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Effect of Alfalfa Plant Extracts on Germination and Early Seedling Growth of Forages

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ABSTRACT: Alfalfa (*Medicago sativa* L.) plants have been reported to contain water-soluble substances that are autotoxic as well as allelopathic. Laboratory experiment through a petri-dish assay with imbibed seeds was conducted to evaluate both autotoxic and allelopathic effects of alfalfa leaf extracts on the germination and early seedling growth of alfalfa, red clover, crested wheatgrass, and Russian wildrye. Alfalfa seed germination was delayed dependent on extract concentration, with no difference in final germination at 72 hours. Root growth of alfalfa was stimulated up to 14% above control at very low concentrations of both leaf and stem extracts of alfalfa and was significantly reduced at extract concentration of more than 0.5 g dry tissue/L (g L^{-1}). Leaf extracts were generally more autotoxic for root growth than were stem extracts. Hypocotyl growth was not affected by all the concentrations of both leaf and stem extracts. Root length of legumes was more sensitive to the autotoxic chemicals from leaf extracts than was germination or shoot length. Hypocotyl growth of two legume plants and plant height of two grasses were not influenced by extracts. Seed germination and root growth of legumes were more inhibited by aqueous extracts of alfalfa leaf than were those of grasses. This result indicates autotoxic effect of alfalfa leaf extracts seems to be greater than allelopathic effect.

Keywords : alfalfa, leaf extracts, petri-dish assay, autotoxicity, allelopathy

Allelopathy is a chemical interaction between plants or between microbes and higher plants that includes stimulatory as well as inhibitory influences (Molisch, 1937). It was later defined as any direct or indirect harmful or beneficial effect of one plant as a donor plant on another as a recipient plant through the production of chemical compounds that escape into the environment (Rice, 1984). It plays a significant role under both natural and agro-ecosystems (Rice, 1984). Autotoxicity is a intraspecific form of allelopathy in which the donor and receptor plants are the same species and have a detrimental effect (Putnam, 1985).

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Alfalfa (*Medicago sativa* L.) has been known to contain water-soluble substance(s) that are autotoxic to the same species (Chung and Miller, 1995a and 1995b; Hall and Henderlong, 1989; Hedge and Miller, 1992; Jensen *et al.*, 1981; Klein and Miller, 1980; Li, 1981; Miller, 1983) as well as allelopathic to bladygrass (*Imperata cylindrica*) (Abdul-Rahman and Habib, 1989), weed species (Chung and Miller, 1995c), cucumber (*Cucumis sativa*) (Ells and McSay, 1991), sorghum (*Sorghum bicolor*) (Hedge and Miller, 1990), corn (*Zea mays*), and soybean (*Glycin max*) (Miller, 1983).

Autotoxicity of alfalfa during and after alfalfa establishment was first described by Jensen *et al.* (1981). They concluded autotoxicity exists where alfalfa has lower germination, poorer establishment, and lower productivity when grown after alfalfa compared with those after another species or after fallow. Jensen (1984) reported better germination achieved when alfalfa was germinated in 7 days rather than 2 or 4 days after seed treatment with an alfalfa-foliage extract in the laboratory. The common field recommendation is to delay seeding of alfalfa after alfalfa for at least 2 weeks and in some cases up to 2 years (Jennings *et al.*, 1996).

Although many of allelochemicals are secondary products of plant metabolism, several are degradation products that occur in the presence of microbial enzymes. Probable major biosynthetic pathways leading to production of autotoxic chemicals could be shikimic acid or acetate pathway. Old alfalfa plants as donor plants release cinnamic acid and derivatives that are derived from aromatic amino acids through shikimic acid or acetate pathway (Rice, 1984). Autotoxic chemicals reported for alfalfa are mainly cinnamic acid and its derivatives such as ferulic acid, vanillic, hydroxybenzoic, *p*-coumaric, *trans*-cinnamic acid, caffeic acid (Hall and Henderlong, 1989; Miller, 1996; Newby et al, 1980; Read and Jensen, 1989), saponin (Guenzi *et al.*, 1964; Miller, 1996), and medicarpin (Dornbos *et al.*, 1990). However, the causative chemicals have not been clearly identified.

Recently, some researchers have attempted to develop new ideas for reducing autotoxicity as well as for exploiting

it as an additional weed management strategy. One approach has been to utilize rotational crops or companion plants in annual or perennial cropping system, especially, utilizing tolerant or resistant rotational cultivars to the auto-allelochemicals (Klein and Miller, 1980; Miller, 1983). Miller (1983) reported that the best preceding crop for establishing alfalfa is corn, followed by various small grains, soybeans and the worst preceding crop is alfalfa. Another approach has been to screen for tolerant or resistant types in germplasm collections of crops, the idea being to transfer this character into cultivars by either conventional breeding or genetic transfer technique (Chung and Miller, 1995b; Miller, 1992).

Red clover (*Trifolium pratense* L.) is the most widely grown of all the true clovers. Grown alone and with grasses, it constitutes the most important legume hay crop in the northeastern U.S. It is used for hay, pasture, and soil improvement, and it fits well into three- and four-year rotation. Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) is perennial grass commonly seeded in the western United States. Hycrestwas developed by ARS in Logan, Uta by crossing cristatum and desertorum type of crested wheatgrass (Asay *et al.*, 1985a). Crested wheatgrass is commonly recommended for forage production. Bozoisky Russian wildrye (*Psathyrostachys juncea*, (Fisch.) Nevski) (Asay *et al.*, 1985b), long-lived, cool-season, drought-resistant, perennial bunchgrass, is well adapted to the northern plains and intermountain states where it grows best on loam and clay and tolerate saline soil. Lawrence and Kilcher (1961) reported that water-soluble substances from alfalfa roots are toxic to several species of wildryegrass (*Elymus* spp.) and wheatgrass (*Agropyron* spp.).

The objective of this research was to evaluate autotoxic or allelopathic effect of alfalfa leaf extracts on the germination and early seedling growth of alfalfa, red clover, crested wheatgrass, and Russian wildrye. This research will be useful for better understanding the mechanisms of alfalfa allelopathy in forage cropping system.

MATERIALS AND METHODS

Sampling and preparation of extracts

Fresh topgrowth of 3-year-old alfalfa 'Cody' plants grown at a field of West Plains, Missouri was harvested at a vegetative stage in November 1995. The plant samples were directly oven-dried at 40° C for 5 days. The dried samples were separated into leaves and stems. The samples were ground with a Wiley mill to pass a 1-mm screen and then stored in a refrigerator at 2° C until used. Twenty grams of dried leaves and stems were separately extracted by soaking in 1 L deionized water at 22° C for 24 hours in a lighted room to give a concentration of 20 g dry tissue L^{-1}. The extract was filtered through four layers of cheesecloth to remove the fiber debris, and centrifuged at 3,000 rpm for 4 hours. The supernatant was vacuum filtered through Whatman No. 42 paper. Stock extracts were made fresh for each experiment. EC and pH of leaf extracts were measured at 0, 0.5, 1, 2, 4 and 8 g L^{-1} and ranged from 0.01 to 1.75 mmho, and from 5.17 to 6.19, respectively. Osmotic potentials of the extract in concentrations of 0 (deionized water, control), 1, 2, and 4 g L^{-1} measured by the method of Boyer and Knipling (1965) were 0.0062 (control), 0.013, 0.024, and 0.048 MPa, respectively. EC and osmotic potentials of leaf extract were increased as the extract concentration increased while pH was not influenced by concentration of extracts (Table 1).

Table 1. Electric conductivity (EC), pH and osmotic potentials of 'Cody' alfalfa leaf extracts as affected by different concentrations.

Extract concentration	EC	pH	Osmotic potential
g L^{-1}	----mmho---	-	--- MPa---
0	0.01	5.17	-0.006
0.5	0.16	6.12	-
1	0.30	6.19	-0.013
2	0.54	6.08	-0.024
4	0.96	5.91	-0.048
8	1.75	5.90	-

Bioassay and data analysis

Difco Bacto agar at a concentration of 16 g L^{-1} was autoclaved at 125° C for 30 min and then equilibrated in a water bath at 50° C along with a flask of stock extract and another of sterile distilled water. The stock extracts were diluted appropriately with sterile warm water to twice each of the desired test concentration, then mixed with the agar solution in a 1 : 1 ratio to give the final concentrations. About 10 mL of extract-agar or water-agar (control) were poured into a 9-cm-diameter plastic petri dish, and allowed to solidify at room temperature. For all experiments seeds were surface sterilized with 0.525 g L^{-1} sodium hypochlorite for 15 min. Seeds were rinsed four times, imbibed in deionized water at 22°C for 12 hours, and carefully blotted using a folded paper towel. Seeds were evenly placed on agar containing extract in each petri dish. The petri dishes were covered, sealed by wrapping in Parafilm, and placed flat in a growth chamber held at 24° C during the14-hour light period and 22° C during the 10-hour dark period. Plates were illuminated at 400 μmol photons m^{-2} s^{-1} PAR provided by a mixture of incan-

descent and fluorescent lamps.

Data were transformed to percent of control for evaluation as used. When the F-test was significant ($P<0.05$), means were separated on the basis of least significant difference (LSD). The experiments were duplicated, each with four replications.

Concentration effects on germination and seedling growth of alfalfa 'Cody'

Fifty seeds of alfalfa 'Cody' were imbibed in distilled water for 12 hours and placed on the agar surface with extract concentrations of 0.5, 1.0, and 4 g L^{-1}. Agar containing distilled water was used for the control. Four replications were used in a randomized complete block design. Cumulative germination was determined by counting the number of germinated seeds at 12-hour intervals over an 72-hour period and transformed into percent germination.

To compare the concentration effect of leaf and stem extracts on seedling growth of alfalfa 'Cody', twenty five seeds were imbibed in distilled water for 12 hours and placed on the agar surface with both leaf and stem extract concentration of 0, 0.1, 0.3, 0.5, 1.0, 4.0 g L^{-1}. Root and hypocotyl lengths of all seedlings in a petri dish were measured 120 hours after transfer of seed to agar.

Allelopathic effect of leaf extracts on alfalfa 'Vernal' and red clover

To know the response of legume plants against alfalfa leaf extracts, twenty imbibed seeds of Alfalfa 'Vernal' and red clover 'Red star' were separately placed on each extract-agar medium with alfalfa leaf extract concentration of 0, 1, and 4 g L^{-1}. Cumulative germination was determined by counting the number of germinated seeds at 12-hour intervals over 144-hour period and transformed into percent germination and GT50, the time needed to reach 50% of final germination. Root and hypocotyl lengths or plant height of all seedlings were measured in 144 hours after transfer of seed to agar, and transformed into I50, the dose required to reach 50% final root and shoot length reduction.

Allelopathic effect of leaf extracts on crested wheatgrass and Russian wildrye

Twenty imbibed seeds of crested wheatgrass 'Hycrest' and Russian wildrye 'Bozoisky-Select' were separately placed on each extract-agar medium with alfalfa leaf extract concentration of 0, 1, and 4 g L^{-1}. Cumulative germination was determined by counting the number of germinated seeds at 12-hour intervals over an 144-hour period and transformed into percent germination and GT50, the time needed to reach 50% of final germination. Root length and hypocotyl length or plant height of all seedlings were measured 144 hours after placing seed on agar containing extract, transformed into I50, the dose required to reach 50% final root and shoot length reduction, and finally compared the values with other species.

RESULTS AND DISCUSSION

Concentration effects on germination and seedling growth of alfalfa 'Cody'

Seed germination of alfalfa Cody was delayed but not stopped. Depending on extract concentrations there were no differences in final germination at 72 hours. This indicates that final germination was little affected but was delayed by the extract depending on concentration. Seed germination was little affected at extract concentrations of 0.5 and 1 g L^{-1} but was significantly reduced by 4 g L^{-1} extract compared to control (Fig. 1).

Root growth of alfalfa 'Cody' was enhanced in autotoxin concentration of both leaf and stem extracts, with 14% stimulation at extract concentration of 0.1 g L^{-1}. This confirms the earlier findings of Einhellig (1986) and Chon *et al.* (2000) that root growth was stimulated at very low concentration of allelopathic plant extracts. Agar-containing extracts from both leaves and stems showed increased inhibitory effects on root growth with increasing of extract concentration. Extracts of alfalfa leaves at the same concentration were more inhibitory than extracts of stems. This corroborates the results of Chung and Miller (1995a) that autotoxic effects of aqueous extracts of plant parts of alfalfa were

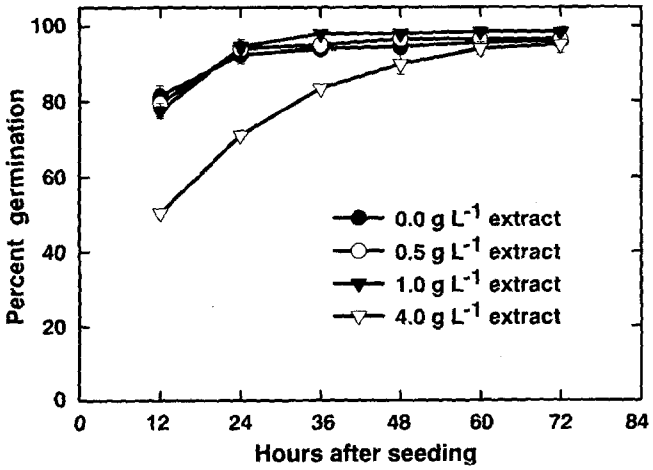

Fig. 1. Effect of extract concentration on cumulative germination of alfalfa seed that had been imbibed for 12 hours then transferred to the extract treatments in agar. Response was concentration dependent.

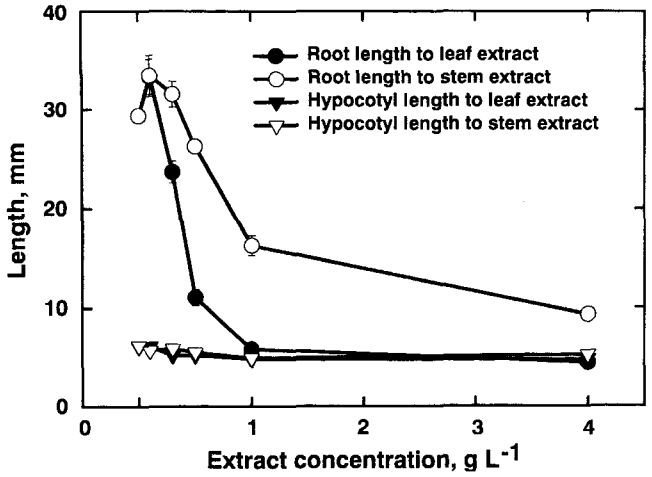

Fig. 2. Effect of extract concentrations from leaves and stems on hypocotyl and root length of alfalfa seedlings 120 hours after seeding. Root growth was stimulated at very low concentration of leaf extracts.

ranked with leaf (greatest), seed, root, flower, and stem (least). Hypocotyl length was not sensitive at all concentrations of both leaf and stem extracts. In this experiment, root length was more sensitive to the extracts than was hypocotyl length or germination (Fig. 2). These results are similar to those of previous laboratory studies by Hedge and Miller (1990), Chung and Miller (1995a), Chon and Choi (2000), and Chon *et al.* (2000). However, we don't know yet if the chemicals from different plant parts that causes autotoxicity are the same or not. Long-term autotoxicity and allelopathy of alfalfa were verified at Urbana, IL, by comparing the germination and growth of alfalfa and sorghum (*Sorghum bicolor* (L.) Moench) on silt loam soil previously cropped to alfalfa (alfalfa-soil) and sorghum (sorghum-soil) (Hedge and Miller, 1990). Plant height and fresh weight per plant of alfalfa and fresh weight per plant of sorghum were lower on alfalfa-soil than on sorghum-soil. Recently, the common field recommandation to avoid autotoxicity is to delay seeding of alfalfa after alfalfa for at least 2 weeks (Tesar, 1993), and in some cases upto 2 years or more (Jennings, 1996).

Allelopathic effect of extracts on alfalfa Vernal and red clover

Seeds of alfalfa and red clover in control were all germinated within 36 hours. Seed germination of two legume plants was delayed when treated at 4 g L^{-1} leaf extract-agar medium. The GT50 values of two species at control were 7.2 and 7.3 hours, respectively, indicating similar speeds in germination of two species. Although seeds of alfalfa Vernal were a little more delayed by extracts than were red clover, final seed germination of each extract concentration or of

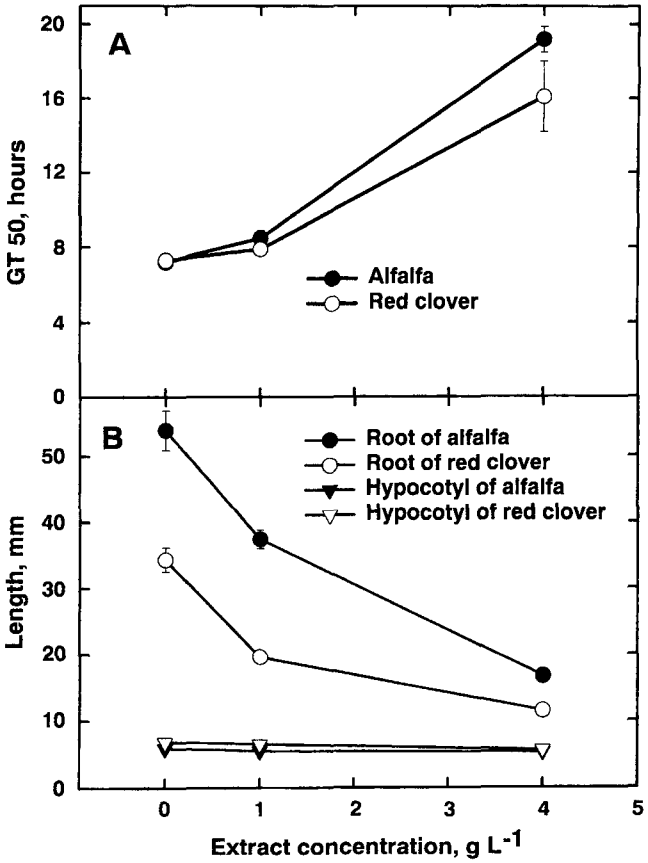

Fig. 3. Effect of extract concentrations on the time to reach 50% of the final seed germination (A) and hypocotyl and root length (B) of alfalfa and red clover seedlings at 144 hours after placing on extract-agar medium. Root growth of two species was significantly reduced with increasing concentrations of extract.

each species remained similar. Hypocotyl lengths of both legumes were not affected by extracts while root growth was significantly reduced as the extract concentration increased. Recently, Chon *et al.* (2000) and Chung and Miller (1995) demonstrated that root elongation was more sensitive to the autotoxin than germination or shoot length. Both aqueous extracts and residues of nine forage grasses affected germination, growth and development of alfalfa because of effects of allelochemicals present in grass residues (Chung and Miller, 1995). Our experience also showed that the extracts of rice and oat straws at 10 and 20 g L^{-1} inhibit root growth of alfalfa (Unpublished data, 1999).

Allelopathic effect of leaf extracts on crested wheatgrass and Russian wildrye

Germination of two grass species was little delayed with increasing of extract concentration of alfalfa leaf extracts. GT50 of control in crested wheatgrass and Russian wildrye

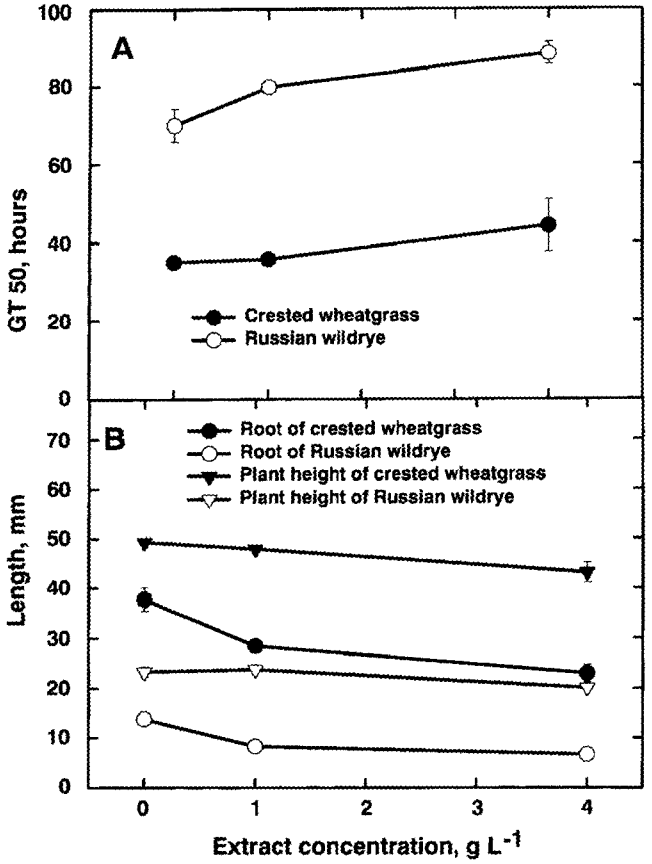

Fig. 4. Effect of extract concentrations on GT50, the time to reach 50% of the final seed germination (A) and hypocotyl and root length (B) of crested wheatgrass and Russian wildrye seedlings at 144 hours after placing on extract-agar medium. Responses of grasses to extracts were less sensitive than were legumes.

were 35 and 70.6, respectively, and of 4 g L^{-1} leaf extract were delayed by 17 and 19 hours, respectively. Plant height of two species was not influenced by extracts while root length was reduced with increasing concentrations of extract. Compared with water-agar control, root and hypocotyl lengths were inhibited by 38~52%, respectively, by highest concentration of extracts (Fig. 4).

We compared values of I50, rates needed to reach 50% inhibition of seed germination, hypocotyl and root growth among four species. Alfalfa was the most sensitive species and crested wheatgrass was the least sensitive species. Response of alfalfa root growth to alfalfa extracts was sensitive and was very similar to that of red clover. Legume plants were more sensitive to the autotoxic chemicals from leaf extracts than were grasses (Table 2). This is supported by the report of Miller (1983) that the best preceding crop for alfalfa establishment is corn (*Zea mays* L.), followed by various small grains, soybeans and the worst preceding crop

Table 2. I50 values, extract concentrations of alfalfa leaves required to reach 50% inhibition of the final shoot and root length, of four different forage crops 7 days after seeding.

Plants tested	Shoot length	Root length
	g L^{-1}	
Alfalfa	22.3	2.4
Red clover	11.3	3.0
Crested wheatgrass	15.8	5.4
Russian wildrye	13.3	4.0

is alfalfa. However, the result contrasts with earlier conclusion based on the field study on the effects of preceding crops on alfalfa autotoxicity and allelopathy (Hedge and Miller, 1990) that alfalfa autotoxicity seems to be more severe than allelopathy. Autotoxic chemicals reported for alfalfa are mainly phenol compounds such as cinnamic acid and its derivatives, but there is no chemical that has been proven unequivocally to be primary cause. This makes it very difficult to understand the problem and full develop sound solution of alfalfa autotoxicity or allelopathy. In 1992, Hedge and Miller reported that coumarin and trans-cinnamic acid at 60 μg mL^{-1} were the most inhibitory among the suspected phenolic allelochemicals assayed for their phytotoxicity on root and shoot growth of alfalfa. However, mixtures of five or more phenolics were more phytotoxic than their respective individual components. A fundamental question is "does the same chemical(s) that cause autotoxicity contribute to allelopathy from other plant parts, or vice versa?" If the chemicals are different, are they produced in the same plant part? Answers to these questions and more will be forthcoming.

CONCLUSIONS

The bioassay study showed that root growth was apparently more sensitive to the autotoxic chemicals than germination or hypocotyl and stimulated at very low concentrations of both leaf and stem extracts. Leaf extracts were stronger than stem extracts. The leaf extracts of alfalfa did not affect the root growth of two grasses although the alfalfa leaf extracts did markedly reduce root growth of two legumes. In this experiment, grass plants such as crested wheatgrass and Russian wildrye were more suitable in an alfalfa cropping system as following rotation crops than were legumes such as alfalfa and red clover. It means that alfalfa autotoxicity seems to be greater than allelopathy with water-soluble inhibitory compounds from aboveground portion of the plant. However, we need in-depth studies on long-term allelopathy and autotoxicity through field assay to better under

stand the mechanism of alfalfa autotoxicity or allelopathy in forage cropping system.

REFERENCES

Abdul-Rahman, A. A. and S. A. Habib. 1989. Allelopathic effect of alfalfa (*Medicago sativa*) on bladygrass (*Imperata cylindrica*). *J. Chem. Ecol.* 15 : 2289-2300.

Asay, K. M., D. R. Dewey, F. B. Gomm, D. A. Johnson, and J. R. Carlson. 1985a. Registration of Hycrest crested wheatgrass. *Crop Sci.* 25 : 368-369.

Asay, K. M., D. R. Dewey, F. B. Gomm, D. A. Johnson, and J. R. Carlson. 1985b. Registration of Bozoisky-Select Russian Wildrye. *Crop Sci.* 25 : 575-576.

Boyer, J. S., and E. B. Knipling. 1961. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proc. N.A.S.* 54 : 1044-1051.

Chon, S. U. and S. K. Choi. 2000. Evaluation of alfalfa autotoxicity on germination and early seedling growth of 3 cultivars. *Plant Res.* 3(1) : 66-70.

Chon, S. U., J. H. Coutts, and C. J. Nelson. 2000. Effects of light and growth media on a seedling assay of alfalfa autotoxicity. *Agron. J.* 92 : 715-720.

Chung, I. M., and D. A. Miller. 1995a. Effect of alfalfa plant and soil extracts on germination and seedling growth. *Agron. J.* 87 : 762-767.

Chung, I. M., and D. A. Miller. 1995b. Difference in autotoxicity among seven alfalfa cultivars. Agron. J. 87:596-600.

Chung, I. M., and D. A. Miller. 1995c. Natural herbicide potential of alfalfa residue on selected weed species. *Agron. J.* 87 : 920-925.

Dornbos, D. L., Jr., and G. F. Spencer. 1990. Natural products phytotoxicity. A bioassay suitable for small quantities of slightly water-soluble compounds. *J. Chem. Ecol.* 16 : 339-351.

Einhellig, F. A. 1986. Mechanisms and modes of action of allelochemicals. p. 171-178. *In* A.R. Putnam and C. S. Tang (ed.) The Science of Allelopathy. John Wiley, New York.

Ells, J. E., and A. E. McSay. 1991. Allelopathic effects of alfalfa plant residues on emergence and growth of cucumber seedlings. *HortScience* 26 : 368-370.

Guenzi, W. D., W. R. Kehr, and T. M. McCalla. 1964. Water-soluble phytotoxic substances in alfalfa forage: Variation with variety, cutting, year, and stage of growth. *Agron. J.* 55 : 499-500.

Hall, M. H., and P. R. Henderlong. 1989. Alfalfa autotoxic fraction characterization and initial separation. *Crop Sci.* 29 : 425-428.

Hedge, R. S., and D. A. Miller. 1990. Allelopathy and autotoxicity in alfalfa: characterization and effects of preceding crops and residue incorporation. *Crop Sci.* 30 : 1255-1259.

Hedge, R. S., and D. A. Miller. 1992. Concentration dependency and stage of crop growth in alfalfa autotoxicity. *Agron. J.* 84 : 940-946.

Jennings J. A. 1996. Rotational interval, soil texture, and zone of influence studies on alfalfa autotoxicity. Ph.D. Diss. University of Missouri, Columbia, MO.

Jensen, E. H. 1984. Problems of continuous alfalfa. *In* Proc. Western Nevada Alfalfa Symposium, Nevada Coop. *Ext. Publ.* 165, Fallon, NV.

Jensen, E. H., B. J. Hartman, F. Lundin, S. Knapp, and B. Brookerd. 1981. Autotoxicity of alfalfa. Max C. Fleischmann College of Agric., Univ. of Nevada Agric. Exp. Stn. Bull. R 144.

Klein, R. R., and D. A. Miller. 1980. Allelopathy and its role in agriculture. *Comm. Soil Sci. Plant Anal.* 11 : 43-56.

Lawrence, T., and M. R. Kilcher. 1961. The effect of fourteen root extracts upon germination and seedling length of fifteen plant species. *Can. J. Plant Sci.* 42 : 308-313.

Li, H. Y. 1981. The autotoxic effect of alfalfa extracts on seed germination and seedling growth. M. S. Thesis, The Ohio State Univ., Columbus, OH.

Miller, D. A. 1983. Allelopathic effects of alfalfa. *J. Chem. Ecol.* 9 : 1059-1071.

Miller, D. A. 1992. Allelopathy and establishment. Alfalfa Talk. Vol. 12, No. 1. Certified Alfalfa Seed Council, Davis, C. A.

Miller, D. A. 1996. Allelopathy in forage crop systems. *Agron. J.* 88 : 854-859.

Molisch, H. 1937. Der Einfluss einer Pflanze auf die andere-Allelopathie. Fischer. Jena.

Newby, V. K., R. M. Sablon, and R. L. Synge. 1980. Free and bound phenolic acids of lucerne (*Medicago sativa* cv. Europe). *Phytochem.* 19 : 651-657.

Putnam, A. R. 1985. Allelopathic research in agriculture: Past highlights and potential. p. 1-8. *In* A. C. Thompson (ed) The Chemistry of Allelopathy. Am. Chem. Soc., Washington, DC.

Read J. J., and E. H. Jensen. 1989. Phytotoxicity of water-soluble substances from alfalfa and barley soil extracts on four crop species. *J. Chem. Ecol.* 15 : 619-628.

Rice, E. L. 1984. Allelopathy. 2nd ed. Academic Press, NY.

Tesar, M. B. 1993. Delayed seeding of alfalfa avoids autotoxicity after plowing or glyphosate treatment of established stands. *Agron. J.* 85 : 256-263.