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Alfalfa soil sickness and autotoxicity

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ABSTRACT

Alfalfa is a perennial legume cultivated worldwide for animal feeding as hay and pasture. Low stand density and yield often occur in alfalfa re-establishment under monoculture leading to decline in forage yield. Literature indicates that this problem is due to "alfalfa soil sickness". Detrimental changes in soil physico-chemical properties, proliferation of soil-borne phytopathogenic fungi and inhibition of symbiotic alfalfa-microorganisms interactions are associated with the phenomenon. The intra-specific allelopathy (autotoxicity) is major component of alfalfa soil sickness. The impact of autotoxicity depends on soil characteristics, time interval between ploughing (killing) the old alfalfa stand and the sowing of new alfalfa, weather (primarily rainfall) and alfalfa cultivar. Many phenolic compounds have been identified as alfalfa autotoxins and can be released by plant leachate and/or residue decomposition. Autotoxins affect the seedling radicle length in seedlings more than germination or hypocotyl elongation. They reduce taproot growth and increase root branching, which adversely affect the crop performance. More studies are needed to elucidate the real ecophysiology of alfalfa autotoxins and the role of soil microorganisms in alfalfa soil sickness.

Key words: Alfalfa monoculture, autotoxicity, phytopathogenic fungi, *Rhizobium*, soil sickness.

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Alfalfa or lucerne (*Medicago sativa* L.) is herbaceous perennial legume cultivated for hay and pasture for animals (60). It is an distributed worldwide due to its adaptability to broad range of soil and climatic conditions and its origin in the northern and eastern coasts of Mediterranean Sea (48). The productive lifespan of an alfalfa field is up to 5 years but in some regions it may be as long as 20 years. Sowing alfalfa seeds over a declining alfalfa stand and no till-systems based on killing the previous old stand before sowing has also been practiced in alfalfa cultivation. These practices, however, were often detrimental for alfalfa re-establishment (35). Alfalfa stands decline considerably after 4-5 seasons making stand renovation necessary due to very low plant population for economical production (36). Its stand density and yield are low, when alfalfa is resown immediately following alfalfa (10). This phenomenon is soil-borne problem that involves several interacting soil biotic and abiotic factors, generally known as 'soil sickness' (52). Extensive research also suggests that alfalfa phytotoxins remaining in the soil, inhibits the growth of the alfalfa and called 'alfalfa autotoxicity' (55).

2. SOIL SICKNESS CAUSES

The intensive alfalfa monoculture without crop rotations or fallows leads to unsuccessful re-establishment of alfalfa stands, reducing forage yield and quality in Argentina, Australia, Canada and United States (13,49,40). Several authors have indicated that autotoxicity is important, if not the major, component of alfalfa soil sickness (6,49,70). However, the participation of other soil factors should not be underestimated (27,43,60). In fact, no single cause has been identified as responsible for alfalfa soil sickness. The following biotic and abiotic factors partially contributes to the alfalfa soil sickness.

2.1. BIOTIC FACTORS

2.1.1. Soil-borne Pests (insects, nematodes, pathogens)

Early studies showed that soil sterilization by steam, Vapam fumigation, or gamma radiation substantially increase alfalfa yield (70). Further studies also suggested that microorganisms from alfalfa soils play role in alfalfa soil sickness (25). Alfalfa monoculture leads to accumulation of soil pathogenic fungi such as *Pythium ultimum* Trow, *P. sylvaticum* Buis., *P. irregulare* Campbell and Hendrix (20), *Phytophthora megasperma* f. sp. medicaginis Kuan and Erwin (43) and *Aphanomyces euteiches* Drechs. (57). These pathogens produce severe seed-rotting and damping-off in alfalfa seedlings at pre-emergence and post-emergence (26,73,67). They can also cause subiethal infections of root systems that may reduce overall plant growth, vigour and ability to withstand several environmental stresses during plant establishment and beyond (26,32). Soil acidification and losses in soil drainage favours the proliferation of these fungi. *Fusarium oxysporum* Schl. f sp. *Medicaginis* (Weimer) Syn. and several species from *Fusarium* genus are also found in alfalfa soils causing root rot and seedling wilt (60). Soil-borne fungi and insects can interact reducing the alfalfa yield and stand density (15,16). In spring-sown alfalfa following alfalfa, soil-borne insects [(*Hypera postica* (Gyllenhall) and

Sitona hispidulus F.)] and soil-borne fungi reduces the stand density of alfalfa seedlings by 40 % and 44 %, respectively (17). *Sitona hispidulus* also increases the incidence of *Fusarium* wilt (33,42).

Long term alfalfa cultivation can also lead to nematode soil infestation. The presence of *Ditylenchus dipsaci* (Kuhn) Filipjev, *Meloidogyne hapla* Chitwood, *M. incognita* (Kofiod and White) Chitwood and *Pratylenchus penetrans* (Cobb) Filipjev & Schuumans Stekhoven in soil was associated with poor stand re-establishment and yield decline (45,60,63). These nematodes also increase the severity of injury caused by Fusarium wilt (21,44).

2.1.2. Biological nitrogen fixation

The symbiosis between Gram-negative Rhizobium bacteria of *Rhizobiaceae* family (e.g. genus *Rhizobium*, *Sinorhizobium* and *Bradyrhizobium*) and alfalfa provides nitrogen needed for the plant growth and development (13). These bacteria might play a protective role in protecting the alfalfa and soybean roots from *P. medicaginis* (64). The bacteria-legume interaction is sensitive to environmental factors and soil acidity is most important. In Argentina, Australia and other countries, long term alfalfa cultivation has led to less nodulation of alfalfa roots related with soil acidification and the associated nutrient disorders, decreasing the forage yield (58,40). Under such conditions, addition of calcium by soil liming, (i) it not only increases soil pH but also improves the growth of the symbiotic bacteria and nodulation ability, (ii) stimulates the production of bacterial polysaccharides involved in root infection and the ability to fix atmospheric nitrogen (13,28,66) (Table 1). Soil nutrient deficiencies, especially phosphate and micronutrients (zinc, molybdenum, copper and cobalt) can limit nodulation and nitrogen fixation (27).

Table 1. Effect of liming of acid soil on the interaction *S. meliloti* – alfalfa in Córdoba (Argentina)

Treatments during bacterial growth		Lime dose	Number of	Shoot dry matter
pH	Ca (mM)	(t/ha)	nodules / plant	(mg/ plant)
7.0	0.14	0	1.00 ± 0.00a	4.23 ± 0.74a
7.0	0.14	1	4.50 ± 0.86a	6.36 ± 0.21b
5.5	0.14	0	0	3.08 ± 0.57a
5.5	0.14	1	3.50 ± 0.29b	6.43 ± 0.84 b

The actual pH values for unlimed and limed Córdoba soil were 5.5 and 6.4, respectively. Data are means ± SE of four independent determinations. Different letters in a column indicate significant differences ($P < 0.05$), according to the LSD test (13).

In certain situations, fungicides applied to soil or to alfalfa seeds can be detrimental for alfalfa re-establisment through growth inhibition of the symbiotic bacterium strains. Tu (65) assayed five fungicides (Benomyl, Captan, Maneb, Thiram and Zineb) on strains of *Rhizobium meliloti* in controled conditions. High fungicide levels suppressed nodulation of *R. meliloti* in alfalfa roots, when compared with no treated plants or plants treated with low levels of fungicides. Alfalfa plants grown from seeds inoculated with *R. meliloti* strains resistant to Thiram, grow better in presence of the fungicide (5.0 mg/g seed) than those inoculated with susceptible strains (52). Koopman *et al.* (40) showed that applications of herbicide chlorsulfuron at doses used in the field can also severely restrict alfalfa nodulation.

2.1.3. Mycorrhiza

Alfalfa associates with mycorrhizal fungi that provides a better nutrient uptake to the plant, especially phosphate and trace elements such as zinc, copper and molybdenum, providing better conditions for symbiotic bacteria nodulation and nitrogen fixation (27). Fungicides and fumigants used to control soil-borne pathogens also kill or inhibit mycorrhizal fungi with a negative impact on alfalfa yield (47). Even where mycorrhizal infection is not completely prevented by fungicide, the infection is unable to function and fails to enhance plant growth (1).

2.2. ABIOTIC FACTORS

Alfalfa improves the soil structure (60). However, alfalfa monoculture involves use of heavy machinery during soil preparation for sowing, fertilization and harvest operations. Traffic often causes soil compaction (22,56) (Table 2) with reduction in the water-use efficiency and infiltration rates (46,56), an increase in soil bulk density (44) and detrimental effects on soil porosity and aeration (56). These changes in soil physical properties negatively affect the ability of alfalfa root to explore the soil environment for water and nutrients.

Table 2. Dry matter yield (t/ha) and water use characteristics of alfalfa at Shafter (California), when soil was subjected to different levels of soil compaction before sowing

Year	Compaction treatment	Yield (t/ha)	Drainage (mm)	Evapotranspiration (mm)	Water use efficiency (kg /ha mm)	R^2
1986	LI (light)	16.5a	161	1115	18.0a	0.98
	MD (medium)	14.4b	68	1059	16.3a	0.97
	HV (heavy)	12.4c	<1	953	16.8a	0.96
	HVTR (heavy+traffic)	11.2c	<1	939	14.7a	0.97
1987	LI	32.2a	108	1412	24.1a	0.99
	MD	30.4ab	20	1363	23.3a	0.99
	HV	27.7b	<1	1283	23.7a	0.99
	HVTR	22.8c	<1	1299	19.1b	0.99
1988	LI	23.3a	466	1152	18.7	0.99
	MD	23.3ab	159	1283	16.4	0.99
	HV	23.7a	<1	1207	19.0	0.99
	HVTR	19.1b	<1	1301	12.5	0.99

Different letter within a row, within year, are significant ($P < 0.05$) according to LSD test (53).

Alfalfa is able to supply its own nitrogen through nitrogen fixing bacteria (41), so generally nitrogen fertilizers are required only during early establishment of seedlings. Long term alfalfa cultivation contributes to soil acidification (pH < 6.5) because large amounts of plant biomass are removed from the field each at harvest resulting in the major removal of basic cations (calcium, magnesium and potassium) and micronutrients (e.g. zinc) from the soil (23,72), hence, inorganic fertilizers are necessary to sustain yields (3,61). In acidic situations, some elements (e.g. aluminium and manganese) can reach

phytotoxic levels in the soil reducing root depth and branching and predisposing plants to drought injury (18,19).

2.3. ALFALFA AUTOTOXICITY

Autotoxicity occurs when a plant species releases chemical substances that inhibit or delay germination and/or growth of the same plant species. Alfalfa autotoxicity has been amply proved (35,36,37,49,50,69,70). Working on alfalfa stand re-establishment, Webster *et al.* (70) reported dwarfed, spindly and yellowish green plants with irregular brown lesions on the tap and lateral roots and a few ineffective nodules. Field and Greenhouse experiments indicated that neither macro- nor micronutrient deficiencies were responsible for the stunted alfalfa growth (69,70). Miller (49) reported low seedling population and second-year dry matter yield in continuous cropping of alfalfa, even though P and K deficiencies were corrected and seeds were treated with Captan to protect seedlings against fungal attack. Alfalfa seedlings are smaller, when grown in alfalfa sick soil compared to fallow soil, even though both soils were steam sterilized. Ground foliage or roots of alfalfa added to both soils significantly inhibited alfalfa seedling size and density (38).

2.3.1. Causative chemicals

Although several compounds have been isolated from alfalfa, but the causative chemicals of alfalfa autotoxicity is not been identified. Saponins were early suggested as the water-soluble "autotoxic principle" released from alfalfa roots (24). Residues from several alfalfa cultivars, were equally toxic to alfalfa seed germination, regardless of saponin contents. These saponins are involved in toxicity, but are not the only autotoxic agents in alfalfa (49). Phytochemical analysis of alfalfa aqueous extracts led to the identification of cinnamic acid and its derivatives (11,51,55). Further studies indicated that aqueous extracts from different plant parts did not equally affect germination, radicle length, or hypocotyl length. Radicle elongation was more sensitive to aqueous extracts than germination or hypocotyl length. Autotoxic effects of water extracts from alfalfa plant parts followed the order from more to less inhibitory to radicle length: leaf > seed > root > stem (7,8,10) (Table 3).

Table 3. Influence of aqueous extracts from alfalfa plant parts or soil extracts on the root length of 5-days old alfalfa seedlings (10)

Tissue	Concentration (g dry weight/100 ml)				LSD (0.05)
	3	6	9	12	
			Root length (cm)		
Leaf[a]	5.0	4.7	3.2	2.6	0.4
Stem[a]	6.6	5.7	5.6	4.9	0.5
Flower[b]	5.1	4.9	4.7	4.4	0.1
Seed[b]	5.3	5.1	3.7	3.0	0.1
Root[a]	6.6	4.9	3.4	2.2	0.3
Soil[a]	6.0	5.5	5.0	4.3	0.8
Control	7.7				
LSD (0.05)	0.4	0.4	0.3	0.5	

[a] Collected from plants in the vegetative growth stage. [b] Collected from plants in the reproductive stage.

Chon and Kim (7) determined the content of several phenolic compounds in alfalfa leaves, stems, roots and seeds. Aqueous extracts of the plant parts and the identified phenolic compounds were also assayed on alfalfa root elongation in laboratory assays. Leaves contained higher contents of allelochemicals (6.99 mg/g dry tissue) than the stems (4.26 mg/g dry tissue), roots (1.77 mg/g of dry tissue) and seeds (0.11 mg/g of dry tissue). Coumarin, *trans*-cinnamic acid and *o*-coumaric acid were the most inhibitory to alfalfa seedling growth and their highest contents were found in alfalfa leaves. Mixtures of five or more phenolic acids were more phytotoxic than their respective individual components, except for *trans*-cinnamic acid and coumarin, suggesting that alfalfa autotoxicity may be caused by interaction of many, yet uncharacterized compounds present in shoots (31). The isoflavonoid medicarpin, isolated from fresh alfalfa leaves, was also identified as possible autotoxin. It inhibited the alfalfa seedling growth at concentrations very close to those found in alfalfa soils, after extraction with ethanol or ethyl acetate (14). As can be concluded, attention on alfalfa autotoxicity has emphasized the identification and quantification of growth inhibitors. In fact, the phytotoxicity of plant leachate after soil incorporation depends on both their toxic and no toxic components and their microbial use as carbon source (59). For example, soil incorporation of a non toxic compound (e.g. glucose) or a toxic compound (e.g. methionine) together with a given concentration of *p*-coumaric acid, increases the phytotoxic activity of this acid respect to when incorporated alone (4,54,59). This is because soil microorganisms prefer the alternative toxic or non-toxic compounds as carbon source. As alfalfa leachates are complex organic mixtures, further studies on alfalfa growth inhibition should be done with mixtures of both toxic and no toxic organic alfalfa constituents in soil, to provide more realistic meaning of the possible participation of the identified phytotoxic compounds in alfalfa autotoxicity.

2.3.2. Ecophysiological aspects

Field and laboratory experiments suggest that alfalfa autotoxins may be released by leaching from mature plants or/and decomposition of plant residues (50). These compounds reduces seedling emergence mainly through inhibition of radicle elongation, with least effect on germination and shoot elongation (10,25,55). Persistance of alfalfa autotoxicity strongly depends on the soil texture and rainfall pattern interactions. Jennings and Nelson (35) simulated the leachate of alfalfa autotoxins by rainfall in both light-textured and heavy-textured soils. They found that a similar amount of alfalfa "autotoxic principle" in light-textured soil had greater influence on alfalfa seedling growth than in heavy-textured soil. Furthermore, the inhibitory activity was quickly eliminated from the light-textured soil, while only diluted in a heavy-textured soil. Failures in renewal of alfalfa plants through direct sowing over old stands could be due to a zone of autotoxic influence around the mature plants (37). An influence zone of 20-25 cm was determined around alfalfa dead and live mature plants, although it effectively do not separate autotoxicity from competition. Furthermore, soil autotoxin contents could depend on the growth stage of alfalfa plants. Soils collected from old alfalfa stands during the reproductive stage, inhibit alfalfa seedling growth more than those collected during the vegetative stage (9).

The yield of alfalfa plants, subjected to autotoxic stress did not increase beyond the first year after stand re-establishment (39,49). Jennings and Nelson (36) observed that roots of plants established after 12 month interval following the previous alfalfa stand,

had more predominant taproot and much less branching than those established after a 2 week interval (Fig. 1). They suggested that autotoxins were responsible for the morphological changes observed in the shorter interval assayed. Chon and Nelson (8) exposed alfalfa seed and seedlings of different ages to alfalfa leaf extracts. Roots from older seedlings had more branched roots and were more tolerant to the extracts than those from seeds or younger seedlings. Root branching would allow to older seedlings to share a dose of autotoxins among several root branches, diluting the autotoxic effect on root growth. In this way, seedling would escape from allelochemical phytotoxicity but would be less vigorous and more sensitive to stress competition (6,69). In laboratory studies, alfalfa leaf extracts reduced the number of root hairs per unit area by 46% and length of root hairs by 54%, compared to control (30). Furthermore, extract-treated alfalfa roots had larger-diameter cortical cells particularly at the periphery, thereby resulting in a greater bulging of the primary root than in distilled-water control. Hedge (29) proposed that the extract-treated roots could mobilize less carbohydrate reserves stored in the root cortex. In this way, an inhibition in the root energetic metabolism could lead to reduction in root elongation and hair formation. Anatomical studies on root tips from alfalfa seedlings treated with coumarin and aqueous leaf extracts, showed that roots were stunted and swollen (6). The inhibition in longitudinal root elongation was also associated with an increase in cell layers of the vascular cylinder and cortex (Fig. 2).

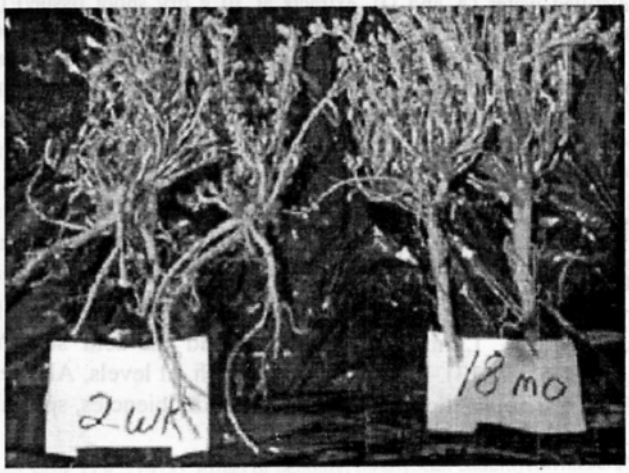

Figure 1. Effects of short (left) and long (right) fallow periods between ploughing and re-establishment of alfalfa following alfalfa on its root development (68).

When collected during the alfalfa reproductive stage, unsterilized soil was more inhibitory than sterilized soil suggesting role of soil microorganisms (9). Microorganisms could contribute to phytotoxicity through microbial transformation products of alfalfa components or synthesis, *de novo*, of microbial phytotoxins. On the other hand, alfalfa leachates and/or residue decomposition could modify soil chemical properties also causing growth inhibition (34). These ecophysiological aspects of alfalfa autotoxicity need further research.

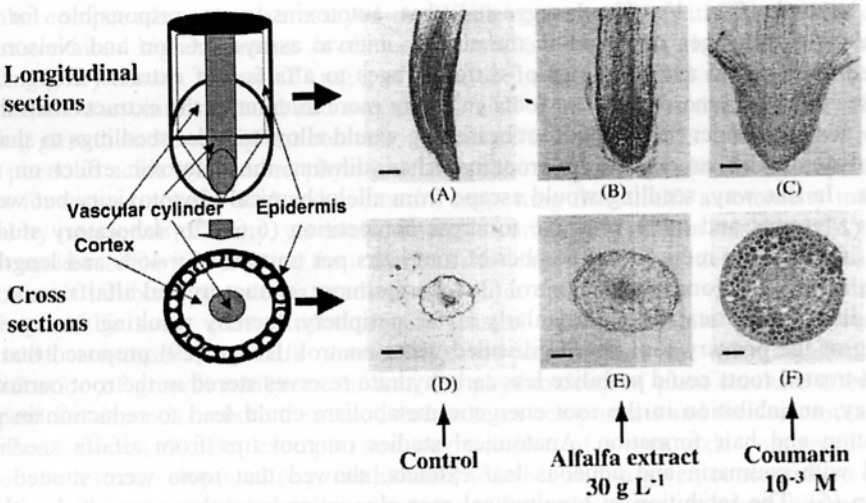

Figure 2. Cross (A, B, and C) and longitudinal sections (D, E, and F) through the root tips of alfalfa grown on distilled water (A and D), extracts at 30 g dry shoot tissue/l (B and E) and coumarin at 10^{-3} M (C and F) on filter paper for 6 days. Note the vascular cylinder, containing procambium and metaxylene, is surrounded by cortex and epidermis. Note also diameters of alfalfa roots treated were larger due to expanding vascular cylinder and cortex cell layers. Photograph is about 100 x. Cross sections (A, B, C) are made at 1 mm distance from the root tip (6).

3. ALFALFA SOIL SICKNESS CONTROL

3.1. SOIL PHYSICO-CHEMICAL PROPERTIES

Harvest traffic should be minimized to increase crop water use efficiency and reduce soil compaction (56). Liming is usually applied on acid soils under alfalfa monoculture to reach pH 6.5-7 (13). In acid soils with high Al levels, Al-tolerant cultivars are suggested (5). Fertilizer application to correct nutrient deficiencies, specially phosphate should be done (3).

3.2. SOIL-BORNE PESTS (INSECTS, NEMATODES, PATHOGENS)

Establishing disease-resistant cultivars is the most effective mean of managing most diseases to minimize the yield losses in alfalfa (42). Improvement of soil physical and chemical properties can substantially reduce the incidence of several soil-borne diseases. Incorporation of green manures in soil and crop rotations increases the yields and reduces the infestation of soil pathogens (71). Antibiotic-producing *Streptomyces* has been used to control *Phytophthora* and other soil-borne pathogens in alfalfa (73). Fallowing for one growing season and rotations with non-host crops successfully reduces soil nematode population (63). Changes in cutting intervals of alfalfa stand, control of alternative host weed and chemical control reduces the incidence of soil-borne insects on alfalfa yields (60).

3.3. MANAGEMENT PRACTICES

Direct sowing on the existing alfalfa stand is not ideal practice to increase alfalfa yield, because too few seedlings usually emerge and survive the existing competitive and autotoxic environment (35). Successful establishment require a time interval between killing or ploughing the old alfalfa stand and the new alfalfa seed sowing. This interval allows the autotoxic substances to dissipate from the soil and may vary according to soil type, seedbed preparation/planting practices and weather. No-till sowing methods based on killing old alfalfa stand should delay 12 month before the next seed sowing, since the lack of tillage prevents an accelerated breakdown of the previous alfalfa crop's residue (12,36). Conventional tillage may reduce this interval to only 6 months by ploughing under the alfalfa in the fall before re-establishing it in the spring. The presence of light-textured soil and high winter rainfall may reduce the interval due to faster leaching of autotoxins. Rotation with crops such as corn or tobacco for at least one year will improve the chances of successful stand establishment, especially in no-till systems. Miller (49) suggested that the autotoxic problem in alfalfa might be solved by selecting new cultivars that do not produce several allelochemicals or those that are resistant to these compounds. Although alfalfa cultivars showed variable autotoxic susceptibility (6,10), yet no cultivars are currently recommended to overcome alfalfa autotoxicity.

4. CONCLUSIONS

Alfalfa soil sickness entails modifications in physico-chemical and biological soil properties that are detrimental to root growth. The provided evidences indicate that alfalfa allelochemicals are also important component of its soil sickness. Autotoxins seem to change root morphology of alfalfa seedlings, reducing the competitive ability of the plant. Autotoxicity may be the result of complex interactions among several compounds. Interaction studies among toxic and non-toxic components from alfalfa leachates and determination of soil microbial utilization of compound mixtures from this origin should be done. Possible microbial transformation of plant phytotoxic compounds or synthesis *de novo* of microbial toxins need also a deeper study. This will provide a better comprehension of the role of soil microorganisms in alfalfa autotoxicity.

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