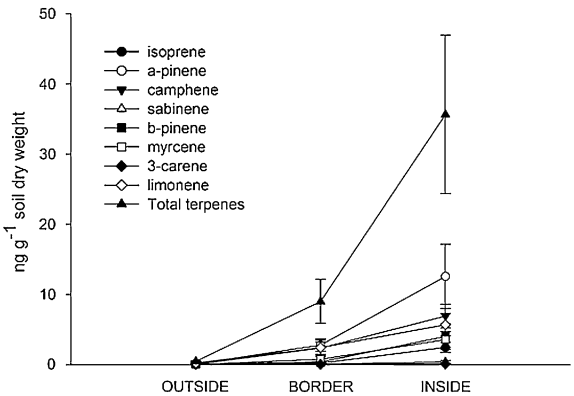

Fig. 1 BVOC concentrations in soil samples collected from inside, bordering, and outside an *Artemisia vulgaris* population

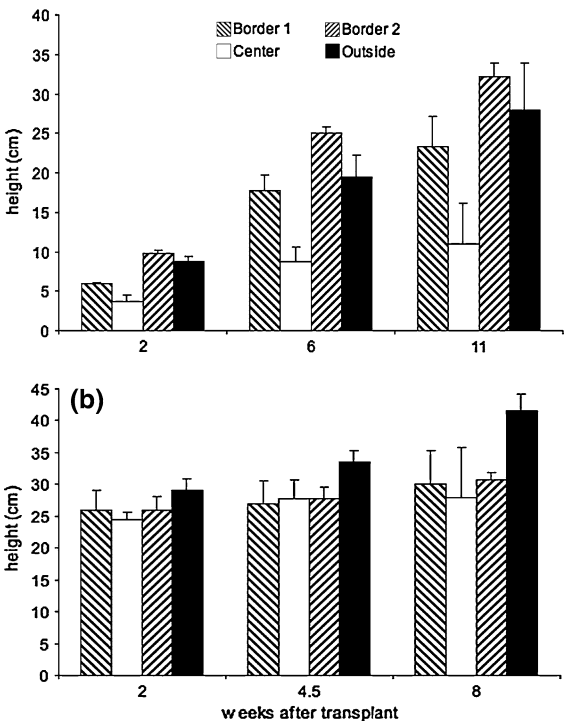

Fig. 2 Stem height of *Solidago canadensis* grown in (**a**) pots or (**b**) resident soil at three times after planting at the East and West border, center, and outside of an *Artemisia vulgaris* monoculture in 2005. Means are not significantly different

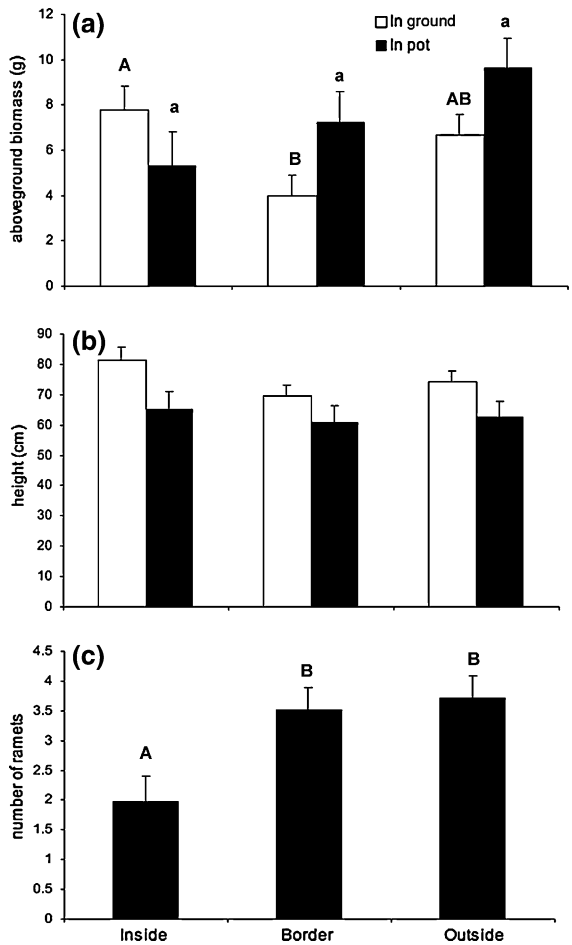

Fig. 3 *Solidago canadensis* (**a**) aboveground biomass, (**b**) height, and (**c**) number of ramets along a transect through an *Artemisia vulgaris* monoculture grown in resident soil or in adjacent pots in 2006. Bars with different letters are significantly different ($P < 0.05$) within a treatment (in pot or in ground)

Fig. 3a). In contrast, *S. canadensis* grown in resident soil at the invasion front had 40% less aboveground biomass than those grown either inside or outside the *A. vulgaris* stand ($F = 4.11$, $P = 0.03$, Fig. 3). *Solidago canadensis* final height did not differ among locations when grown in resident soil ($F = 2.32$,

$P = 0.13$), but differed when grown in pots ($F = 0.14$, $P = 0.87$) (Fig. 3). *Solidago canadensis* grown in resident soil in all three locations produced a single ramet. However, ramet production of *S. canadensis* grown in pots inside the *Artemisia* stand was reduced by nearly 50% relative to the open grown control ($F = 5.4$, $P = 0.01$; Fig. 3c).

Greenhouse activated carbon-competition experiment

Activated carbon had no effect on *S. canadensis* height ($F = 1.0$, $P = 0.31$), aboveground biomass ($F = 2.53$, $P = 0.11$), belowground biomass ($F = 1.64$, $P = 0.20$), or the root-to-shoot ratio ($F = 0.27$, $P = 0.60$). In addition, activated carbon did not affect *A. vulgaris* height ($F = 0.25$, $P = 0.61$), aboveground biomass ($F = 1.29$, $P = 0.26$), belowground biomass ($F = 0.16$, $P = 0.69$), or the root-to-shoot ratio ($F = 0.09$, $P = 0.77$).

Discussion

Throughout the growing season, gaseous monoterpene concentrations within *A. vulgaris* canopies, with or without substantial physical canopy disturbance, were >1,000-fold lower than those concentrations found to induce phytotoxicity in laboratory assays (Barney et al. 2005b). Inside the *A. vulgaris* monoculture, monoterpene concentrations in the top 1 cm of the soil profile were up to 11 ng g $(SDW)^{-1}$, >70-fold higher than a few meters outside the stand. Some measures of *S. canadensis* performance paralleled monoterpene soil concentrations—taller and larger individuals outside the *A. vulgaris* stand as compared to inside—especially when grown in resident soil (i.e., *A. vulgaris* "conditioned" soil). Additionally, *S. canadensis* grown in resident in situ soil (above and belowground interaction) produced only single ramets, while adjacent pot-grown (aboveground interaction only) *S. canadensis* was more productive. However, reduced *S. canadensis* performance does not appear to be the result of BOCs derived from *A. vulgaris* roots, as *S. canadensis* performance was unaffected by activated carbon in the soil matrix. Therefore, our results suggest that *A. vulgaris* leaf-derived BVOCs never reach phytotoxic concentrations in their gaseous state, but through accumulation in the soil matrix they may achieve levels that reduce interspecific performance within and bordering an *A. vulgaris* clone, fostering advancement along the clonal invasion front (Fig. 4). Additionally, *A. vulgaris* has evolved an 'invasive phenotype' in the introduced range of North America, producing fewer, relatively short ramets with vast underground rhizome networks that exhibit enhanced competitive ability as compared to native-range populations (Barney et al. 2008). The combination of increased competitive ability and utilization of

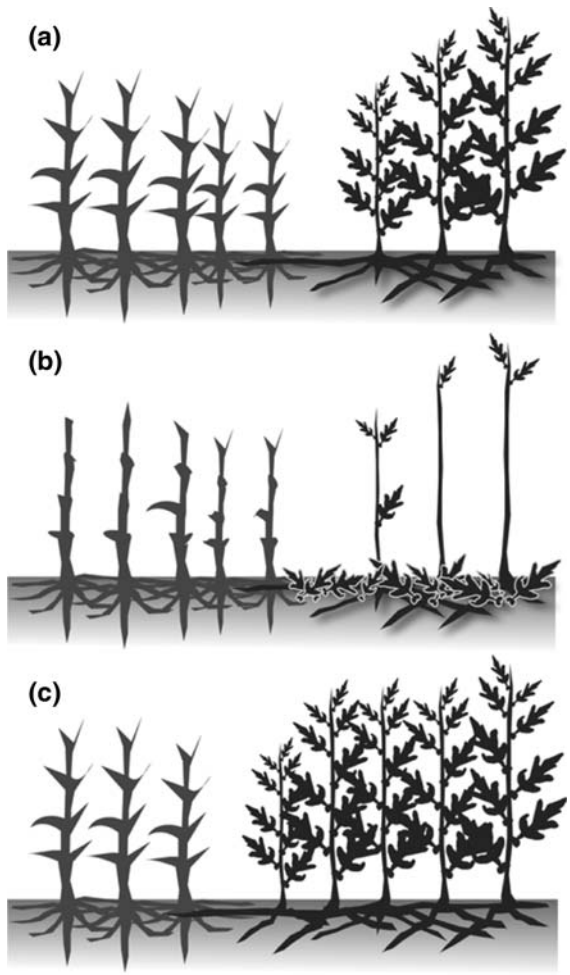

Fig. 4 Conceptual diagram illustrating the advance of an *Artemisia vulgaris* clone through an old field. **a** Roots and rhizomes of *A. vulgaris* (right) and *Solidago* (left) overlap though aboveground ramets remain distinct stands. **b** Leaf senescence and drop in late fall and winter, contributing BVOCs to soil matrix. **c** Advance of *A. vulgaris* along invasion front from ramets emerging from existing underground rhizomes

monoterpene phytotoxins has contributed to the invasiveness of *A. vulgaris* in North America.

Unlike the light-dependent emission of many terpenoids, monoterpenes are generally stored in specialized ducts or glands; thus emission is typically correlated with increasing temperature (Lerdau and Gray 2003). Monoterpene concentrations inside storage organs are often greatest in young tissue and decrease with age (Maffei et al. 1989). Barney et al. (2005b) found individual monoterpene concentrations up to 29-fold greater in young (60 days old) *A. vulgaris* tissue as compared to old (120 days old) tissue. In this study, we found monoterpene concentrations in undisturbed *A. vulgaris* canopies to be greatest in May (26.82 ppbv total monoterpenes) when plants were short (15 cm tall) and new foliage was being produced, but not August (3.66 ppbv) as would be expected when ambient temperatures are highest and plants were tallest (>160 cm), though foliage production had ceased. We were unable to calculate BVOC emission fluxes for *A. vulgaris*, but the related *A. tridentata* has been documented at <0.2 μg g $(LDW)^{-1}$ h^{-1} (Guenther et al. 1996) and *A. californica* has documented fluxes between 9.6 and 47.0 μg g $(LDW)^{-1}$ h^{-1} (Arey et al. 1995)—which might be artificially high due to mechanical disturbance of the enclosure. The BVOC concentrations found in this study are within the range of those expected −0.02–200 ppbv (Arey et al. 1995; Guenther et al. 1996). Nevertheless, even with major canopy disturbance—which would produce maximum BVOC effusion within the canopy—increasing total volatile monoterpene levels to 27 ppbv, the phytotoxic concentration observed in laboratory assays (Barney et al. 2005b) was never achieved at any point during the study, regardless of tissue age or ambient temperature. Additionally, we did not detect the two most phytotoxic monoterpenes, cineole and camphor, within the canopy. Either these populations do not emit these compounds or, more likely, the rate of mixing in the canopy and surrounding atmosphere is of a magnitude that makes the partial pressures of these compounds below the detection limit of GC-MS. Based on ambient and disturbed canopy monoterpene concentrations, our results suggest that, *A. vulgaris* monoterpenes in their gaseous state play a negligible direct role in plant–plant competitive interactions.

Solidago canadensis performance (height) was reduced when grown in pots inside an *A. vulgaris* stand experiencing only aboveground (atmospheric) interactions. The reduction in *S. canadensis* aboveground productivity (up to 60% shorter in center of stand, and 50% fewer ramets) is likely not the result of interactions with gaseous *A. vulgaris* BVOCs (see above), but could be the result of the accumulation of monoterpenes through dry or wet (i.e., rainfall) deposition to the potting mix, as hydrocarbon monoterpenes (e.g., α-pinene) are water soluble up to 32 ppm (Weidenhamer et al. 1993). Barney et al. (2005b) demonstrated that soil indirectly exposed to *A. vulgaris* tissue for 24 h (i.e., dry deposition) reduced *Lepidium sativum* L. seedling shoot length by 50%. Additionally, the action of raindrops breaking BVOC-storage structures combined with moderate water solubility could allow for in-season BVOC accumulation in the upper soil horizon. However, as *A. vulgaris* leaves senesce and drop, the unruptured trichomes and sequestered BVOCs become components of soil organic matter, which is the likely source of the majority of soil BVOCs. Several years of this cycle of in-season BVOC wet and dry deposition during the growing season, followed by aboveground biomass incorporation into the soil matrix, could increase monoterpene concentrations to phytotoxic levels (note: bioassays using field soil have been unsuccessful to date in this system due to the resident seed bank precluding identification of the *Solidago* test species, data not shown). This could explain the poor performance of resident soil grown *S. canadensis*, which only yielded single ramets (Fig. 3c). Hayward et al. (2001) demonstrated the sequestration and subsequent release of plant-derived BVOCs in soils when they quantified monoterpene emission rates from Sitka spruce (*Picea sitchensis*) forest floor at 33.6 μg m^{-2} h^{-1} for undisturbed soil, and an astounding 199 μg m^{-2} h^{-1} with the duff removed. We found up to 34 ng g $(SDW)^{-1}$ total terpenoids in the top 1 cm inside the *A. vulgaris* stand and 9 ng g $(SDW)^{-1}$ at the border, representing total monoterpene levels in the soil matrix and not an ambient flux. It should be noted that pot-grown *S. canadensis* were on average smaller than adjacent resident soil-grown *S. canadensis* (Figs. 2 and 3), likely an artifact of the lower water-holding capacity of the media used in the pots (we noted quicker drying of the pots than the resident soil between rain events). We made only within-treatment (pot-grown vs. pot-grown)

comparisons to avoid different relative growth potentials in soil or pots. Additionally, differences in *S. canadensis* performance are not attributable to variation in growing environment (e.g., light quantity, relative humidity) inside versus outside the *A. vulgaris* stand, as stem density, canopy height, and canopy structure were similar in the adjacent community.

The decline in terpenoid levels in the soil matrix from the invasion front to 2 m outside the *A. vulgaris* stand suggests that aboveground litter (leaves and inflorescences) is the primary source of soil BVOCs (Fig. 4). Emission of BVOCs from the soil matrix would occur over subsequent growing seasons as a function of soil temperature (van Roon et al. 2005, Hayward et al. 2001), but would also solubilize in the soil solution (Weidenhamer et al. 1993), and adsorb to soil particles (Barney et al. 2005b). Germinating seedlings and emerging rhizome buds of competing species will experience BVOCs in aqueous solution and as vapors in the soil matrix, potentially reducing growth and fostering *A. vulgaris* advancement from existing rhizomes (Fig. 4). Up to 5% of the underground rhizome architecture exists beyond the aboveground invasion front in *A. vulgaris* clones (Barney et al. 2005a), which produce new ramets the following year with reduced interspecific competition (Fig. 4). The aboveground invasion front of single clones of *A. vulgaris* have been documented to increase at a linear rate of 60 cm $year^{-1}$ with a concomitant 10-fold increase in ramet production annually (Barney et al. 2005a). This rapid expansion of *A. vulgaris* clones is likely fostered by the sequestration of leaf synthesized-BVOCs in the internal and bordering soil environment, which reduce interspecific performance (Fig. 4). Our results using activated carbon suggest that BOCs in the soil matrix are foliar-derived.

Several studies have demonstrated that activated carbon can mitigate the negative effects of carbon-based root exudates on recipient species (Callaway and Aschehoug 2000; Prati and Bossdorf 2004; Siemens and Blossey 2007). In our study, the presence of activated carbon did not enhance *S. canadensis* performance when grown in pots with *A. vulgaris*, suggesting that *A. vulgaris* does not produce root-derived BOCs likely to be eliminated by active charcoal (monoterpenes or other phytotoxins) in the time frame of that experiment (i.e., 8 weeks). However, this does not preclude the potential sequestration of BOCs in underground structures that are released upon root turnover or rhizome senescence (e.g., Kovacevic et al. 2002). Our soil BVOC assay was unable to definitively determine if terpenoids, which accumulate in the rhizosphere over the life of the population, originate from leaf or underground tissue. However, there is little evidence to suggest that root/rhizome tissue exudes/emits terpenoids (Charlwood and Charlwood 1991).

Future studies investigating the existence of secondary compounds in plant–plant interactions in invasive plant species would benefit by investigating whether there has been positive selection for genotypes over-yielding phytotoxic chemicals. A handful of studies have begun to document the evolution of more competitive phenotypes in the introduced range (Bossdorf et al. 2005 and references therein), but little attention has been paid to the evolution of enhanced phytotoxic phenotypes (Callaway and Ridenour 2004). Additionally, the 'training' of soils by invasive plants (e.g., Klironomos 2002) could be an indirect artifact on interspecific plants via exudation/emission of unique phytochemicals. The more unique an introduced chemical, or suite of compounds, is to a naïve ecosystem, the increased likelihood of those compounds having unexpected ecological consequences and enhancing the invading species performance.

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