The allelopathic potential of an invasive species, *Microstegium vimineum*, and a native species, *Eupatorium rugosum* and the effects of microbial organisms in allelopathic studies

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Abstract

The success of invasive species can have profound effects on ecosystems. One crucial factor which may be involved in this success is the allelopathic potential of an invasive species. Our study focused on the allelopathic potential of *Microstegium vimineum*, an invasive annual grass in forests of eastern North America, and a native herb layer species, *Eupatorium rugosum*. In one experiment we assessed the allelopathic effect of each species on germination of two common test species, radish and lettuce, by applying aqueous extracts from the roots and shoots of *M. vimineum* and *E. rugosum* to test seeds in Petri dishes. All extracts inhibited seed germination, relative to water controls. In a second experiment, we planted seeds from lettuce and radish in a factorial pot experiment with 1) non-sterile forest soil or heat-sterilized forest soil; 2) activated carbon added or not added; and 3) watered with *M. vimineum* extract, *E. rugosum* extract or water. Neither species' extracts reduced the biomass of test seedlings, but they both either inhibited seed germination or increased seedling mortality. Allelopathic effects against seeds and seedlings of competitors may therefore be one mechanism of *M. vimineum* invasiveness, but this strategy is also available to a native species. Biomass of plants grown with carbon in non-sterile soil decreased relative to controls, while it increased with carbon in sterile soil. A model is provided which offers an explanation for complex microbiological effects in allelopathic studies and plant growth in both sterilized and non-sterilized soil.

Microstegium vimineum

Eupatorium rugosum

Introduction

Allelopathy is the negative effect of chemicals produced by one species of plant on the survival, growth, and/or reproduction of another species. Allelopathic chemicals can inhibit seed germination and reduce the biomass of competitors. Recent studies suggest that allelopathy may be an important mechanism of invasion by non-native species. However, if allelopathy is also employed equally by co-occurring native competitors, it is less likely to be a factor in non-native invasion. Therefore, we compared the allelopathic potential of a non-native invasive species and a co-occurring native species.

Microstegium vimineum is an annual C_4 grass that was introduced into eastern North America from east Asia. It is invasive in the mid-Atlantic region, where the herb layer of invaded forests can become dominated by *M. vimineum*. Morphologically, its roots and lower shoots are rather nondistinct; plants grow with the lower portion of their shoots nearly horizontal, in close proximity to the soil. A common native species that co-occurs with *M. vimineum* is the perennial herb *Eupatorium rugosum*. It has well established roots which allow the herb to stand upright, and is known to be toxic. Both species may grow together as co-dominants, but only the non-native *M. vimineum* is considered "invasive."

Our study also focused on the indirect effect of allelopathy on plants via direct effects on the soil microflora, which can have complex influences on plant growth. Negative influence may come from resource competition, pathogens, or the release of toxic chemicals upon microbe death ("lysotoxins"). Positive effects may include mutualistic relationships and fertilization of soil upon microbe death.

Methods

We collected *M. vimineum* (MIVI) and *E. rugosum* (EURU) samples from Washington Crossing State Park, Titusville, New Jersey, USA. Roots and shoots were separated, cleaned and dried for three days at 60º C. We made plant extracts by combining 1 g (dry weight) of plant tissue per 10 mL deionized water in a blender for 90 sec, stirring for 16 hrs, and filtering through cheesecloth. Deionized water was the control solution. Commercially available lettuce and radish were used as test species.

Effect of EURU and MIVI extracts on seed germination in Petri Dishes

- 25 seeds per Petri dish in filter paper with 1 cm between seeds.
- Treated once with extract from MIVI shoots or roots, EURU shoots or roots, or water.
- Plates sealed with parafilm.
- 12 replicates, randomized into blocks and maintained at room T.
- Number of germinated seeds counted each day for 7 days.
- On day 7, all germinated seedlings were air dried and weighed.

Effect of EURU and MIVI extracts on seedling survival and biomass in soil

- Seeds planted in film can pots: half in normal forest soil, half in forest soil heat-sterilized in an autoclave; half of each had activated carbon added (to adsorb possible allelochemicals).
- Pots watered with whole-plant MIVI or EURU extract or water, 1 x per week for 3 weeks.
- Survival and dry biomass recorded after 3 weeks growth.

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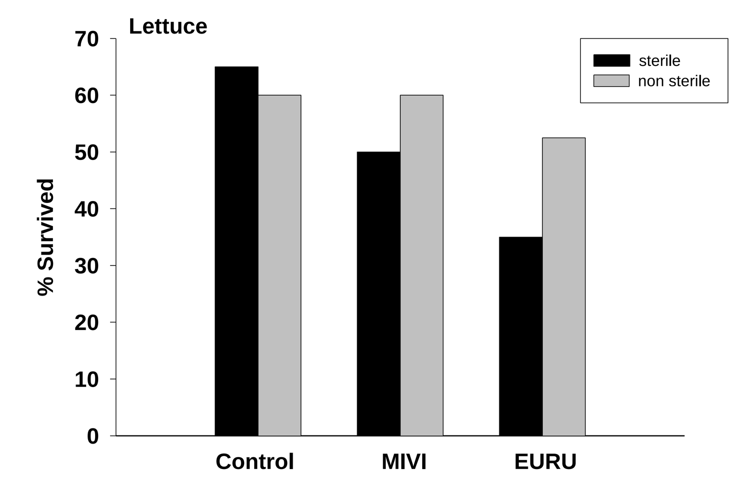
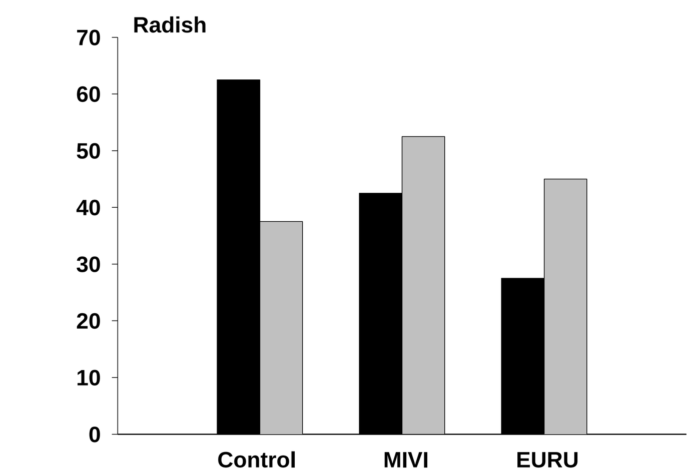
Results

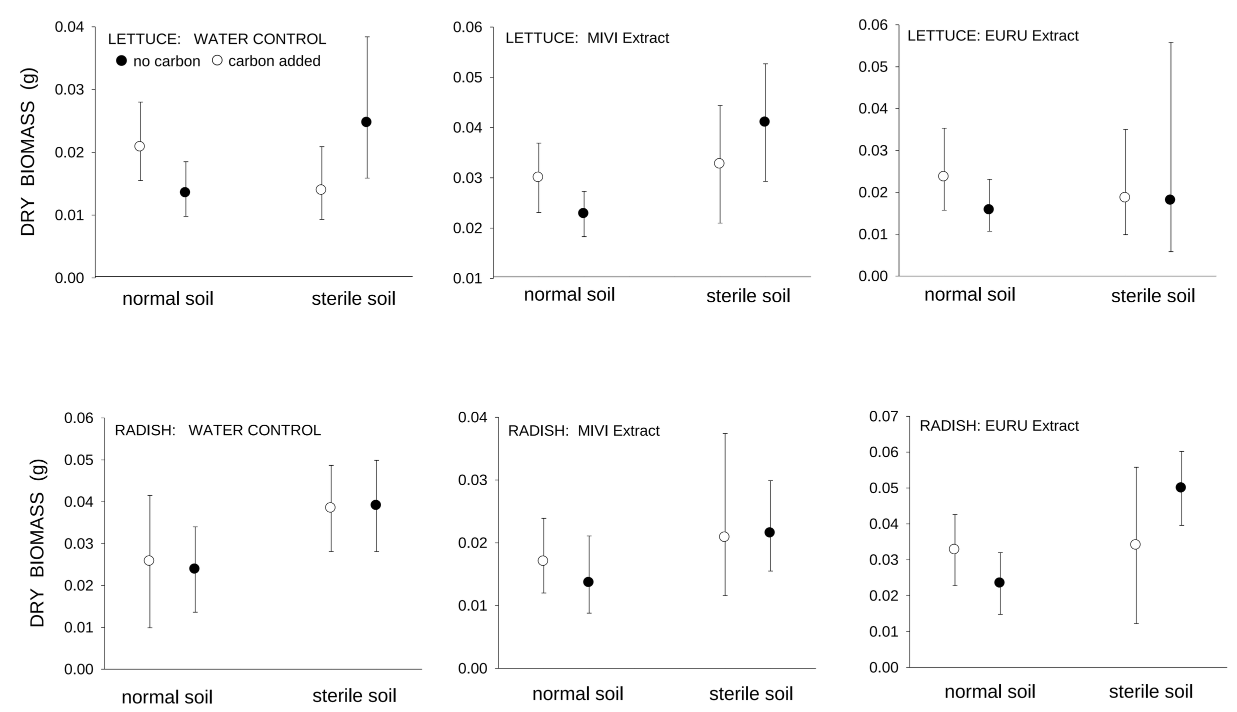
Table 1. Mean percent germination on Days 2, 4, and 7 and total biomass of germinated seedlings on Day 7.

Lettuce					
mean % germination	**Water control**	**MIVI roots**	**MIVI shoots**	**EURU roots**	**EURU shoots**
Day 2	97 % [A]	16%	7.33%	39.70%	8%
Day 4	98.67%	28.67%	36.33%	72%	34%
Day 7	99%	37.01%	54%	80.33%	38%
total dry biomass of germinated seedlings	3.041 g	0.223 g	0.282 g	1.280 g	0.121 g

Radish					
mean % germination	Water Control	MIVI roots	MIVI shoots	EURU roots	EURU shoots
Day 2	97%	64%	29.33%	35.33%	15%
Day 4	98%	92.67%	86.67%	82%	45%
Day 7	98.33%	94.33%	95.33%	91.33%	56%
total dry biomass of germinated seedlings	20.999 g	19.510 g	14.949 g	12. 736 g	6.940 g







Discussion

Effect of EURU and MIVI extracts on seed germination in Petri Dishes

All extracts proved to be allelopathic to some extent on seed germination. When compared to the seeds treated with the control, each delayed and inhibited seed germination and simultaneously limited the biomass of germinated seedlings. The shoots of *E. rugosum* proved to exhibit the strongest allelopathic effect on both radish and lettuce seeds when compared with the other extracts. *Eupatorium rugosum* appears to thrive in forests where deer have decimated many other plant species, perhaps due to high levels of secondary chemicals in the shoots; or results suggest that another benefit may be allelopathy against plant competitors; allelopathic chemicals are often non-specific. The roots and shoots of *M. vimineum* had very similar allelopathic effects on both radish and lettuce seeds when compared to each other. Due to their similar morphology, it is possible that they share similar levels of secondary chemicals that may inhibit seed germination and seedling growth. Additionally, because the roots and shoots are both oriented in close proximity to the soil, there may be no evolutionary advantage to specifically allocating allelopathic chemicals to either region of the plant.

Effect of EURU and MIVI extracts on seedling survival and biomass in soil

Our results showed that *M. vimineum* and *E. rugosum* extracts inhibit seedling survival in the soil, yet do not reduce biomass. This is strong evidence that if allelopathy is affecting these two species, it occurs only at the earliest life history stages of seed germination and/or seedling survival. This is in agreement with the results from the Petri dish seed germination experiment. It would be more economical, and therefore evolutionarily favored, for plants to gain a dominant advantage by producing allelopathic chemicals which inhibit seed germination specifically. By doing this, competitors are inhibited before they have a chance to exert any negative effect on the allelopathic plant. Due to the extremely sensitive nature of lettuce and radish seeds, a future experiment is planned which will more directly test the allelopathic effects of MIVI and EURU on other native and invasive species forest species, which will simultaneously offer insight into the effects of a shared evolutionary history on allelopathic potential.

Contrary to our expectation, the addition of activated carbon, which has been shown to inactivate allelochemicals in other studies, did not increase the growth of plants growing in the soils we watered with plant extracts. Rather, carbon addition had a negative effect on plant growth, except when the soils were sterilized. We offer a model to explain the complex effects that experimental soil sterilization may have on plant growth (Figure 3.)

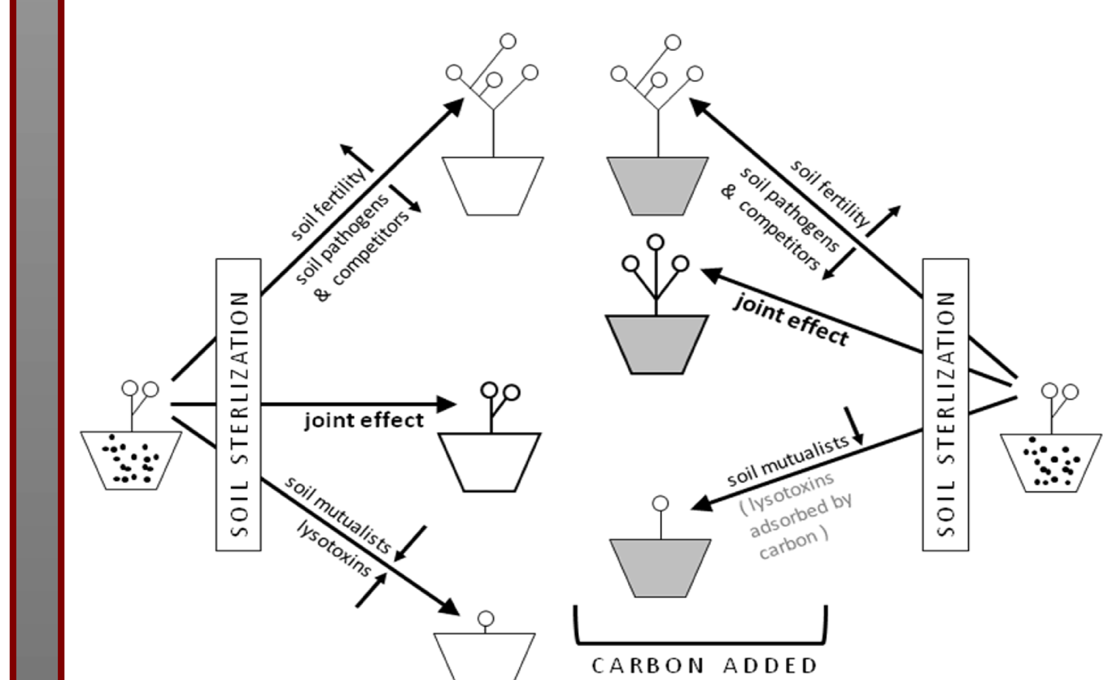


Figure 3. Model hypothesizing the sterilization-induced microbial effects on plant growth. It explains the greater biomass observed in plants grown in sterilized soil compared to non-sterilized soil when activated carbon is present (the **"joint effect"** pots in the model).

We suggest that when soil is sterilized and the soil flora are killed, plants may lose the benefit of soil mutualists, and gain from the death of soil pathogens and competitors. In addition, the breakdown of microbes may release toxins into the soil ("lysotoxins") but may also add fertility to the soil. The joint outcome of these positive and negative effects will be no change in plant growth between normal and sterilized soil, as we saw in the pots without carbon added.

However, when carbon is added the lysotoxins may be adsorbed, so that the joint effect effect of sterlization becomes somewhat positive, as we saw in our data from carbon-added pots.