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American Midland Naturalist, Vol. 146, No. 1. (Jul., 2001), pp. 146-152.

Stable URL:
http://links.jstor.org/sici?sici=0003-0031%28200107%29146%3A1%3C146%3AEOGM%5BP%3E2.0.CO%3B2-N

American Midland Naturalist is currently published by The University of Notre Dame.

Am. Midl. Nat. 146:146–152

Effect of Garlic Mustard [Alliaria petiolata (Beib. Cavara & Grande)] Extracts on Plants and Arbuscular Mycorrhizal (AM) Fungi

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ABSTRACT.—We examined the effects of garlic mustard (*Alliaria petiolata*) leachates on germination of arbuscular mycorrhizal (AM) fungal spores, colonization of plant roots by AM fungi and germination and root growth of monocot and dicot plants under laboratory conditions. In the field we examined the effect of garlic mustard on AM inoculum potential (MIP). Water leachates of garlic mustard prevented germination of spores of the AM fungus *Gigaspora rosea*, inhibited the formation of AM associations with tomato (*Lycopesicum esculentum*) and significantly reduced germination of tomato seeds. Garlic mustard leachates also reduced root length of tomato and sorghum (*Sorghum bicolor*) seedlings. Under field conditions we found a significant negative correlation between the density of garlic mustard and the mycorrhizal inoculum potential of the soil in which the plants grew. These results suggest that garlic mustard may reduce the competitive abilities of native plants by interfering with the formation of mycorrhizal associations and root growth.

INTRODUCTION

Garlic mustard [*Alliaria petiolata* (Beib.) Cavara & Grande] is an invasive, exotic biennial member of the Brassicaceae. It is native to Eurasia (Clapham *et al.*, 1952) and is now naturalized in much of the eastern United States and adjacent Canada. Garlic mustard was first noted in North America in 1868 on Long Island, New York. By 1990 the plant had spread to three Canadian Provinces (Scoggan, 1978; Cavers *et al.*, 1979), 27 Midwestern and Northeastern states in the United States, the District of Columbia and Oregon and Utah (Nuzzo, 1991, 1993). Resource managers recognize garlic mustard as a threat to forested areas of the Northeastern and Midwestern United States and in southern Ontario and Quebec (Scoggan, 1978; Cavers *et al.*, 1979; Nuzzo, 1991; Yost *et al.*, 1991). In relatively undisturbed forest this species can form monospecific stands that dominate the understory, displace the native understory flora and reduce the species diversity of these forests (Nuzzo, 1991; Yost *et al.*, 1991). McCarthy (1997) reported that the diversity of forest understory species increased when garlic mustard was removed from plots. However, garlic mustard is frequently found near disturbed areas, especially those adjacent to trails or openings in forests or at the edges of forests. Byers and Quinn (1998) suggested that the ability of garlic mustard to invade undisturbed forests and out compete native species is not clear. Garlic mustard, like many other invasive species (Jonstone, 1986; Knops *et al.*, 1995), may require some degree of disturbance before it can colonize a site.

Garlic mustard has been thoroughly studied in terms of its life history (Lhotska, 1975; Baskin and Baskin, 1992; Anderson *et al.*, 1996), and ecological impact on both plant (Yost *et al.*, 1991; Nuzzo, 1998) and animal (Porter, 1994; Huang *et al.*, 1995) populations in forests in which it has become established. Research findings regarding the allelopathic activity of garlic mustard have been mixed. McCarthy and Hanson (1998) found little evi-

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dence of allelopathic effects of water-based garlic mustard extracts on germination and seedling biomass of radish (*Raphanus sativus* L.), winter rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* Roth) and lettuce (*Lactuca sativa* L.), attributing the success of this plant to competitive rather than allelochemical attributes. Kelley and Anderson (1990), however, isolated water-soluble compounds through thin layer chromatography which suppressed the germination of several plant species. Isolation of the hydrolysis products of glucosinolates present in garlic mustard extracts and subsequent seedling radical elongation assays performed by Vaughn and Berhow (1999) also revealed phytotoxic effects on plants including wheat (*Triticum aestivum* L.) and, to a lesser degree, on garden cress (*Lepidium sativum* L.), another member of the Brassicaceae.

Garlic mustard, like many other members of the Cruciferae, produces glucosinolates, a class of organic molecules which, when acted upon by the enzyme myrosinase (α-thioglucosidase glucohydrolase; VanEtten and Tookey, 1983) after plant cell disruption are degraded to form products including substituted isothiocyanates, thiocyanides, nitriles, and oxazolidinethiones (Vaughn, 1999). Isothiocyanates in particular have been studied extensively in terms of pesticidal (Borek *et al.*, 1994; Brown and Morra, 1995; Vaughn, 1999) and antifungal (Vaughn, 1999) activity. Shreiner and Koide (1993a, b) reported that 4-hydroxybenzyl isothiocyanate, derived from the hydrolysis of glucosinalbin found in wild mustard [*Brassica kaber* (DC.) Wheeler], inhibited the germination of the arbuscular mycorrhizal (AM) fungus *Glomus etunicatum*. This is of interest because garlic mustard, like all other members of the Brassicaceae, is nonmycorrhizal. This suggests that isothiocyanates and/or other glucosinolate derivatives may influence invasive and competitive ability of garlic mustard through allelopathy and inhibition of mycorrhizal formation of herbaceous deciduous forest understory species, 71–84% of which have been shown to form arbuscular mycorrhizal associations (Brundrett and Kendrick, 1988; Berliner and Torrey, 1989; Roberts, 1997).

The objective of this study was to examine the effects of exposure to garlic mustard extract on the germination of AM fungal spores, colonization of plant roots by AM fungi and germination and root growth of monocots and dicots.

METHODS

The effect of garlic mustard leachate on the germination of Gigaspora rosea azygospores.—*Gigaspora rosea* inoculum was obtained from INVAM (International Vesicular Mycorrhizal collection facility, University of West Virginia) and cultured in a sterilized medium with a sorghum (*Sorghum bicolor* L.) host for 60 days. Azygospores were isolated using a wet sieving density gradient methods (Anderson and Liberta, 1992; Roberts, 1997). To determine if garlic mustard produces chemicals that inhibit germination of AM fungal spores, azygospores of *G. rosea* were placed on the surface of 1% Bacto-agar containing a defined plant culture medium prepared with or without a garlic mustard leachate (Allen *et al.*, 1979). Garlic mustard leachate was prepared by soaking 50 g of air-dried whole plant material in 1000 ml sterile distilled water for 24 h. The leachate was passed through a series of coarse sieves to remove large particulate matter and then through a 0.2 μm millipore filter to disinfect (Cote and Thibault, 1988). In the first trial a single 10 ml plastic petri dish was prepared containing medium in which one-half of the water was replaced with garlic mustard leachate. A 10 ml dish containing only water in the medium was prepared as a control. Ten azygospores were placed on the surface of the medium in each dish. All petri dishes were incubated at 30 C for 5 d, then removed. Treatment and control plates were then examined microscopically and the number of germinated spores was counted. In a second trial 5 azygospores were placed on the surface of medium contained in each of 5 petri

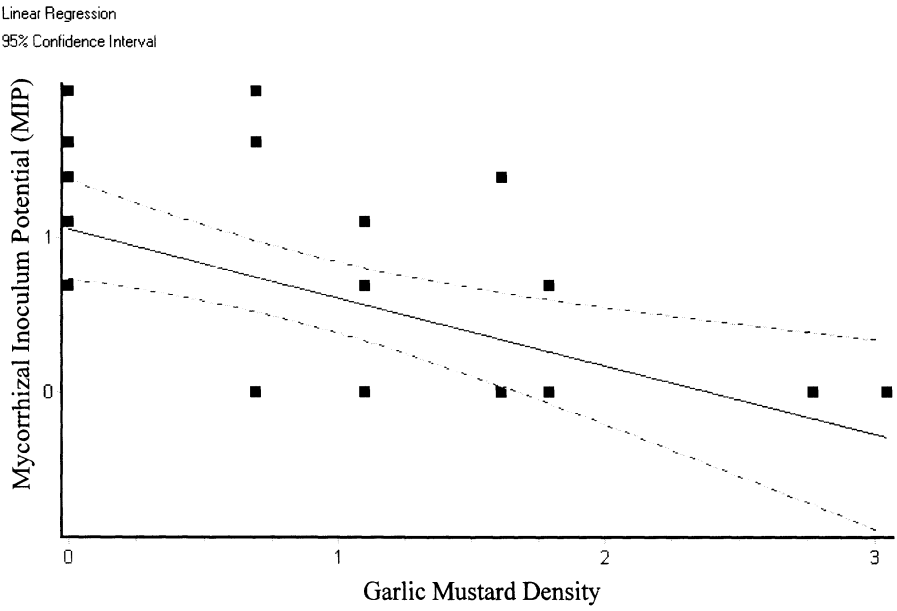

FIG. 1.—The effect of variation in mycorrhizal inoculum potential (MIP) inside of garlic mustard colonies measured as density (plants/dm^2) on mycorrhizal inoculum potential (MIP). All data were natural log transformed. Note: three pairs of data points overlap, reducing the number of visible points to 17

dishes for the treatment and control. The number of spores that germinated in each trial was recorded.

The effect of garlic mustard leachate on the colonization of tomato (Lycopersicum esculentum Mill).—Tomato seeds were surface sterilized in 0.05% sodium hypochlorite for 2 min and germinated on Bacto-agar to insure sterility. *Gigaspora rosea* azygospores were surface sterilized for 2 min in 0.05% sodium hypochlorite, then rinsed 5 times in sterile distilled water. One seedling and 4 spores were aseptically transferred under a laminar flow hood to 10 ml of agar-based semi-solid plant culture medium in a 100 ml bottle. There were 10 replicates for the treatment (containing 50% leachate) and control. All replicates were sealed in a surface-sterilized, dust-free Plexiglas chamber, which was placed in a greenhouse for 20 d. Spores were then examined to determine if germination had occurred, and the number of germinated and non-viable spores was recorded. Each root was examined microscopically for AM colonization, and root lengths were measured. Data for differences in root length were analyzed by Student's *t*-test.

The effect of garlic mustard leachate on the germination and root growth of monocot and dicots.—To test for the effect of the leachate on seed germination and root growth of a monocot plant, 10 ml of plant culture medium were poured into sterile 100 ml glass bottles with ten replicates for the treatment (50% leachate in the medium) and the control. Two surface-sterilized sorghum (*Sorghum bicolor* L.) seeds were aseptically transferred to the surface of the medium in each bottle. For comparison with a dicot, the same procedure was followed, except that 5 ml of defined culture medium with or without leachate were poured into five 10 ml petri dishes and 5 surface-sterilized tomato seeds were placed on

the agar surface of each. Seeds were incubated at 30 C, and after 5 d the number of germinated seeds was recorded and root lengths of tomato and sorghum were measured.

The effect of garlic mustard on the mycorrhizal inoculum potential of soil.—Variation in mycorrhizal inoculum potential (MIP) was examined inside and outside of garlic mustard colonies in 1992 at two sites: ParkLands Foundation Merwin Nature Preserve (July 28), located in McLean County, Illinois, 25 km from Normal, Illinois, and Indiana Dunes National Lakeshore, Porter, Indiana (July 30). The ParkLands site is a second growth oak-hickory forest dominated by white oak (*Quercus alba*) and shagbark hickory (*Carya ovata*) that was grazed until 1967, but has been undisturbed since then. The Indiana Dunes site is a stabilized dune forest along the southern edge of Lake Michigan typified by red oak (*Quercus rubra*), red maple (*Acer rubrum*) and white pine (*Pinus strobus*). At both sites a 30 m transect was located inside of a garlic mustard stand. At 3 m intervals along the transect, a dm^2 quadrat was located using a stratified random sampling procedure. Within each quadrat the number of garlic mustard plants was counted and 4 soil cores 2.5 cm in diameter and 10 cm deep were extracted. Soil cores from the ParkLands and Indiana Dunes sites were taken from inside (n = 10) large garlic mustard stands. Each sample replicate was placed in a separate plastic bag and transported to the laboratory to determine MIP using the corn root bioassay technique described by Moorman and Reeves (1979). Corn seeds (*Zea mays* L., Funk Seed International, hybrid G4444) were surface disinfected for 3 min in 90% ethanol, then placed in vermiculite moistened with deionized water for 48 h at 30 C to initiate germination. The germinated seeds were placed one each on a substrate in separate 165 cm^3 plastic conical containers (Ray Leach "Cone-tainers" Nursery, Canby, Oregon) to be assayed. The corn seedlings were grown for 15 d in a Conviron growth chamber with light/dark periods of 14/10 h at temperatures of 24/22 C, respectively, with an irradiance level of 1400 $\mu mol\ m^{-2}\ s^{-1}$. Plants were harvested, the roots of each excised and washed free of soil. A 0.25 g fresh weight root sample was taken from each plant and cut into 1 cm segments and cleared in 5% KOH and stained with 0.05% trypan blue prepared in an acidic glycerol solution (Koske and Gemma, 1989). One hundred stained root segments from each plant were then scored for colonization using the gridline-intersect method of Giovanetti and Mosse (1980). Soil MIP calculated from the 20 quadrats within the garlic mustard stands was regressed against the number of garlic mustard plants per quadrat. The MIP and counts of garlic mustard were log transformed to linearize the data.

RESULTS

The effect of garlic mustard leachate on the germination of Gigaspora rosea azygospores.—In the first trial none of the 10 *G. rosea* azygospores germinated in the petri dish with Bacto-agar plus garlic mustard leachate, while 7 of 10 germinated in the petri dish containing Bacto-agar alone. In the second trial, when *G. rosea* azygospores were placed in dishes containing Bacto-agar alone or Bacto-agar plus garlic mustard leachate, more azygospores (Mean ± SE = 2.8 ± 0.66 vs. 0) germinated in the control than in the treatment plates.

The effect of garlic mustard leachate on the colonization of tomato.—None of the *Gigaspora rosea* azygospores placed on Bacto-agar containing garlic mustard leachate with tomato seedlings germinated, while 13 spores (Mean ± SE = 0 vs. 1.3 ± 0.30) germinated in the control. Seven of the 10 tomato seedlings grown in the control were colonized by *G. rosea* while none of the seedlings in the treatment were colonized. Plants grown in the control produced significantly longer root lengths (8.05 ± 1.88 cm vs. 2.91 ± 0.76 cm) than those grown in the treatment ($t = -2.66$, df = 18, $P < 0.05$) which contained leachate.

The effect of garlic mustard leachate on the germination and root growth of monocot and dicots.—While there were no significant differences between the number of sorghum seeds

which germinated between the treatment and control (1.35 ± 0.11 vs. 1.35 ± 0.11), the mean root length of sorghum seedlings in the treatment containing garlic mustard leachate was shorter (2.91 ± 0.77 cm vs. 8.05 ± 1.88 cm) than those grown in the control. Tomato also produced shorter root lengths when grown in the treatment than in the control (6.62 ± 1.30 cm vs. 11.20 ± 1.12 cm). More tomato seeds germinated in the control than in the treatment (0.72 ± 0.09 vs. 0.45 ± 0.11) containing garlic mustard leachate.

The effect of garlic mustard and tomato on the mycorrhizal inoculum potential of soil.— Regression analysis revealed a significant negative correlation ($r^2 = 0.2940$; $P < 0.05$, df = 1) between the density of garlic mustard in dm^2 quadrats and MIP (Figure 1).

DISCUSSION

Garlic mustard is common in areas where soil disturbances have taken place, such as ditches, shaded roadsides, at the edges and along trails in woodlots and forests (Cavers *et al.*, 1979). Burke and Grime (1996) suggested that natural areas are more likely to be subject to invasion by non-native species if they have been recently subjected to intense disturbance events. Many studies have shown that soil disturbance caused by strip mining, selective cutting and clearcutting of forests reduces the level of AM fungal inoculum in soil (Reeves *et al.*, 1979; Gould and Liberta, 1981; Jasper *et al.*, 1991; Moffat, 1993; Gould *et al.*, 1996). Reeves *et al.* (1979) reported that, while mycorrhizal plants comprise the majority of the herbaceous plant species in subclimax and climax ecosystems, disturbed habitats having low levels of mycorrhizal fungal propagules are likely to support non-mycorrhizal plants, which may further reduce soil inoculum levels. Allen and Allen (1984) hypothesized that non-mycorrhizal plants growing in soil having low MIP slow the rate of succession of mycorrhizal species. Establishment of garlic mustard on a site and its potential to disrupt mycorrhizal systems may have profound affects on deciduous forests in eastern North America because of the high percentage of understory species forming mycorrhizae (Brundrett and Kendrick, 1988; Berliner and Torrey, 1989; Roberts, 1997).

In an examination of the release of allelochemicals from green manure composed of a related mustard, rape (*Brassica napus* L.) Gardiner *et al.* (2000) found that nine different products of the degradation of glucosinolates, including five isothiocyanates, three nitriles and one oxazolidinethinone were observed in maximum concentration in soil 30 hours after initial plow down, and that those compounds derived from rape seed root where dominant as compared to those of shoot and leaf. This suggests that the roots were most likely to be the source of phyotoxic and fungitoxic compounds. Borek *et al.* (1994) found that myrosinase decomposition of the glucosinolate sinigrin in water extracts from six soils with pH values above 4.0 produced primarily allyl isothiocyanate. In a later study Borek *et al.* (1996) reported that extracellular myrosinase can exist in soil, particularly in the rhizosphere of mustards. The release of glucosinolate compounds from mustard roots owing to exudation or root tissue damage could result in enzymatic degradation of these compounds into a number of different allelochemicals.

The release of the products of glucosinolate degradation from the roots of garlic mustard may inhibit germination of the seeds of native mycorrhizal plants and AM fungal spores in close proximity to the rhizosphere. The inhibitory effect of such allelochemicals would help to maintain a microsite environment amenable to only garlic mustard plants and a few other species capable of tolerating such compounds. Further research investigating the presence of glucosinolates, their breakdown products and soil-borne myrosinase in and around the rhizosphere of living first- and second-year garlic mustard plants is warranted.

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SUBMITTED 28 SEPTEMBER 2000 ACCEPTED 26 FEBRUARY 2001