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Can the negative plant–soil feedback of *Jacobaea vulgaris* be explained by autotoxicity?

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Abstract

Field and bioassay studies with *Jacobaea vulgaris* (ragwort) have shown that plants grow poorly in soil originating from the rhizosphere of this species and that this can influence the dynamics of ragwort populations during secondary succession. In the present study we examined whether the negative effect of ragwort on conspecifics may be due to autotoxicity. First, we experimentally established that ragwort exerts negative plant–soil feedback. We subsequently examined the inhibitory effects on germination and seedling performance of different strengths of aqueous extracts made from shoot and root tissues of ragwort, and from soil in which ragwort had been growing. The effects of the extracts were tested for seedlings growing in sterilised soil or in glass beads with water. Finally, the inhibitory effect of entire root fragments on seedling performance was tested. We observed that performance of seedlings growing in glass beads was significantly reduced by the high and medium strength root and shoot extracts. Extracts made from soil did not differ significantly from the control, and seedlings growing in sterilised soil were also not affected by ragwort extracts. Seed germination was significantly reduced by the high strength shoot extract only. The root length of seedlings growing in water with root fragments was reduced significantly. We conclude that under laboratory conditions ragwort can be autotoxic and discuss the role that autotoxicity may play in influencing the dynamics of ragwort populations during secondary succession.

Zusammenfassung

Freiland- und Biotest-Untersuchungen an *Jacobaea vulgaris* (Jakobs-Greiskraut) haben gezeigt, dass Pflanzen schlecht in Erde wachsen, die aus der Rhizosphäre dieser Art stammt und dass dies die Populationsdynamik des Greiskrauts während der Sekundärsukzession beeinflussen kann. Hier untersuchten wir, ob der negative Effekt des Greiskrauts auf Artgenossen auf Autotoxizität zurückzuführen sein könnte. Zuerst erbrachten wir den Nachweis, dass das Greiskraut ein negatives Pflanze-Boden-feedback bewirkt. Wir untersuchten dann die hemmende Wirkung von unterschiedlich starken wässrigen Extrakten aus Spross und Wurzeln des Greiskrauts und aus Erde, in der Greiskraut gewachsen war, auf die Keimung und die Wuchsleistung von Sämlingen. Die Effekte der Extrakte wurden an Sämlingen getestet, die in sterilisierter Erde oder in Glaskugeln mit Wasser wuchsen. Schließlich wurde der inhibitorische Effekt von ganzen Wurzelstücken getestet. Wir beobachteten, dass die Wuchsleistung der Sämlinge, die in Glaskugeln wuchsen, signifikant von starken und mittelstarken Extrakten aus Spross und Wurzeln

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reduziert wurde. Bodenextrakte unterschieden sich nicht signifikant von den Kontrollen, und Sämlinge, die in sterilisierter Erde wuchsen, wurden ebenfalls nicht von Greiskraut-Extrakten beeinflusst. Die Samenkeimung wurde nur durch den starken Sprossextrakt signifikant reduziert, und die Wurzellänge von Sämlingen, die in Wasser mit Wurzelfragmenten wuchsen, wurde signifikant reduziert. Wir schließen daraus, dass das Jakobs-Greiskraut unter Laborbedingungen autotoxisch sein kann, und wir diskutieren die Rolle, die die Autotoxizität für die Populationsdynamik des Greiskrauts während der Sekundärsukzession spielen kann.

Keywords: Allelochemicals; Allelopathy; Phytotoxicity; *Senecio jacobaea*; Soil sickness; Succession

Introduction

Ragwort (*Jacobaea vulgaris*, syn *Senecio jacobaea*) is an early successional species that is highly dominant in old-fields early after cessation of agricultural practices. However, after a few years of dominance, its abundance sharply declines (Bezemer, Harvey, Kowalchuk, Korpershoek, & van der Putten 2006; van de Voorde, van der Putten, & Bezemer 2012). This boom-bust pattern is characteristic for many wild plant species that occur in succession gradients (Halpern, Antos, Geyer, & Olson 1997; Meiners, Rye, & Klass 2009; Olff and Bakker 1991). Bioassay studies have shown that ragwort plants grow poorly in soil collected from areas where it is a dominant species in the vegetation (Bezemer et al., 2006; van de Voorde, van der Putten, & Bezemer 2011), suggesting that negative feedback from the soil contributes to the temporal pattern of this plant species during secondary succession. The negative soil effects have been attributed to soil fungal pathogens that build-up in the presence of this plant species (Bezemer et al. 2006). However, it is also possible that the negative effects on plant growth are due to autotoxic allelopathic effects (Bonanomi, Legg, & Mazzoloni 2005; Rice 1984; Wilson & Rice 1968).

Allelopathy is the effect of one plant on another through the production of chemical compounds that are released into the environment (Lambers, Chapin, & Pons 2008; Rice 1984). A special form of allelopathy is autotoxicity, which occurs when the released chemical compounds inhibit the growth of plants of the same species (Lambers et al. 2008; Miller 1996). Through autotoxic effects seed germination and seedling establishment can be reduced near full-grown plants, which minimises intraspecific competition for resources (Liu, Zeng, An, Mallik, & Luo 2008; Perry et al. 2005). Although allelopathy and autotoxicity are predominantly studied within an agricultural context (Liu et al. 2008; Miller 1996), they can also play an important role in natural systems, for example during succession (Bonanomi et al., 2005; Wilson & Rice 1968) or exotic plant invasions (Callaway & Aschehoug 2000; Hierro & Callaway 2003).

In nature, plant chemical compounds can enter the soil through foliar leaching, root exudation, decomposition of plant tissues, or volatilisation (Inderjit & Nilsen 2003; Lipinska & Harkot 2007; Rice 1984). However, these chemical compounds are not equally distributed amongst plant organs and the concentration and composition can differ considerably between aboveground and belowground parts (Hol, Vrieling, & van Veen 2003; van Dam et al. 2003). Typically, leaf extracts are a more consistent source of allelochemicals than root extracts (Lipinska & Harkot 2007). Plant responses to allelochemicals are generally stronger in roots than in aboveground tissues (Blum, Shafer, & Lehman 1999; Tawaha & Turk 2003). Moreover, the effects of chemicals often become stronger with increasing concentrations of the extracts (i.e. Pergo et al. 2008), while at very low concentrations phytotoxins might even have positive effects on plants (Calabrese & Blain 2008).

A well-studied group of chemical compounds that are known to exhibit allelopathic effects are alkaloids (Wink, Latz-Brüning, & Schmeller 1999). Ragwort and other species in the *Senecio* family produce a variety of pyrrolizidine alkaloids (PAs) that can act as toxins and deterrents against other plants (Fujieda, Shoyama, Matsunaka, & Nishioka 1988) and against plant antagonists, such as insects, nematodes and soil fungi (Hol & van Veen 2002; Kowalchuk, Hol, & van Veen 2006; Macel et al. 2005; Thoden, Boppré, & Hallmann 2009). Ahmed and Wardle (1994) tested the effects of extracts of ragwort tissues on co-occurring pasture plant species and showed that these extracts inhibited seed germination, seedling emergence and growth of these species. They also showed that extracts made from leaf material inhibited growth of other plant species more than root extracts.

We first carried out a plant–soil feedback experiment to confirm that ragwort develops a negative soil feedback. Subsequently, we tested the hypothesis that reduced ragwort performance in soil in which it has been grown previously is due to autotoxicity. In order to test this hypothesis, we examined the autotoxic potential of ragwort in a series of laboratory experiments. We tested the inhibitory effects of aqueous extracts made from root and shoot tissues on seed germination and seedling performance of ragwort. We also examined the effects of extracts taken from soil conditioned by ragwort, as well as the effect of root fragments on seedling performance.

Materials and methods

In November 2007, ragwort seeds were collected from approximately 100 plants in an old field (see below) where ragwort is abundant. Pappus was carefully removed and the seeds were surface-sterilised for 2 min in 0.4% chloride solution and rinsed. Seeds were germinated in demineralised water on glass beads of 1 mm diameter in a growth cabinet at 16 h 25 °C light and 8 h 20 °C dark, in order to mimic natural day/night conditions. One-week-old seedlings were used for all experiments.

Plant–soil feedback experiment

To examine the effect of ragwort on subsequent ragwort performance via changes in the soil, we carried out a plant–soil feedback experiment. The experiment consisted of two phases: a conditioning phase in which ragwort was growing in 'new' soil, and a feedback phase in which the effect of the conditioned soil on ragwort performance was measured.

In the conditioning phase, ragwort plants were grown in irradiated field soil or in irradiated field soil inoculated with live field soil. All field soil had been collected from a depth of 5–20 cm below the soil surface in an old-field that was taken out of agricultural production in 1995 and where ragwort was present (approximately 10% cover). The field is located in a nature reserve at the Veluwe, the Netherlands (52°04′N, 5°45′E). The soil type is sandy loam (van der Putten et al. 2000). Pots (0.9 L) were filled with irradiated field soil (>25 KGray gamma irradiation, Isotron, Ede, The Netherlands), which was inoculated in a 6:1 ratio with live field soil. A second set of pots was inoculated in a 6:1 ratio with autoclaved (3 consecutive days, 20 min at 120 °C) field soil. Each pot contained 1.2 kg of soil. Three one-week-old seedlings were planted per pot and both treatments were replicated five times. The pots were placed randomly in a greenhouse at 70% RH, at 21 °C during the day (16 h) and 16 °C during the night (8 h) at 17% soil moisture content (based on dry weight). Natural day light was supplemented by metal halide lamps (225 μmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). After 10 weeks aboveground biomass was harvested. The soil and roots of each pot were subdivided into four equal parts. From two parts of the pots the roots were rinsed. Shoots and roots were oven-dried for 5 days at 70 °C and weighed. The other two parts of soil and roots per pot were used as inoculum for the feedback phase of the experiment. Large roots were removed as they may re-sprout. These two parts per pot were homogenised in a 1:1 ratio with 640 g of irradiated soil, on a dry weight basis, ensuring that soil and roots from each pot were kept separate. Besides the two treatments from the first phase, we included a new sterile soil treatment, which was autoclaved field soil homogenised in a 1:1 ratio with sterilised soil, as was done for the other treatments in the feedback phase. Three one-week-old ragwort seedlings were planted in each pot. After one week the seedlings were randomly thinned to two seedlings per pot. Greenhouse conditions were as in the conditioning phase. After six weeks, aboveground biomass was harvested and the roots were rinsed. Shoots and roots were oven-dried for 5 days at 70 °C and dry weight was determined.

Preparation of plant and soil extracts for autotoxicity experiments

To obtain shoot, root, and soil material for the extract preparation, three seedlings were planted in a pot (1 L) filled with 950 g irradiated field soil on a dry weight basis. Greenhouse conditions were similar to conditions in the plant–soil feedback experiment. There were three replicate pots. After 12 weeks the pots were harvested. Biomass and soil from each pot was kept separate and for each pot a root, a shoot and a soil extract was made. On the day of the harvest, the soil moisture content of each pot was 17%. For each pot all leaf material was clipped and cut into pieces of approximately 1 cm to be used for extractions. The soil of each pot was sieved through a mesh of 2 mm to separate roots from soil. The roots were collected from the mesh, rinsed in demineralised water for 20 s, and cut into 1 cm pieces.

For each pot, twelve g of fresh leaf or root material (corresponding with 3 g dw) or soil (corresponding with 10.5 g dw) was soaked in 40 ml demineralised water, stirred for 20 s and left in the dark for 18 h. Twelve grams of shoot tissue corresponds with roughly the total foliar biomass from a pot; twelve grams of roots corresponds roughly with one fourth of the total root biomass per pot. The solutions were filtered (125 μm mesh size, Omnilabo, Breda, the Netherlands), filter-sterilised (0.2 μm, Whatman, Puradisc FP 30) directly after extraction, and kept at 4 °C until further use. Solutions were tested using three concentrations: pure (high strength), diluted 1:1 with demineralised water (medium strength), or diluted 1:19 with demineralised water (low strength).

Test 1: Autotoxic effects in glass beads

The experiment was carried out using 24-well microplates with 3.3-ml wells (16 mm diameter). Each well was filled with 3 g glass beads (1 mm diameter) that had been sterilised for 48 h at 110 °C. At the start of the experiment, each well received 0.7 ml high, medium or low strength shoot, root or soil extract. Control seedlings received 0.7 ml demineralised water. All treatments were replicated 8 times, so that there were 224 seedlings (3 replicate pots × 3 extract types × 3 concentrations × 8 seedlings + 8 control seedlings). All seedlings received 0.1 ml half-strength Hoagland solution (Hoagland & Arnon 1950) and five times 0.2 ml demineralised water during the experiment. The microplates were placed in plastic boxes (13 cm × 18 cm × 6 cm) with transparent lids to prevent evaporation and kept in a greenhouse at conditions described above. The experiment was harvested after 19 days. For each seedling maximum root length was measured. Seedlings were then dried for two days at 50 °C and weighed.

We also compared seedling growth in demineralised water with growth in acidified demineralised water, and in demineralised water with additional nutrients. The experiment was carried using the conditions as described above. The pH of the acidified control (pH = 5.4) equals the pH of the root extracts, which had the lowest pH. Seedlings in the nutrient addition treatment received 0.7 ml demineralised water and 0.1 ml half-strength Hoagland solution. The seedlings in the pH control treatment received 0.8 ml acidified demineralised water, and the seedlings in the control treatment received 0.8 ml demineralised water. All treatments were replicated 7 times. The experiment lasted for 19 days, during which the seedlings received five times an additional 0.2 ml demineralised water.

Test 2: Autotoxic effects in autoclaved soil

Seedlings were grown individually in 10 ml glass vials (22 mm diameter) filled with 12 g soil that had been autoclaved for 20 min at 110 °C during 2 consecutive days. The vials were kept in plastic boxes (13 cm × 18 cm × 6 cm) with transparent lids and placed in a greenhouse at conditions as described above. Each seedling received 1 ml low or high strength of the shoot or root extract. Control plants received 1 ml of demineralised water. There were 8 replicates for each treatment resulting in 104 vials (3 replicate pots × 2 extract types × 2 concentrations × 8 seedlings + 8 control seedlings). During the experiment the seedlings received three times 0.2 ml of demineralised water. After 21 days all seedlings were harvested as described above.

Test 3: Autotoxic effect of root fragments

The experiment was carried out using 24-well microplates (3.3 ml wells, 16 mm diameter). The wells were filled with 3 g sterilised glass beads. The glass beads were mixed with 0.25 g fresh root fragments, corresponding with 0.06 g dw root material per well. One seedling was planted into each well. Control seedlings were grown on glass beads and received no root fragments. All treatments were replicated 10 times, which resulted in 40 seedlings in total (3 replicate pots × 10 seedlings + 10 control seedlings). The microplates were placed in plastic boxes (13 cm × 18 cm × 6 cm) with transparent lids to prevent evaporation and kept in a greenhouse at conditions described above. During the experiment seedlings received four times 0.2 ml water. After 12 days seedlings were harvested as described above.

Test 4: Autotoxic effects on seed germination

Twenty surface sterilised ragwort seeds (2 min in 0.4% chloride solution and subsequently rinsed) were placed on a filter paper (diameter 8.5 cm, ref. 0/971510) in a Petri-dish. Each dish received 4.5 ml high or low strength extract made from shoots or roots. Control seeds received 4.5 ml of demineralised water. Each treatment contained five replicate Petri-dishes. In total the experiment comprised 65 Petri-dishes (3 replicate pots × 2 extract types × 2 concentrations × 5 dishes + 5 control dishes). Petri-dishes were placed in a germination cabinet at 20 °C in the light (16 h) and 15 °C in the dark (8 h). After 10 days each dish received an additional dosage of 0.25 ml of extract and 1.5 ml demineralised water, whereas the controls received 1.75 ml of demineralised water. Germination was checked daily for 19 days and total percentage germination per Petri-dish was calculated.

Data analyses

Data were analysed using analysis of variance (ANOVA; Genstat 12; Payne et al. 2008). Data were checked for homogeneity of variances using Levene's tests ($P > 0.05$) and for normality using Shapiro–Wilk's test ($P > 0.05$). Results from the first and second growth phase of the plant–soil feedback experiment were analysed separately, and individual comparisons per growth phase were based on a Tukey HSD post hoc test. For all autotoxicity experiments, replicates for treatments originating from the same extract (from the same pot) are strictly speaking pseudoreplicates and were therefore averaged prior to analyses, so that there were three replicates for each extract type and concentration.

Data from tests 1, 2 and 4 were analysed using an one-way ANOVA. Individual comparisons were based on a Tukey HSD post hoc test. Subsequently, the extract type and strength effects were compared using planned comparisons. In Test 3, the effects of addition of root were compared using a two sample *T*-test. To fulfill requirements of normality, biomass and root length data were log-transformed and percentage data were arcsin-transformed prior to statistical analyses.

Results

Plant–soil feedback experiment

Total biomass in the conditioning phase of the greenhouse experiment was significantly lower in pots inoculated with live field soil than in the sterilised control (38% reduction; $F_{1,8} = 33.1$, $P < 0.001$; Fig. 1). Ragwort biomass in the feedback phase differed significantly between the three treatments ($F_{2,12} = 12.4$, $P = 0.001$) and was significantly lower (66%) in both soils that originated from the conditioning phase than in the new sterile soil (Fig. 1). In the feedback phase, biomass did not differ between the two conditioned soils (Fig. 1).

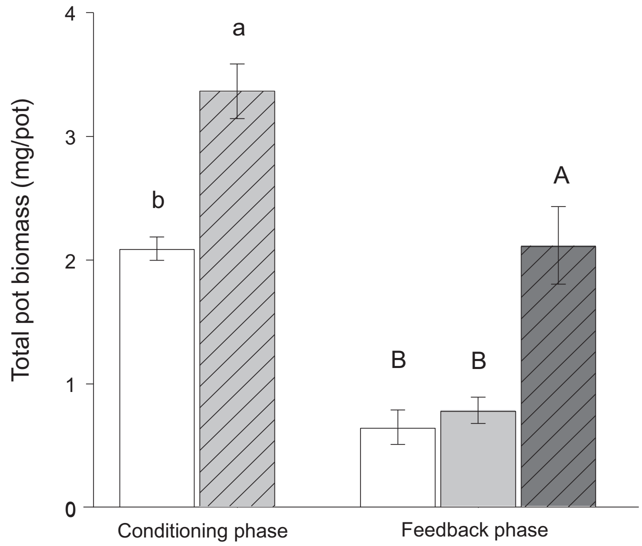

Fig. 1. Mean (± SE) total pot biomass (dry weight) of plants growing in irradiated field soil inoculated with live field soil (white bars) or with autoclaved field soil (grey bars). The soil used in the feedback phase was either soil inoculated with live (white) or with autoclaved (light grey) field soil during the conditioning phase, or in a new sterile soil (dark grey hatched bar). The hatched bars indicate the control treatment per phase. The conditioning phase lasted for 10 weeks and the feedback phase for 6 weeks. Different letters indicate significant differences ($P < 0.05$) based on a Tukey's HSD post hoc test for each growth phase.

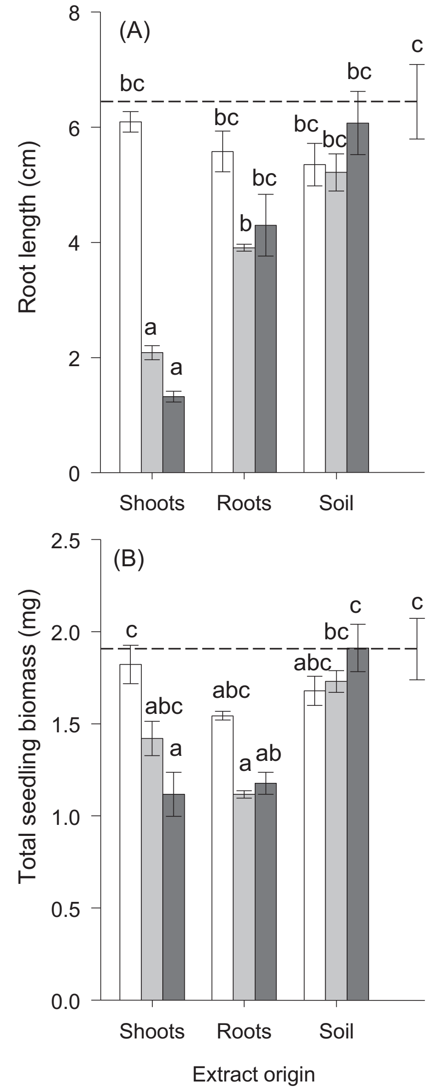

Fig. 2. Mean (± SE) maximum root length (A) and total biomass (dry weight) (B) of seedlings growing in glass beads inoculated with low (white bars), medium (gray bars) or high (black bars) concentrations of shoot, root or soil extracts. The hatched line indicates the control treatment with demineralised water. Different letters indicate significant differences ($P < 0.05$) based on a Tukey's HSD post hoc test.

Test 1: Autotoxic effects in glass beads

Maximum root length (RL) and total biomass (BM) differed significantly between treatments (RL: $F_{9,24} = 26.48$, $P < 0.001$; BM: $F_{9,24} = 7.03$, $P < 0.001$; Fig. 2). As compared to the control, root length was reduced in the treatments with the high and medium strengths of the shoot extract and by the medium strength root extract. Biomass was reduced by the high strength shoot extract and the medium and high strength root extracts. Seedlings that received soil extracts did not differ significantly from the control. When root and shoot extracts were compared, root length was more reduced by shoot extracts than by root extracts ($F_{1,30} = 9.21$, $P = 0.005$) while there was no difference for biomass ($F_{1,30} = 1.54$, $P = 0.22$). The medium and high strength extracts had a more negative effect on root length than the low strength extract ($F_{1,30} = 8.26$, $P = 0.007$). Only the high strength extract reduced total biomass, as compared to the low strength extract ($F_{1,30} = 4.45$, $P = 0.04$). The methodological check showed that both seedling biomass and root length did not significantly differ between the control treatment with water only and the treatments with the lowered pH or additional nutrients (Table 1).

Table 1. Mean (±SE) root length and biomass (dry weight) of seedlings growing in wells that received demineralised water, Hoagland nutrient solution (Nutrients), or an acidified water solution (pH). *F* and *P* values of a one-way ANOVA are also presented.

	Root length (cm)	Total biomass (mg)
Water	6.8 ± 0.8	1.9 ± 0.1
Nutrients	6.7 ± 0.7	1.6 ± 0.1
pH	5.6 ± 0.5	1.6 ± 0.1
$F_{2,18}$	1.18	0.60
P	0.33	0.56

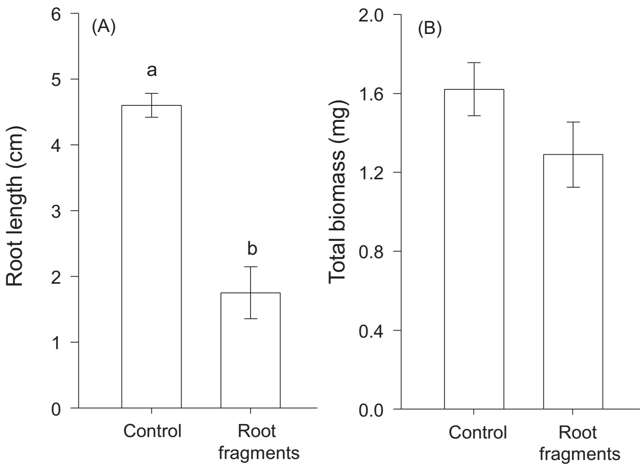

Fig. 3. Mean (±SE) maximum root length (A) and total biomass (dry weight) (B) of seedlings growing in glass beads (control) or in glass beads with root fragments. Different letters indicate significant differences ($P < 0.05$) based on a two sample T-test.

Test 2: Autotoxic effects in autoclaved soil

In autoclaved soil, there were no significant autotoxic effects of root or shoot extracts on root length or biomass (RL: $F_{4,14} = 0.72$, $P = 0.59$; BM: $F_{4,14} = 0.68$, $P = 0.61$).

Test 3: Autotoxic effect of root fragments

Addition of root fragments to the glass beads did significantly reduce maximum root length ($T = 7.21$, $P < 0.001$; Fig. 3A), but not total seedling biomass ($T = 0.56$, $P = 0.59$; Fig. 3B).

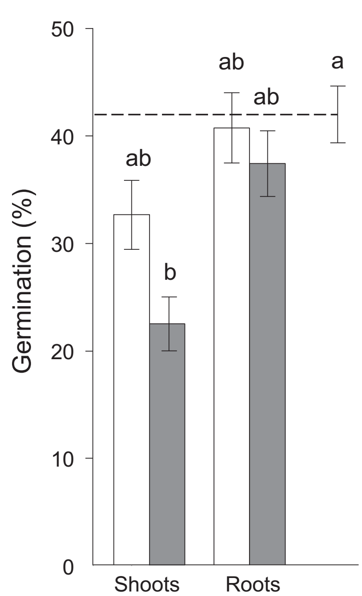

Fig. 4. Mean (±SE) percentage germination of seeds that received demineralised water (control, dashed line), or high (white bars) or low (black bars) strength leaf or root extracts. Different letters indicate significant differences ($P < 0.05$) based on a Tukey's HSD post hoc test.

Test 4: Autotoxic effects on germination

Seed germination differed between the five treatments ($F_{4,12} = 3.23$, $P = 0.04$) and between root and shoot extracts ($F_{1,8} = 6.4$, $P = 0.03$). Germination was only significantly lower (53%) than the control following addition of high strength shoot extracts (Fig. 4).

Discussion

In a series of laboratory experiments we tested the autotoxic potential of ragwort as a possible explanation for the negative plant–soil feedback that this species exhibits. Our results show that ragwort exhibits autotoxic properties, but that the substrate in which the plants grow, the type of plant extract used, and the extract concentration are important determinants of the autotoxic effect. In our study, extracts made from ragwort tissues reduced germination and seedling growth. Seedling performance was not affected when seedlings received the acidified control solution or only water, which strongly reduces the possibility that side effects, such as acidity or nutrient deficiency, may have caused the observed growth reduction.

Our results allow us to make three conclusions about autotoxicity of ragwort. First, extracts made from shoots inhibit germination and root growth more than root extracts. Ahmed and Wardle (1994), who studied the allelopathic effects of ragwort on other species, also found that extracts from shoots had the strongest allelopathic effects on other pasture species, and this appears a general observation in studies on allelochemical effects (Rice 1984; Lipinska & Harkot 2007). Second, the autotoxic effects of ragwort are dosage-dependent, being strongest for the most concentrated extracts. This is in line with studies on allelopathic effects of other plant species (Chon & Kim 2002; Tawaha & Turk 2003; Dorning & Cipollini 2006). Third, extracts do not exhibit autotoxic effects when applied to soil. This is interesting, as it has been suggested that soil biota may reduce the ecological consequences of released plant chemicals (Inderjit & van der Putten 2010), whereas our results show that also sterilised soil may have such an effect.

It is possible that the absence of growth reduction that we observed in soil was because the chemical compounds that cause the autotoxic effect were absorbed to the soil particles, which reduces their mobility and buffers their negative effect (Krogmeier & Bremner 1989; Wardle, Nilsson, Gallet, & Zackrisson 1998). This possibility is supported by our observation that addition of extracts made from soil did not reduce seedling performance. Another possible explanation is that nutrient availability was higher in soil than in glass beads, however, this appears unlikely, because we corrected for nutrient differences by adding Hoagland solution. Other studies have shown that increasing nutrient availability can reduce the negative effects of allelopathy (Rice 1984; Inderjit & Weiner 2001). It could also be that the autotoxic compounds in ragwort are hydrophobic and that they were therefore not present in the aqueous extracts that we applied. In contrast to our study, Ahmed and Wardle (1994) found relatively strong effects of extracts made from soil in which ragwort had been growing on the performance of other plant species. However, these authors provided the soil extracts more regularly and for a longer period of time than we did, which could explain why they found growth inhibition. Also, they made extracts from field plants, which were older and therefore often more autotoxic than the plants we used for extract preparation. Alternatively, ragwort could be less sensitive to soil extracts than the other pasture species that were studied by Ahmed and Wardle (1994).

While our study shows that extracts from ragwort tissues potentially exhibit autotoxic effects, it is unlikely that these autotoxic effects are the only cause of strong growth reduction in soils where the plant previously has been grown. In our study, we used extracts from damaged plant tissues, which typically have stronger allelopathic effects than extracts made from intact tissues (Orr, Rudgers, & Clay 2005). In addition, we included concentrations that were higher than what would occur under natural conditions. Still we did not find autotoxic effects when these extracts were applied to seedlings growing in soil. Therefore, our results suggest that under field conditions, with probably much lower concentrations of allelochemicals, tissue extracts are unlikely to have a strong effect on seedling performance. However, shoot extracts did reduce ragwort germination and this could consequently affect ragwort establishment and population dynamics on a longer time scale. Whether shoot leachate concentrations in the field are strong enough to cause negative effects on germination should be tested under field conditions.

In contrast to the addition of extracts, when we incorporated entire root pieces into the soil, we found strong growth reduction effects on ragwort. Roots of those seedlings were also much shorter and thicker than the roots from control seedlings (TFJ van de Voorde, personal observation). Altered root morphology is a commonly observed allelopathic effect (Chon & Kim 2002; Gatti, Ferreira, Arduin, & Perez 2010). Eventually, these changes in morphology can limit nutrient uptake and reduce seedling performance and fitness (van der Putten, Breteler, & van Dijk 1989). Therefore, in the field, such root deformations may have large consequences, especially when plants that co-occur with ragwort are not affected by presence of these root fragments. It is important to note that while it is possible that the growth effects that we observed after addition of root pieces are due to allelochemical effects, the effects may also have been caused by micro-organisms present on or in the root fragments, which could also explain the morphological changes (van der Putten et al. 1989). Alternatively, soil-microorganisms might have released or activated certain chemicals from the decomposing root fragments. These chemicals may have different phytotoxic effects than the allelochemicals present in the sterile plant extracts. Further, as root fragments decompose slowly, it is possible that the allelochemicals were released over a longer time period. Incorporating plant material into soil can also have indirect inhibitory effects by influencing for example nutrient mobilisation, pH or microbial activity (Facelli & Pickett 1991). Future studies should focus on disentangling the mechanisms through which root fragments cause this strong reduction in growth of ragwort.

In conclusion, our experiments show that ragwort can be autotoxic under laboratory conditions. However, the effects were dependent on the extract type and concentration applied, and autotoxicity was not observed when seedlings were growing in sterilised soil. In contrast, incorporation of root fragments reduced ragwort performance considerably, although the role of soil (micro-) organisms should be investigated in more detail. These results suggest that autotoxicity does not play an important role in the decline of ragwort abundance in old-fields and growth reduction in greenhouse experiments. Future studies should address the role of autotoxicity for the dynamics of ragwort in the field and to what extent allelopathic effects of other co-occurring species affect the performance of this plant in the field.

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