Reprinted from *Crop Science*
Vol. 30, No. 1

6308

Purchased by U. S. Dept. of
Agriculture for Official Use

Medicarpin Delays Alfalfa Seed Germination and Seedling Growth

David L. Dornbos, Jr., Gayland F. Spencer, and Roger W. Miller

Medicarpin Delays Alfalfa Seed Germination and Seedling Growth

David L. Dornbos, Jr.,* Gayland F. Spencer, and Roger W. Miller

ABSTRACT

The objective of this study was to identify a compound(s) that may be responsible, at least in part, for alfalfa autotoxicity. We found that medicarpin (3-hydroxy-9-methoxypterocarpan) is a compound produced by alfalfa (*Medicago sativa* L.) that contributes to this. A clay loam soil from a declining alfalfa stand with two plants per 930 cm^2 and its 95% ethanol extract inhibited alfalfa emergence and seedling growth whereas extracted soil did not. The soil extracts contained at least 10 mg kg^{-1} medicarpin. Medicarpin, 4-methoxymedicarpin, sativan, and 5′-methoxysativan were isolated from alfalfa foliage with 95% ethanol, purified, identified by gas chromatography-mass spectroscopy, and then exogenously applied to alfalfa and velvetleaf (*Abutilon theophrasti* L.) seeds using an agar bioassay. Medicarpin at 1×10^{-7} mol/seed reduced both alfalfa and velvetleaf seedling length by 39% after 72 h. Sativan and both methoxy derivatives had no affect on germination or seeding growth. In a timecourse study, 2×10^{-7} mole/seed medicarpin delayed germination 12 h and seedling growth 44 h, during this time the alfalfa seedlings metabolized 85% of the medicarpin. Seedling growth then resumed at the control rate. Medicarpin is produced by mature alfalfa, is present in soil from a declining alfalfa stand, and is absorbed by alfalfa seedlings where it is metabolized and exerts a transient phytotoxic effect. Medicarpin may permit established alfalfa plants to control the ecology in their immediate proximity by inhibiting seedling establishment of nearby plants, thereby gaining a competitive advantage.

ALLELOPATHY is any direct or indirect harmful effect on a recipient plant caused by a compound produced by the donor plant (Rice, 1984). Alfalfa is a perennial agronomic crop produced for its high forage yield and high-quality forage, but the autoallelopathic (autotoxic) nature of alfalfa limits long-term productivity. Alfalfa plants in autotoxic fields are dwarfed, spindly, yellowish, and have few effective nodules (Webster et al., 1967). Mature alfalfa stands exhibit reduced yields and poor reseeding success (Kiesselbach et al,. 1934; Miller, 1983), even after accounting for differences in soil fertility and pathogen levels (Miller, 1983). Alfalfa often cannot be re-established without an intervening crop or without waiting at least 2 wk after plowing (Tesar, 1984). Reduced forage yield and high erosion rates on erodable soils represent two subsequent losses.

A key requirement for the demonstration of an allelopathic interaction, allowing separation of allelopathy from other mechanisms of plant interference, is the identification of a specific, causative, phytotoxic compound. In alfalfa, the search for the purported allelochemical(s) has focused on water-soluble extracts of plant and soil material. Water extracts of 'Buffalo' and 'Ranger' alfalfa forage reduced the shoot and root growth of corn (*Zea mays* L.) seedlings (Guenzi et al., 1964). Aqueous extracts that were phytotoxic were obtained from the leaves, stems, and crowns of fresh alfalfa, dry hay, old roots, soil, and soil residues (Hall and Henderlong, 1984; Klein and Miller, 1980; McElgunn and Heinricks, 1970; Nielsen et al., 1960). Prior to this study, however, specific chemical compounds responsible for alfalfa autotoxicity have eluded identification.

Isoflavanoid phytoalexins are a major class of low molecular weight inhibitory compounds synthesized by certain plants, notably members of the *Leguminosae* (Smith and Banks, 1986). In particular, medicarpin is synthesized de novo in alfalfa (Smith et al., 1971), is present in red clover (*Trifolium pratense* L.) (Higgins and Smith, 1972), and is a component of the natural chemical defense mechanism against several fungal pathogens. In red clover leaves challenged with *Helminthosporium carbonum*, medicarpin is one of the compounds found in sufficient concentration to inhibit fungal growth (Duczek and Higgins, 1976). *Stemphylium botryosum*, *S. sarcinaeforme* (Duczek and Higgins, 1976), and *Ascochyta rabiei* (Kraft et al., 1987) are pathogenic to alfalfa and degrade medicarpin.

In addition to their postulated role in antimicrobial chemical defense, phytoalexins are not uniquely toxic to fungi, but have inhibitory effects across much of the biological spectrum (Smith and Banks, 1986). Of 11 isoflavonoids tested for inhibitory activity against eight *Rhizobium* strains, medicarpin was strongly inhibitory, and at higher doses, bactericidal towards slow-growing *R. japonicum* and two fast-growing *Lotus* rhizobia (Pankhurst and Biggs, 1980). Seed germination, root and hypocotyl growth of red, white (*T. repens* L.), and alsike (*T. hybridum* L.) clover were inhibited by several isoflavanoid compounds, including trifolirhizin (Chang et al., 1969). Medicarpin supplied exogenously to alfalfa seed reduced their germination by 59% after 6 d when using a filter-paper bioassay (Miller et al., 1988). Phenolics, but not isoflavanoids, were isolated from a soil capable of causing clover sickness (Chang et al., 1969).

For a plant interaction to be considered allelopathic, it is necessary to demonstrate that a chemical species is released from a donor plant, resides in the soil in sufficient quantity, and is taken up by, and has an inhibitory effect on a recipient species. The objectives of this study were to extract medicarpin and several related compounds from alfalfa foliage and soil from an established alfalfa stand, and determine their phytotoxicity on, and metabolism by, alfalfa seedlings.

MATERIALS AND METHODS

Source of Medicarpin. Medicarpin, 4-methoxymedicarpin, sativan, and 5′-methoxysativan were extracted from dry, ground alfalfa foliage with 95% ethanol; fractionated on a silica column using a stepwise gradient with hexane, ethyl acetate, and methanol, and preparative silica thin-layer chromatography with 4% methanol in chloroform (Miller et al., 1989). Medicarpin was purified on a 250 by 4.6 mm

D.L. Dornbos, Jr., 6330 Gandner Rd., Chandler, In 47610; and G.F. Spencer and R.W. Miller, USDA-ARS, Northern Regional Res. Ctr., 1815 North University Street, Peoria, IL 61604. Received 17 Feb. 1989. *Corresponding author.

Published in Crop Sci. 30:162–166 (1990).

Whatman[1] ODS-2 column (DuPont, Wilmington, DE) with curve seven of the Waters' Model 660 gradient programmer and two Waters' M6000-A pumps (Milford, MA). The gradient was run in 30 min from 80% degassed 1:1:6 acetonitrile:ethanol:water (Pump A) and 20% 1:1:1 acetonitrile:ethanol:water (Pump B) to 20% of Pump A and 80% of Pump B. For quantification, each medicarpin sample was evaporated to dryness, the residue dissolved in 0.5 mL of 1 mg/mL phenol (internal standard) in ethanol, and 25 μL injected. The compounds were detected at 254 nm with a Schoeffel Instrument Corp. (Applied Biosystems, Ramsey, NH) variable wavelength detector, and the peaks were integrated with a Spectra-Physics 4290 integrator (San Jose, CA). Peaks corresponding to each compound were collected, and their identity and purity confirmed by gas chromatography-mass spectroscopy (GC-MS) and proton nuclear magnetic resonance (^{1}H-nmr) (Miller et al., 1988).

Bioassay. Alfalfa or velvetleaf seeds were treated exogenously with each test compound using the agar bioassay developed by Dornbos and Spencer (1989). One milliliter of water agar was placed in a 4-mL glass vial and allowed to harden. The test compound was dissolved in 1-mL chloroform and diluted to 100 mL with hexane. A dilution series was made from the stock solution to give the following treatments: 0, 2, 4, 6, 8, and 10 $\times$ 10^{-8} mol/seed of compound. One milliliter of each solution was pipetted onto the hardened agar and the solvent removed by passive evaporation. Alfalfa and velvetleaf seeds were surface sterilized with 10:90 Chlorox®:water (v/v) for 15 min, then allowed to imbibe on moistened filter paper for 4 h. Five alfalfa or velvetleaf seeds per vial were placed on the treated agar, the vials then were capped and placed in a growth chamber at 25 °C. After 72 h, the percentage of germinated seeds, defined as protrusion of the radicle through the seed coat, and seedling length were measured. Each treatment was replicated four times and all bioassays were repeated at least twice.

Medicarpin Uptake and Metabolism. One milliliter of 1-m*M* medicarpin (300 μg) in 1:99 chloroform:hexane (v/v) was added to 45 agar vials prepared as before, 1 mL of the solvent mixture alone was added to another 45 vials, and the solvent was allowed to evaporate. Five imbibed alfalfa seeds were placed on the agar of each vial, then the vials placed in the growth chamber. Three vials (replicates) treated with medicarpin, and three without, were removed at 4- or 8-h intervals during the 120-h experiment. The number of germinated seeds in each vial was counted, the seedlings were rinsed with warm water, and their lengths measured. Medicarpin treatment represented the main plots and the sampling interval the split plots of the experimental design.

The quantity of medicarpin recoverable from the agar of each vial and from the combined seedlings was measured. Each agar sample was dissolved in warm water and extracted three times with chloroform in a separatory funnel. The organic phases were then combined and evaporated to dryness. The residue from each extract was dissolved in ethanol with 1 mg/mL phenol and quantified using high pressure liquid chromatography as before. Beginning at 28 h, the 15 seedlings were combined, placed in a 20-mL test tube, capped, and frozen until extracted. Chloroform (10 mL) was added to the frozen seedlings, which were then homogenized thoroughly using a Polytron homogenizer (Brinkman Instruments, Inc. Westbury, NY.). After shaking for 1 h, the homogenate was filtered and the eluate evaporated to dryness at 25 °C under N. Each extract was dissolved in ethanol and quantified as before.

[1] Mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.

Soil Extraction. A clay loam soil from a declining alfalfa stand established in 1984 that consisted of two alfalfa plants per 930 cm^2 was supplied by Dr. A. Putnam (1987 personal communication). Two independent soil samples were obtained in the Fall of 1987 from the upper 10 cm of the soil profile, dried for 72 h at 100 °C in a convection oven, and stored at room temperature. Two 150-g soil samples were extracted for 8 h in a Soxhlet apparatus with either 95% ethanol or ethyl acetate. Extracts were reduced to dryness and thoroughly washed with 20 mL 1:99 chloroform:hexane. The solvent mixtures were reduced to 9 mL at 25 °C under nitrogen. One milliliter of both extracts was removed, dried, redissolved in ethanol containing 1 mg/mL phenol, and chromatographed. The remaining extract was tested for phytotoxicity with the agar bioassay. One milliliter per vial of both extracts (eight replicates) was used to test phytotoxicity using the agar bioassay with alfalfa seeds as previously described.

Three 150-g soil samples were extracted with 95% ethanol for 24 h in a Soxhlet apparatus. The extracted soil samples were then combined and the remaining solvent allowed to completely evaporate. Ten alfalfa seeds were planted 0.7 cm deep in approximately 30 g each of extracted (13 samples), unextracted (14 samples), and potting soils (7 samples). The soil in each pot was saturated with distilled water, the pots covered with plastic wrap, and then placed in a growth chamber at 25 °C. The number of emerged seedlings were counted at 24-h intervals for 3 d. The seedlings were removed from the soil and their lengths (shoot plus root) measured after 3 d.

Experimental Design. All experiments were conducted as completely randomized designs except for medicarpin uptake and metabolism, which was a split-plot completely randomized design. Treatment effects were considered significant when $P \leq 0.05$ as determined by the General Linear Model and Regression procedures of the Statistical Analysis System (SAS Institute, Inc., 1982).

RESULTS AND DISCUSSION

Inhibition by Soil from an Alfalfa Stand and its Extract. The emergence rate of alfalfa seedlings in extracted soil was 69, 36, and 19% greater than the unextracted soil after 24, 48, and 72 h, respectively (Table 1). Further, alfalfa seedlings from the unextracted soil were 16% shorter after 72 h. Because the pH of both the unextracted and extracted soils was 7.3, the difference in seedling performance was presumed due to the removal of one or more compounds. An ethanol-soluble compound(s), thus, delayed the emergence of alfalfa seedlings and inhibited their growth in the unextracted soil.

The ethanol- and ethyl acetate-soluble compounds from the alfalfa soil were tested for phytotoxicity using the agar bioassay. The germination percentages of alfalfa seeds treated with the ethanol and ethyl acetate

Table 1. Daily emergence percentage and seedling length of alfalfa planted in potting, extracted, and unextracted soils.

Soil	Emergence (d) 0	1	2	3	Seedling length
	%				%
Potting	0	4.3	64.3	88.6	6.1
Extracted†	0	2.3	46.9	78.5	3.8
Unextracted†	0	0.7	30.0	63.6	3.2
LSD (0.05)‡	0	7.2	7.2	7.2	0.5

† From an alfalfa field with two plants per 930 cm^2.
‡ LSD (0.05) for comparison of emergence percentage between days is 8.7.

Table 2. Effect of the extract of 150 g of soil from an alfalfa field with two plants per 930 cm^2 on the growth of alfalfa.

Extraction solvent	Germination	Seedling length	Extracted compounds
	%	cm	mg
Control	98	3.2	–
95% ethanol	85	1.4	107
Ethyl acetate	88	1.7	63
LSD (0.05)	8	0.6	–

extracts were 13 and 10 percentage points lower than the controls, respectively (Table 2). Alfalfa seedling length also was reduced 56% by the 95% ethanol and 47% by the ethyl acetate extract. Compounds extracted from the soil inhibited alfalfa seed germination and seedling growth.

Phytotoxicity of Four Isoflavanoid Compounds from Alfalfa. Medicarpin, 4-methoxymedicarpin, sativan, and 5′-methoxysativan (Fig. 1) were extracted from alfalfa foliage, purified, and identified by GC-MS and ^{1}H-nmr (Miller et al., 1988, 1989). The germination of alfalfa and velvetleaf was not inhibited by these compounds in agar bioassay (Table 3). Miller et al. (1988) found medicarpin inhibited the seed germination of lettuce (*Lactuca sativa* L.) and alfalfa by 59% after 6 d on moistened filter paper.

Seedling growth of alfalfa and velvetleaf was inhibited by medicarpin that was applied exogenously in the agar bioassay (Fig. 2). The minimum toxic dose of medicarpin for these seedlings was approximately 5×10^{-8} mol/seed. At doses greater than the minimum, increased concentrations of medicarpin linearly reduced the seedling length of both plant species. The dosage at which seedling length was inhibited by 50% (ID_{50}) was 10×10^{-8} mol/seed for alfalfa and 6×10^{-8} mol/seed for velvetleaf. In contrast, the seedling growth of alfalfa and velvetleaf was not inhibited by 4-methoxymedicarpin, sativan, and 5′-methoxysativan (Table 4).

To be autoallelopathic, medicarpin must reside in the soil and be taken up by the inhibited species. Chromatographic and mass spectroscopic analysis indicated that the 95% ethanol and ethyl acetate extracts of the soil from the declining alfalfa stand contained at least 10 mg kg^{-1} medicarpin, or approximately 1×10^{-8} mol/seed. As demonstrated in bioassay, the lowest toxic dose of medicarpin was approximately 5×10^{-8} mol/seed (Fig. 2). Medicarpin was present in soil from a declining alfalfa stand at a concentration reasonably close to that phytotoxic in bioassay.

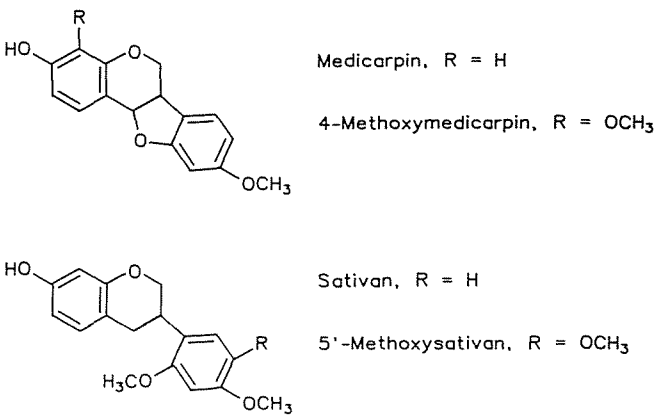

Fig. 1. Chemical structures for four isoflavanoid compounds from alfalfa foliage.

Table 3. Three-day germination percentage of alfalfa and velvetleaf seeds treated exogenously with four compounds from alfalfa foliage.

Dose	Compound: Medicarpin	4-Methoxy-medicarpin	Sativan	5′-Methoxy-sativan
10^{-8} mol/seed	% germination			
		Alfalfa		
0	95	95	95	95
2	85	95	100	95
6	90	85	90	95
10	95	95	100	90
LSD (0.05)	17	14	11	17
		Velvetleaf		
0	95	90	85	95
2	95	100	90	80
6	95	95	90	90
10	100	100	85	100
LSD (0.05)	8	8	20	20

Metabolism of Medicarpin by Alfalfa. Medicarpin inhibited early seedling growth of alfalfa and velvetleaf in bioassay to a greater extent than measured at the conclusion of the 3-d growth period. To describe the dynamics of growth inhibition, the germination percentage and length of alfalfa seedlings were measured at 4- to 8-h intervals using the agar bioassay. When five alfalfa seeds per vial were treated with 300 μg (2.2×10^{-7} mol/seed) of medicarpin, germination was delayed for 12 h in comparison to untreated seeds (Fig. 3a). Seedling growth was evident after 20 h for alfalfa seeds treated with medicarpin, and after 8 h for untreated seeds. Medicarpin-treated alfalfa seedlings increased in length to 0.4 cm by 25 h, but did not increase further until after 44 h (Fig. 3b). The alfalfa seedlings treated with medicarpin resumed growth at rates comparable to control seedlings after 44 h. The length of untreated alfalfa seedlings increased throughout the period from 0 to 6.3 cm. When no alfalfa seeds were placed on the agar, all the medicarpin was recoverable after 120 h. No medicarpin was detected in

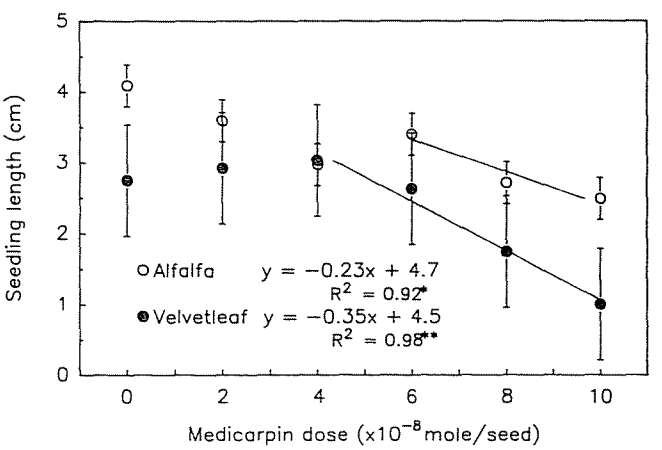

Fig. 2. Dose response of exogenously applied medicarpin on seedling growth of alfalfa and velvetleaf (** and * represent significance at the 0.01 and 0.05 probability levels). Error bars ± SE.

Table 4. Length of alfalfa and velvetleaf seedlings after a 3-d growth period when exogenously treated with three compounds from alfalfa foliage.

Dose	Compound		
	4-Methoxymedicarpin	Sativan	5′-Methoxysativan
10^{-8} mol/seed	seedling length (cm)		
	Alfalfa		
0	4.1	3.8	3.3
2	3.4	4.0	3.3
6	3.6	3.3	4.1
8	3.3	–	–
10	3.6	4.0	4.2
LSD (0.05)	1.1	1.1	1.1
	Velvetleaf		
0	2.3	4.2	3.7
2	2.7	3.9	3.0
6	1.3	3.4	2.3
8	2.7	–	–
10	3.2	2.1	3.1
LSD (0.05)	1.9	1.9	1.9

extracts of agar on which alfalfa seeds were germinated and grown for 120 h in the absence of exogenously supplied medicarpin.

The quantity of medicarpin that was recovered from the agar decreased with time when alfalfa was present, suggesting that it was metabolized by alfalfa. Medicarpin content decreased linearly by 36% after 20 h, and by an additional 27% after 44 h to 108 μg (8×10^{-8} mol/seed), while germination and seedling growth were inhibited (Fig. 3a,b). The data of the dose response for alfalfa (Fig. 2) and timecourse study concur in demonstrating inhibition of seedling growth by 8×10^{-8} mol/seed medicarpin. After 44 h, the medicarpin content continued to decrease slowly from 108 to 42 μg (3×10^{-8} mol/seed), during which time seedling growth resumed at the same rate as the controls. Because the alfalfa seeds were surface sterilized before being placed on the agar, and no microbial growth was evident, it is unlikely that the reduction in medicarpin content was due to microbial contamination.

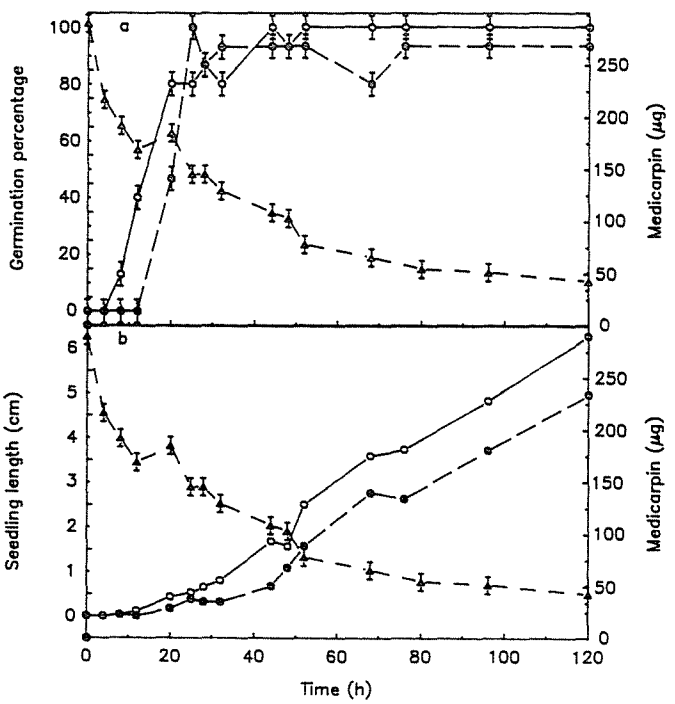

Fig. 3. The quantity of medicarpin (▲--▲) recovered from the agar in bioassay, and it relationship to the germination percentage (a), and seedling length (b) of alfalfa treated with medicarpin (●--●) or without medicarpin (○—○). Error bars ± SE, error bars for seedling length are buried within the data points.

Medicarpin should be absorbed by, and be present in, alfalfa seedlings to elicit a phytotoxic symptom, and to satisfy the definition of an autotoxic compound. At each sampling time after 28 h, alfalfa seedlings were removed from the agar and extracted. Between 1.7 and 3.4 μg/seedling (0.6 and 1.3×10^{-8} mol/seedling) of medicarpin was extracted from seedlings grown in the presence of medicarpin and sampled between 28 and 52 h. Alfalfa seedlings grown on agar in the absence of medicarpin contained no medicarpin. In addition, no medicarpin was detected in extracts from alfalfa seedlings grown in the presence of medicarpin that were sampled after 52 h, when 70 μg of medicarpin (5×10^{-8} mol/seed) remained on the agar. These data suggest that exogenously supplied medicarpin is absorbed and detoxified (metabolized) by alfalfa seedlings. *Stemphylium botrysum* Wallr., a fungus pathogenic to alfalfa, can reduce medicarpin to vestitol (Higgins and Smith, 1972), but the metabolic product(s) of medicarpin in alfalfa seedlings have not been characterized yet.

Medicarpin is responsible, at least in part, for the autoallelopathic characteristics of alfalfa. Medicarpin is produced by mature alfalfa, is present in soil from a declining alfalfa stand, and is absorbed by alfalfa seedlings upon which it exerts a transient phytotoxic effect in bioassay. Uptake and apparent detoxification of medicarpin by alfalfa seedlings suggests that a continued supply of medicarpin may be required for the symptoms of allelopathy to persist. The biosynthesis and release of medicarpin into the environment suggests that a dynamic mechanism may be operative in which mature alfalfa plants are capable of manipulating their ecology to gain a competitive advantage over nearby seedlings (including alfalfa) for the essential components of water, light, and nutrients.

REFERENCES

Chang, C.F., A. Suzuki, S. Kumai, and S. Tamura. 1969. Chemical studies on "clover sickness". Part II. Biological functions of isoflavanoids and their related compounds. Agric. Biol. Chem. 33:398–408.

Dornbos, Jr., D.L., and G.F. Spencer. 1989. Natural products phytotoxicity: A bioassay suitable for small quantities of slightly water-soluble compounds. J. Chem. Ecol. (in press).

Duczek, L.J., and V.J. Higgins 1976. The role of medicarpin and maackiain in the response of red clover leaves to *Helminthosporium carbonum*, *Stemphylium botryosom*, and *S. sarcinaeforme*. Can. J. Bot. 54:2609–2619.

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. 56:499–500.

Hall, M.H., and P.R. Henderlong. 1984. Preliminary isolation of the autotoxic compound in alfalfa. p. 128. *In* Agronomy abstracts. ASA, Madison, WI.

Higgins, V.J., and D.G. Smith. 1972. Separation and identification of two pterocarpanoid phytotoxins produced by red clover leaves. Phytopathology 62:235–238.

Kiesselbach, T.A., A. Anderson, and J.C. Russell. 1934. Soil moisture and crop sequence in relation to alfalfa production. J. Am. Soc. Agron. 26:422–442.

Klein, R.R., and D.A. Miller. 1980. Allelopathy and its role in agriculture. Commun. Soil Sci. Plant Anal. 11:54–56.
Kraft, B., L. Schwenen, D. Stockl, and W. Barz. 1987. Degradation of the pterocarpan phytoalexin medicarpin by *Ascochyta rabiei*. Arch. Microbiol. 147:201–206.
McElgunn, J.D., and D.H. Heinricks. 1970. Effects of root temperature and a suspected phytotoxic substance on the growth of alfalfa. Can. J. Plant Sci. 50:307–311.
Miller, D.A. 1983. Allelopathic effects of alfalfa. J. Chem. Ecol. 9:1059–1072.
Miller, R.W., R. Kleiman, R.G. Powell, and A.R. Putnam. 1988. Germination and growth inhibitors of alfalfa. J. Nat. Prod. 51:328–330.
Miller, R.W., G.F. Spencer, and A.R. Putnam. 1989. (-)-5′-Methoxysativan, a new isoflavan from alfalfa. J. Nat. Prod. 52:634–636.
Nielsen, K.F., T. Cuddy, and W. Woods. 1960. The influence of the extract of crops and soil residues on germination and growth. Can. J. Plant Sci. 40:188–197.
Pankhurst, C.E., and D.R. Biggs. 1980. Sensitivity of *Rhizobium* to selected isoflavanoids. Can. J. Microbiol. 26:542–545.
Rice, E.L. 1984. Allelopathy. 2nd ed. Academic Press, Inc., New York.
SAS Inst. 1982. SAS user's guide. Statistics. SAS Inst., Cary, NC.
Smith, D.A., and S.W. Banks. 1986. Biosynthesis, elicitation and biological activity of isoflavanoid phytoalexins. Phytochemistry 25:979–995.
Smith, D.G., A.G. McInnes, V.J. Higgins, and R.L. Millar. 1971. Nature of the phytoalexin produced by alfalfa in response to fungal infection. Physiol. Plant Pathol. 1:41–44.
Tesar, M.B. 1984. Establishing alfalfa after alfalfa without autotoxicity. p. 39. *In* 29th Alfalfa Improvement Conf., 15–20 July, Lethbridge, Alberta, Canada.
Webster, G.R., S.V. Khan, and A.W. Moore. 1967. Poor growth of alfalfa (*Medicago sativa*) on some Alberta soils. Agron. J. 59:37–41.