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SHORT COMMUNICATION

Allelopathic effects of rye, brown mustard and hairy vetch on redroot pigweed, common lambsquarter and knotweed

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ABSTRACT

In bioassays, the water extracts of rye, brown mustard and hairy vetch biomass were toxic to target species, suggesting the release of phytotoxins from plant biomass. The toxic effects on germination were extracts concentration dependent. Root growth was more affected than shoot growth. We found that allelopathic inhibition of weeds occurred through different toxic mechanisms: (i) reduction in seeds germination, (ii) lengthening the germination process and (iii) slow growth of seedlings. All these factors may reduce the plants population in the field and thus their competitive ability. In terms of inhibition of weeds germination and particularly root growth of seedlings, the test crop followed the order: brown mustard > rye > hairy vetch. Their weed suppressing ability may be used in future weed management strategies. Weed control may be further improved with proper cultural practices, i.e. delaying the time of residue incorporation into soil in spring if soil biota rapidly inactivates toxic allelochemicals and increasing cover crop biomass through seeding density or fertilization.

Key words: *Amaranthus retroflexus,* aqueous extracts, brown mustard, *Chenopodium album,* hairy vetch, plant residues, *Polygonum aviculare,* rye.

INTRODUCTION

One of the alternative strategies of weed management in agroecosystems is controlling weeds through allelopathy, to reduce dependency on herbicides (1,10,29,31). Cover crops are grown to increase soil organic matter, reduce soil erosion, improve soil structure and for weed management (19,30). Use of allelopathic cover crops is a promising application of allelopathy for selective weed control, by direct phytotoxic effects through production and release of allelochemicals in soil (22,24). The cover crop biomass remains on the soil surface or is incorporated into the soil before the following crop is seeded. Allelochemicals present in crop biomass slowly releases into the soil and reduces the germination and the growth of weeds, providing early-season weed control (15,18).

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The allelopathic effects of allelochemicals are selective, species specific and concentration dependent (25). Rye, hairy vetch and brown mustard are common cover crops used for weed-control. Rye reduced early season biomass of *Chenopodium album*, *Digitaria sanguinalis* and *Ambrosia artemisifolia* by 98, 42 and 90 %, respectively (3) and reduced the emergence of *Amaranthus retroflexus* by 95 % and of *Polygonum* spp. by 100 % (23). Rye and hairy vetch reduced total weed densities by over 80 % (21), while vetch reduced total weed density by 55-88 % and *Chenopodium album* emergence by 84-100 % (13). Brown and white mustard decreased total weed biomass by 49 % and reduced height of *Amaranthus* by 33 % (15). It is not clear, however, whether the effect of weed control in the field is from the allelochemicals released from roots of living plants or from the decomposition of their residue in soil. The compounds released from the added plant residues can interact with soil biota, which may decrease, or even increase, the phytotoxicity (9,14). Indeed, timing of phytotoxicity is variable, with some studies reporting greatest toxicity immediately after incorporation or desiccation of residue (2,8) and others reported the increasing toxicity with increasing time after incorporation (13,16).

The objective of this study was (i) to investigate the allelopathic potential of the biomass of the winter cover crops: rye, brown mustard and hairy vetch on germination and early growth of *Amaranthus retroflexus*, *Chenopodium album* and *Polygonum aviculare* and (ii) to eliminate confounding soil sorption effects. The bioassays, were done in petriplates in controlled environment.

MATERIALS AND METHODS

In bioassys, phytotoxic activity of the aqueous extracts from the biomass of three donor species [rye (*Secale cereale* L.), brown mustard (*Brassica juncea* L.) and hairy vetch (*Vicia villosa* Roth.)] on the seed germination and seedling growth of three target weed species [redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarter (*Chenopodium album* L.) and knotweed (*Polygonum aviculare* L.)] was determined. There were 3 treatments [1:10 (w/w) and 1:5 (w/w), control (deionized water)] and were replicated thrice in a randomized complete block design.

Plants of rye, cv. 'Fausto', brown mustard, cv. 'ISCI20', and hairy vetch, cv. 'Villana', were grown in open fields at the Experimental Research Station, Department of Agronomy and Agroecosystem Management, University of Pisa, at S. Piero a Grado, near Pisa, Italy (43° 67'N, 10° 30'E, 5 m above sea level)]. The crops were sown on November 6, 2003 and were harvested at anthesis on April 30, 2004. Standard cultural practices for each cover crop were followed. The biomass dry weight of rye, brown mustard and hairy vetch was 94, 77 and 63 q ha^{-1}, respectively. The fresh biomass of harvested plants were chopped into small pieces (about 5 cm) and then tissues were ground in laboratory grinder with a 40-mesh screen. Aqueous extracts at 1:10 dilution were obtained by mixing 100 g fresh biomass with 1 L deionized water for 24 h at laboratory temperature (about 22° C) (2). Thereafter the solutions were filtered through one layer of filter paper (Whatman No. 1), as per Chon *et al.* (5). Dry matter contents of the extracts was determined by oven drying and was 3.2 g L^{-1} for rye, 3.8 g L^{-1} for brown mustard and 2.5 g L^{-1} for hairy vetch. Aqueous extracts at 1:5 dilution were obtained by oven drying at 35° C. All solutions were stored at below 5° C until bioassays were performed. The osmotic potential of each extract

was determined using a cryoscopic osmometer (OSMOMAT 030, Gonotec). The osmotic potential of extracts at the highest concentration was 0.3 kPa for brown mustard and rye and 0.5 kPa for hairy vetch.

Seeds of *Amaranthus retroflexus*, *Chenopodium album* and *Polygonum aviculare* were collected from maize crop fields on previous summer. The seeds were surface-sterilized in 1:10 (v/v) hypochlorite bleach for 10 min and rinsed several times with distilled water. One hundred seeds were placed on one layer of filter paper (Whatman n. 1) in 17.5 cm Petri dishes, previously sterilized by autoclave. Five mL of extracts were added to each Petri dish. The Petri dishes were placed in a growth chamber at 20° C temperature, 70±5% relative humidity and 450 μE m^{-2} s^{-1} radiation (16 h light/8 h dark) for 14 days. Petri dishes were checked regularly and deionized water was added as needed. For 14 days germinated seeds were counted at 24-hour intervals. A seed was regarded as germinated when the radicle had protruded at least 1 mm. Seedling and root length of germinated seeds was measured on all seedlings in each Petri dish at 14 days after sowing. Mean Germination Time (MGT) was calculated as:

$$MGT = \sum(N \times d) / \sum d$$

Where, N is the number of seeds germinated on day d (number of days from the start of germination test). Germination data was tested for homogeneity of variance using Bartlett test (27). Data were not normally distributed and were $\log_{10}$ transformed; retransformed data are presented in the results. All data were statistically analized by ANOVA, performed separately for each donor species in order to test the main effects of dilution rate of extracts, weed species and their interaction. Duncan's multiple range test was used to separate the means when the ANOVA F-test indicated a significant effect of the treatment (28).

RESULTS AND DISCUSSION

The aqueous extracts of cover crops significantly affected the germination percentage and rate, as well as the seedlings development of target weeds. However, the magnitude of the effect was species- and extract dilution- dependent.

Rye extracts reduced the germination of *Amaranthus* seeds by 23% at both concentrations, of *Polygonum* by 14% at 1:10 concentration and by 49% at 1:5 concentration and had little influence on *Chenopodium* (Figure 1). Accordingly, MGT of *Chenopodium* was not modified by aqueous extract of rye shoots, compared to the control, while that of *Amaranthus* and *Polygonum* was significantly increased by 1.1 and 1.4 days at 1:10 concentration and by 2.3 and 2.6 days at 1:5 concentration. The inhibitory effects of rye extracts were also evident on the root and shoot growth of seedlings. Rootlet length of *Amaranthus* and *Polygonum* was greatly reduced at both concentrations than *Chenopodium*, although significantly affected, was less reduced. Rye extracts significantly reduced the shoot length of *Amaranthus* at both concentrations (25% at 1:10 and 83% at 1:5) and of *Chenopodium* and *Polygonum* only at the higher concentration (35% compared to control). The magnitude of reduction in shoot growth was always lower than on root growth.

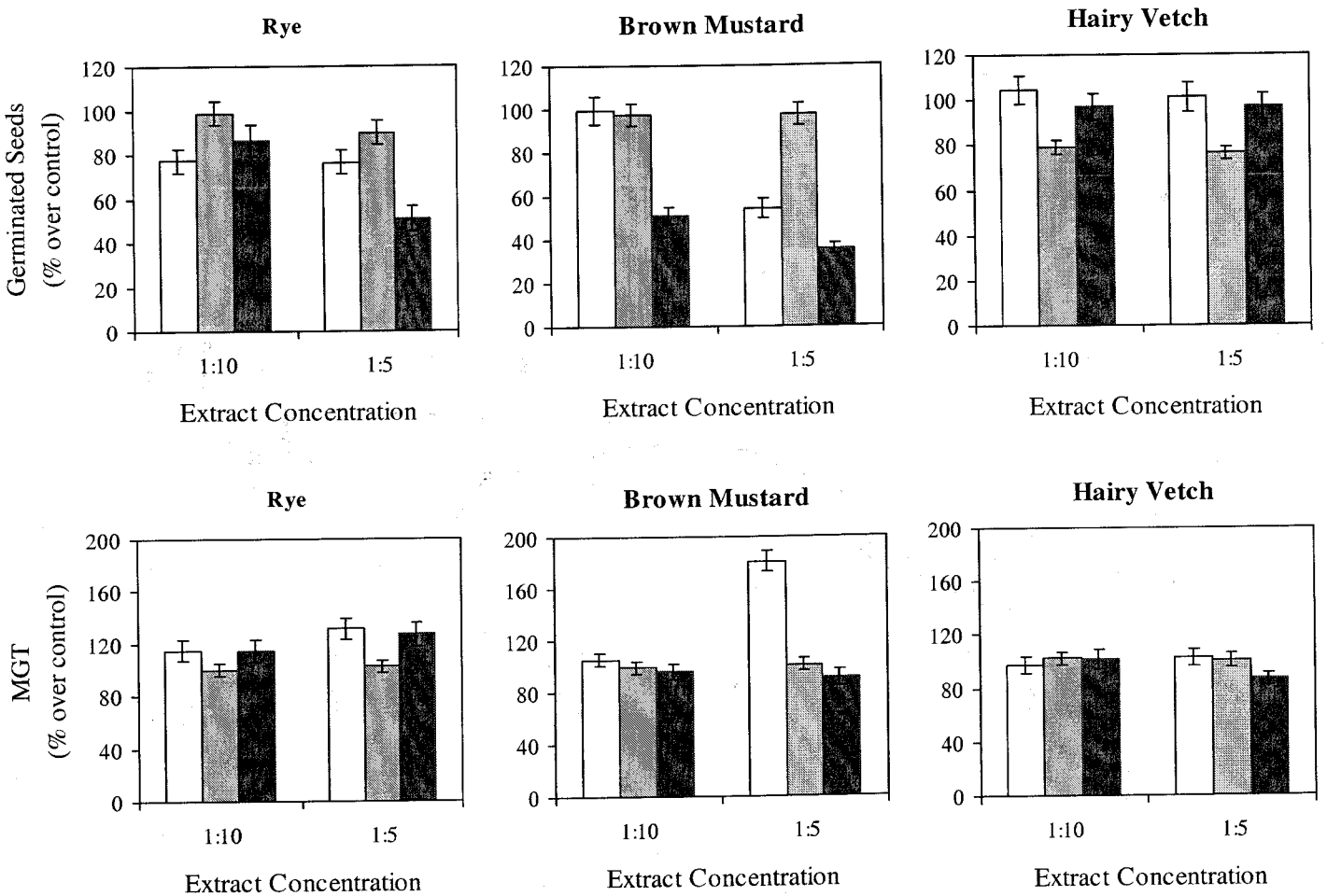

Figure 1. Effect of rye, brown mustard and hairy vetch on germination and mean germination time (MGT) of *Amaranthus retroflexus L.*, *Chenopodium album* L. and *Polygonum aviculare* L. In this and in the following figure, vertical bars indicate standard error.

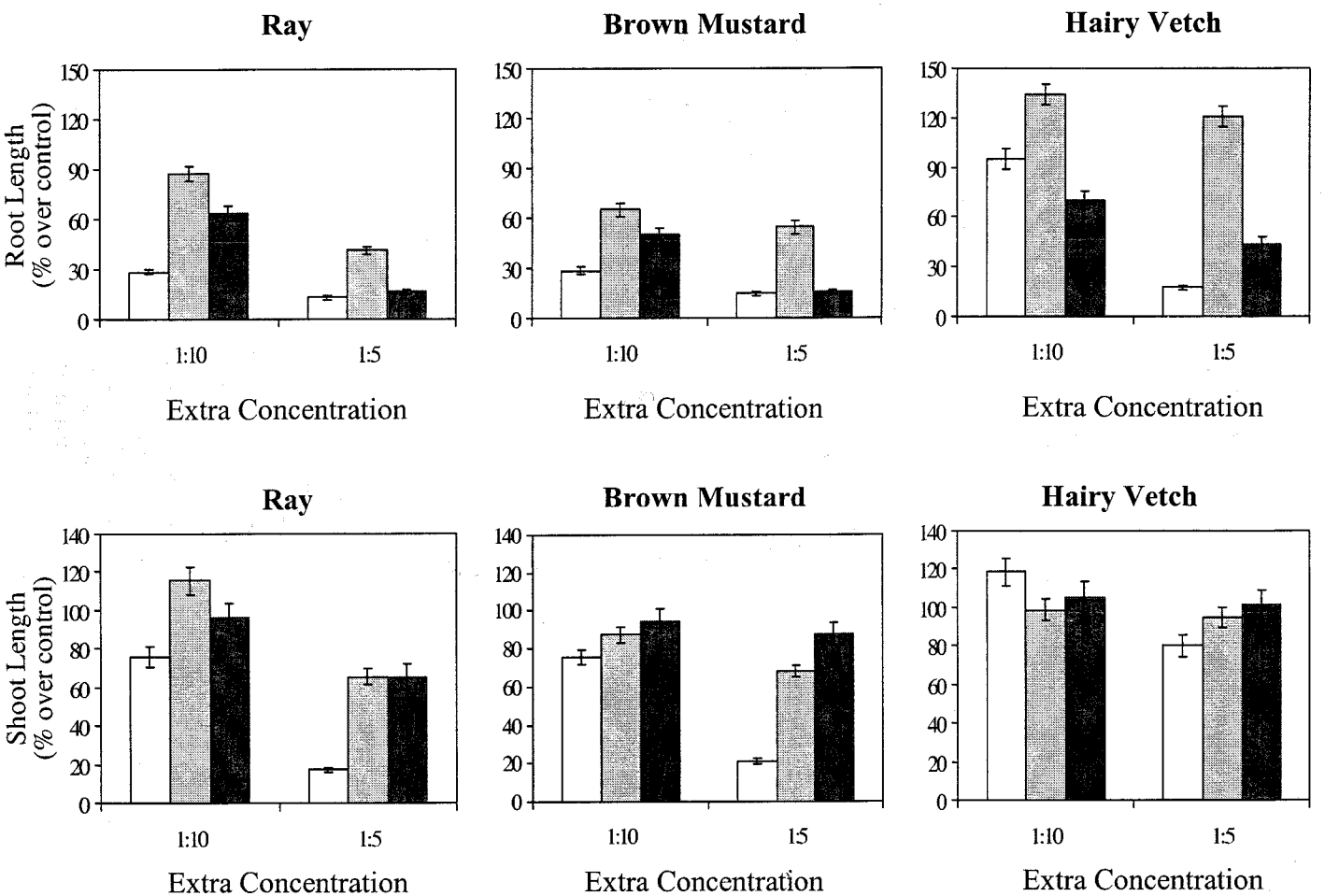

Figure 2. Effects of rye, brown mustard and hairy vetch on root and shoot length of *Amaranthus retroflexus* L., *Chenopodium album* L. and *Polygonum aviculare* L.

The extracts of brown mustard did not affect the germination of *Chenopodium,* but significantly reduced the germination of *Amaranthus* at highest concentration (46%) and of *Polygonum* at both concentrations (49% and 64% at 1:10 and 1:5 concentration) (Figure 2). Brown mustard extracts did not modify MGT of the three weeds, except at 1:5 dilution ratio that significantly increased the MGT of *Amaranthus* by 6 days over control. The extracts greatly reduced the root length both in *Amaranthus* and in *Polygonum* at both concentrations, while that of *Chenopodium* was less reduced. Brown mustard extracts also reduced the shoot growth of all weed species and the reduction was drastic at higher concentrations. Among the weeds, a higher inhibitory effect was observed at both concentrations on *Amaranthus*, followed by *Chenopodium* and *Polygonum*.

Hairy vetch extracts did not inhibit the germination of *Amaranthus* and *Polygonum* seeds at both dilutions, but caused 20% inhibition in *Chenopodium* and did not influence the MGT of all test weeds (Figure 3). Hairy vetch extracts reduced the root length of *Polygonum* seedlings at both concentrations (30% at 1:10 and 57% at 1:5, compared to the control) and that of *Amaranthus* only at the higher concentration (83%), but did not affect the *Chenopodium* seedlings. Finally, hairy vetch extracts did not influence the shoot length of test weeds, except at 1:5 concentration on *Amaranthus* seedlings (20% reduction over control).

Results from field trials showed that growth of *Chenopodium* was completely inhibited by residues of rye, hairy vetch and brown mustard, while *Amaranthus* development was reduced only by hairy vetch (11). The disagreement between our field and laboratory results supports the hypothesis of interactions between allelochemicals and soil biota. Under field conditions, the phytotoxicity of hairy vetch biomass possibly originated from the decomposing activity of soil microorganisms and consequently the inhibitory effect of this cover crop is evident in the field but not in the bioassay. On the contrary, the ineffectiveness of rye and brown mustard against *Amaranthus* in the field possibly depend either on a quick degradation of the toxic compounds or on a low concentration of these compounds in the incorporated biomass. Similar results were obtained by Lehman and Blum (17) and Smeda and Weller (26) who found that the effect of rye residue on weed emergence decreased over time and was practically insignificant within 6-weeks after soil incorporation. The loss of debris toxicity with field ageing was associated with the loss of soluble components, some of which are phenolic acids, whose allelopathic effects are known (3).

In bioassays, germination is less sensitive than seedling growth, especially root growth, thus radicle length is considered the best parameter to determine the allelopathic potential of a species (4,6). The exact concentration and consequently its osmotic potential may also modify the response to allelochemicals: moderate concentrations delay the germination, whereas, high concentrations also reduce final germination (20). In alfalfa, the osmotic potential of its leaf extracts affected the seed imbibition in the first germination phases, but in the following phases of seed germination, the major influence was due to toxic effect; hence correcting the osmotic effects is not necessary (7). Moreover, in our research, the osmotic potential of the extracts was low and may be considered a non-influential factor in the bioassay (12). In our research, root length was more affected than shoot length and a dose related response was observed only in few cases, however, although we cannot completely exclude an osmotic effect of donor crop extracts, we believe that the toxic effects exerted major influence.

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