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The allelopathic potential of alfalfa root medicagenic acid glycosides and their fate in soil environments

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Summary The allelopathic effect of alfalfa (*Medicago media* Pers.) and red clover (*Trifolium pratense* L.) root saponins on winter wheat seedling growth and the fate of these chemicals in soil environments were studied. Seed germination, seedling and test fungus growth were suppressed by water and by alcohol extracts of alfalfa roots, and by crude saponins of alfalfa roots, indicating that medicagenic acid glycosides are the inhibitor. Powdered alfalfa roots inhibited wheat seedling growth when added to sand. At concentrations as low as 0.25% (w/w) the root system was completely destroyed whereas seedling shoots suffered little damage. Red clover roots caused some wheat growth inhibition when incorporated to sand, but their effect was much lower than in the alfalfa root treatment.

Soil textures had a significant influence on the inhibitory effect of alfalfa roots. The inhibition of seedling growth was more pronounced on light than on heavy soils. This was attributed to the higher sorption of inhibitors by heavy soils.

Incubation of alfalfa roots mixed into loose sand, coarse sand, loamy sand and clay loam for a period of 0–8 days resulted in decreased toxicity to both *T. viride* and wheat seedlings. This decrease occurred more quickly in heavier soils than in loose sand, due to the hydrolysis of glycosides by soil microorganisms. Soil microbes were capable of detoxifying medicagenic acid glycosides by partial hydrolysis of sugar chain to aglycone.

These findings illustrate the importance of medicagenic acid glycosides as an inhibitor of wheat seedling growth, and of their fate in different soil environments.

Introduction

Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) are perennial legumes giving good yields of high protein pasture. Furthermore, they contribute large amounts of organic matter to the soil, increase water infiltration rate and improve soil structure. Alfalfa donates twice as much organic dry matter as does red clover, however succeeding crop establishment and yield is frequently much poorer after alfalfa than after red clover or other legume crops[13,15,17]. It is commonly believed that the cause of this phenomenon lies in soil moisture and nutrient depletion, or in weed overgrowth in alfalfa stands[9]. But there is some evidence that phytotoxic substances in alfalfa are involved in this adverse effect[13,15].

Some authors have shown that alfalfa contains water soluble substances toxic to other plants and that alfalfa roots are richer in these

chemicals than the tops[6,9,10,12,21,23]. A 20% (w/w) incorporation of ground alfalfa tops or roots to clay soil resulted in inhibition of cotton seed emergence by 50 and 65% respectively[25].

Water extracts of alfalfa contain a wide spectrum of chemicals including saponins. This group of compounds, especially those of alfalfa tops, are quite well known because of their antinutritional character. It has also been suggested that they play an important role in plant succession[13,15]. Their inhibitory effect on seed germination has been reported[7,14,16,18,20].

Alfalfa root saponins can be separated by chemical methods into cholesterol precipitable and nonprecipitable fractions[5,18]. Only the precipitable fraction, consisting of a mixture of medicagenic acid glycosides, exhibited an inhibitory effect both on *Trichoderma viride* and wheat seedling growth[18]. The nonprecipitable fraction as well as red clover root saponins had little or no effect on seedling growth[18,19].

The quantity of medicagenic acid glycosides in alfalfa roots was found to be around 6% of root dry matter[18]. Accepting the thesis that alfalfa stands can leave in the soil up to 10 T/ha of root dry matter, one can calculate that about 600 kg/ha of highly active allelochemicals are incorporated. This is quite a large amount, and considering the possibility of ununiform saponin distribution in the soil volume they may create hazardous conditions for subsequent crops. Apparently, nothing is known about the behavior and the fate of medicagenic acid glycosides in the soil environment. For this reason laboratory and greenhouse experiments were set up to provide some data on this problem.

In some experiments where alfalfa roots or their extracts were tested for allelopathic potential, red clover roots and their appropriate extracts were used for comparison.

Materials and methods

Experiment 1. The effect of alfalfa and red clover root extracts on Trichoderma viride *and wheat seedling growth*

Roots of alfalfa and red clover were collected from field grown plants in the fall of 1982. Air-dried roots were finely powdered and 1 g samples were extracted by soiling with water or sequentially with methylene chloride, ethanol and water. The methylene chloride and alcohol extracts were evaporated *in vacuo* until solvents were completely removed. The dry residues were dissolved in such a volume of distilled water as to make concentrations equal to that of water extracts (5 ml is equivalent to 50 mg of root dry matter). The same concentrations of water solutions of crude saponins[18,19] were prepared. All these extracts and saponin solutions were used for *T. viride* and wheat seedling growth biotests. Bioassays with *T. viride* were performed according to the method previously described[8]. Wheat seed germination tests were performed in Petri dishes covered with filter paper. Twenty seeds were placed on each plate and the appropriate solution (5 ml) was added. Dishes with distilled water were used as a control. Seeds were allowed to germinate and grow

temperature in natural light for 7 days, at which time germination and seedling length were determined. All combinations were done in 5 replicates.

Experiment 2. Effects of alfalfa and red clover root concentrations on seedling growth in sand

Concentrations of 0; 0.25; 0.50 and 1% (w/w) of finely powdered alfalfa or red clover roots and of alfalfa or red clover roots after ethanol extraction were mixed into silica sand. This soil was placed in 9 × 9 cm plastic pots (400 cm^3). Twenty winter wheat cv. Grana seeds were planted in each pot. Seeded pots were set in a greenhouse with an average daytime temperature of 20°C, and average nighttime temperature of 15°C. Soil moisture was maintained at 60% of full soil water capacity. Seedlings were allowed to grow 14 days, then the length of the roots and shoots were measured. This process was completed in 4 trials.

Experiment 3. Effects of red clover and alfalfa roots on wheat seedling growth in different soil textures

Alfalfa and red clover roots were mixed at the rate of 1% (w/w) into different soil types: loose sand, coarse sand, loamy sand and heavy loam. The data on the physical properties of these soils is given Table 1. The experiment was then set up in the same manner as Experiment 2. Soils with no additives were established as a control. Beginning on the 7th day after planting, the number and percentage of emerging wheat shoots were calculated. The length of roots and shoots were measured after 21 days.

Experiment 4. The relationship between alfalfa root decomposition and toxicity

The same types of soils as in Experiment 3 were used. For each sample 45 g of soil was mixed with 0.5 g of alfalfa root powder and watered to 60% of full water capacity. Each sample was incubated at room temperature for a period of 0–8 days. At the end of incubation, 100 ml of distilled water was added and the soil was shaken for 4 hours. Then samples were centrifuged (30 min. 2500 g) and 10 ml of supernatant was added to *T. viride* growth medium. The diameter of the fungus colony was measured as described above. Every treatment for each soil was repeated four times. For wheat seedling growth trials, alfalfa roots were mixed into coarse sand soil at the rate of 1% (w/w) and incubated at room temperature for 0–8 days. Than wheat was planted, and after 21 days of growth the length of shoots and roots was measured. Soil with no alfalfa roots mixed in was used as a control.

Experiment 5. The interaction of alfalfa root saponins and soil born microorganisms

The objective was to determine the probable fate of cholesterol precipitable alfalfa root saponins[18] in soil conditions. For that purpose 4 kg of coarse sandy soil was extracted by shaking for 4 h with 4 l of distilled water. 300 mg of saponins were added to 3 l of this extract and the solution was thoroughly mixed until saponins were completely dissolved. 1 l of this solution was separated and immediately the saponins were extracted into n-butanol. The butanol was evaporated *in vacuo* and the dry residue dissolved in 100 ml of distilled water. This was used for wheat seedling growth Petri dish tests in the amount of 5 ml per plate. The remaining 2 l of the original 3 l of saponin solution was allowed to stand at room temperature for 2 days. After 2 days 1 l was separated and saponins extracted with butanol as above. The last 1 l was left for incubation for another 2 days and

Table 1. Physicochemical data of used soils

Soil	Particle size distribution %			CEC meq in 100 g of soil	Organic carbon mg/100 g soil
	1–0.1	0.1–0.02	0.02		
Loose sand	99	1	0	0.85	79.20
Coarse sand	86	6	8	5.44	639.22
Loamy sand	57	25	18	6.15	883.29
Heavy sand	16	17	67	21.17	2254.23

then also extracted in the same manner. Seedling growth tests were performed in the same way as in Experiment 1.

In order to check if incubation in soil water extracts is connected with any qualitative changes in saponin fraction, TLC of butanol extracts was done after 0, 2 and 4 days of incubation. Thin layer chromatography was performed on silica Gel 60 plates using ethyl acetate-acetic acid-water (7 : 2 : 2) as a developing system. Saponins were visualized with Liebermann-Burchard reagent. The cholesterol non-precipitable alfalfa root saponins[18] and red clover root saponins[19] were tested for qualitative changes in the same manner.

The butanol extracts were also analysed for the presence of medicagenic acid. Plates were developed with A_1: petroleum ether-acetic acid-water (7 : 2 : 1) and A_2: benzene-methanol (92 : 8) and visualized in the same manner as saponins. Colour development of visualized spots were observed in natural and UV light.

Experiment 6. Evaluation of saponin sorption in soils

The ability of the four used soils to sorb saponins were evaluated according to previously described method[11]. For that purpose 20 mg of cholesterol precipitable saponin fraction was dissolved in 50 ml of distilled water. 10 g of soil was added. This mixture was shaken for 6 h and centrifuged. The supernatant was collected and each soil sample washed three times with 20 ml of water and again centrifuged. All supernatants were combined and saponins extracted into butanol. Then the butanol was evaporated and dry residue dissolved in 10 ml of distilled water. 1 ml of this solution was added to growth medium of *T. viride* and by measuring the diameter of the fungus colony, the saponin content in solution was evaluated. Sorption value was estimated by subtracting the saponin content of the final solution from the saponin content of the solution at the beginning of the procedure. Analyses for every soil were performed four times.

Results

Trichoderma viride growth was strongly inhibited by water and ethanol extracts and crude saponins from alfalfa roots (Table 2). The same extracts had an extreme negative influence on wheat seedling growth. The roots of the seedlings grown in the presence of these extracts were completely damaged. At first browning of root tips was observed, fol-

Table 2. Seed germination, wheat seedling and *T. viride* growth inhibition caused by red clover and alfalfa root extracts

Plant part	Extract	Germination	Growth inhibition %		
			T. viride	Wheat	
				Shoot	Root
Before extraction with ethanol					
Alfalfa root	Water	60	72	67	91
	Ethanol	60	73	70	91
	Crude saponins	55	67	63	86
Clover root	Water	95	7	32	50
	Ethanol	85	5	5	7
	Crude saponins	95	0	0	0
After extraction with ethanol					
Alfalfa root	Water	86	0	30	60
Clover root	Water	90	0	28	40

lowed by decay of whole root system. Shoot growth was also considerably inhibited, but the extent of this inhibition was much lower than that for the root system. Furthermore those extracts strongly inhibited wheat seed germination. Approximately 40% of sown seeds did not germinate under this treatment.

Other extracts has small or no influence on fungus and seedling growth. Water extract from red clover roots retarded both root and shoot growth but to a much lesser extent than alfalfa. The level of this retardance was comparable to that of water extract from red clover roots and alfalfa roots earlier extracted with ethanol. In addition a linear correlation was established between *T. viride* and shoot ($r = 0.95$) and root ($r = 0.93$) growth, and between fungus growth and seed germination ($r = 0.99$).

Similar results to those obtained with extracts were obtained when powdered red clover and alfalfa roots were added to silica sand in which wheat was planted (Table 3). Alfalfa roots added to the sand caused great retardation of wheat shoot growth and complete decay of seedling roots. The level of shoots and root inhibition was dependent on the rate of alfalfa root addition, but it should be pointed out that at a rate of 0.25% seedling shoots did not suffer great damage whereas the root system was completely destroyed. Red clover roots without or after extraction with alcohol had a very small adverse effect on wheat, comparable to the effect of alfalfa roots extracted previously with alcohol. In these cases there was little or no correlation between root concentration and wheat seedling growth.

The soil texture had a significant influence both on seedling emergence (Fig. 1) and on the growth of wheat shoots and roots (Table 4), 1% addition of alfalfa to loose sandy soil caused significant delay of seedling

Table 3. The effect of rate of alfalfa and red clover roots incorporated into silica sand on wheat seedling growth inhibition (%)

Kind of residue	Residue rates (%)							
	0	0.25	0.5	1.0	0	0.25	0.5	1.0
	Wheat root growth inhibition (%)				Wheat shoot growth inhibition (%)			
Before extraction with ethanol								
Alfalfa root	0a	71b	78c	87d	0a	28b	42c	50d
Clover root	0a	30b	29b	28b	0a	23c	15b	7b
After extraction with ethanol								
Alfalfa root	0a	23b	40d	36c	0a	9b	24c	0a
Clover root	0a	0a	15b	10b	0a	5a	15b	19b

Values within a row followed by different letters (separately for roots and shoots) differ (p 0.05).

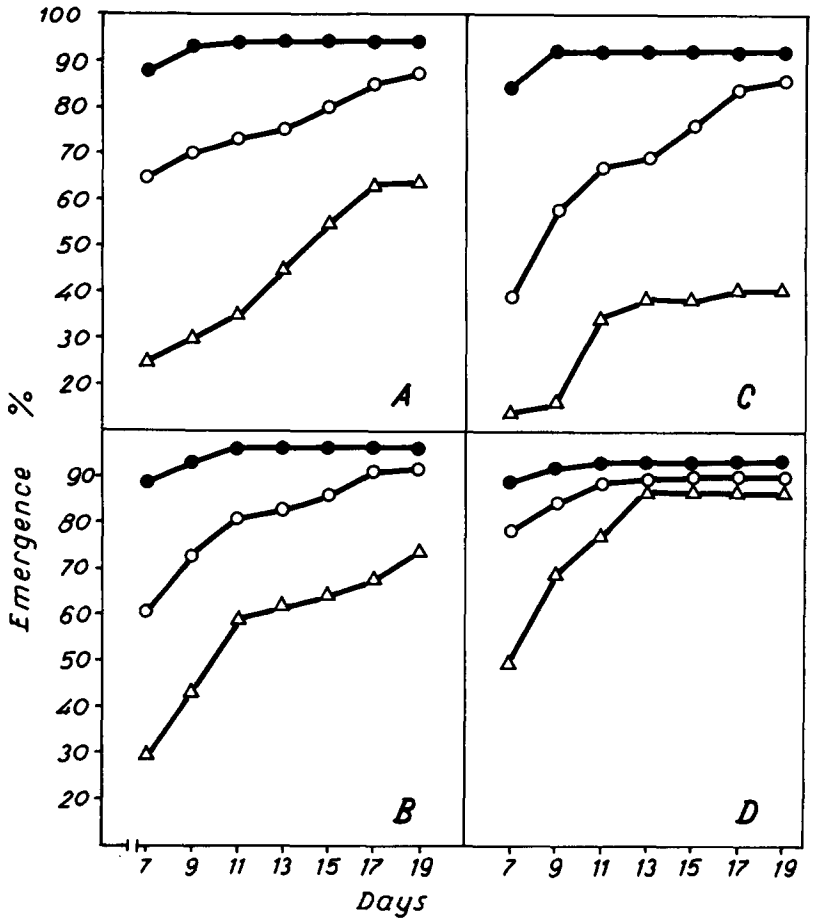

Fig. 1. Effect of alfalfa and red clover root incorporation on wheat seedling emergence in differently textured soils. Symbols: **A** — loose sand, **B** — coarse sand, **C** — loamy sand, **D** — heavy loam, ●—● control, ○—○ clover roots added, △—△ alfalfa roots added.

emergence and approximately a 40% decrease in plants sprouted. Seedling growth conditions in this treatment were much worse than in the same soil with red clover roots or no roots mixed in. But in heavier soils the detrimental effect of adding alfalfa roots was weaker, and in the heavy loam soil was very close to the other soil treatments. An exception was noted in loamy sandy soil, where seedling growth conditions were even worse than in loose sand with the same additions. Seedling emergence under alfalfa root treatment in this soil was very low: 19 days after planting only 40% of the seedlings emerged. Emergence on the soil treated with red clover roots was also delayed, but after 19 days approached the level of the control.

In addition to effects on seed emergence added alfalfa roots caused

Table 4. The effect of alfalfa and red clover root incorporated into soils with different texture on wheat seedling growth

Soil type	Alfalfa		Red clover	
	Wheat seedling growth inhibition (%)			
	Shoot	Root	Shoot	Root
Loose sand	52a	86c	26b	33b
Coarse sand	41c	53b	25b	30b
Loamy sand	41c	47b	24b	31b
Clay loam	27b	17a	15a	7a

Values within a column followed by different letters differ (p 0.05).

significant variations in wheat shoot and root growth on the four tested soils, but the addition of red clover roots did not. Alfalfa root additions to soil greatly inhibited seedling shoot and root growth on loose sandy soil and moderately inhibited growth in coarse sandy and loamy sandy soils. Inhibition was very low in heavy loam and was comparable to that of red clover root treatment.

Incubation of alfalfa roots mixed at the rate of 1% (w/w) into the four soils for a period of 0–8 days caused rapid decrease of toxicity as measured by *T. viride* test (Fig. 2). Water extracts from loose sandy soil with alfalfa roots mixed in were still very toxic to fungus after 8 days of

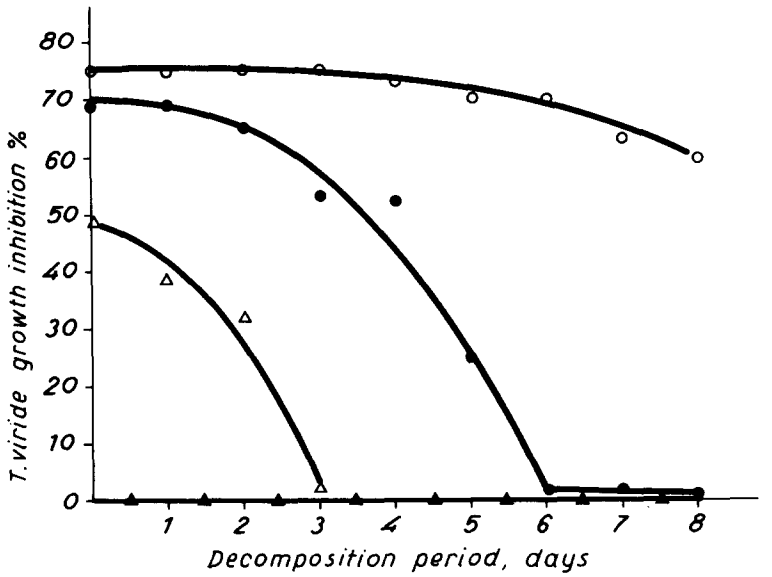

Fig. 2. *T. viride* growth inhibition as affected by water extracts of alfalfa roots decomposed in differently textured soils. Symbols: o—o loose sand, ●—● coarse sand, △—△ loamy sand, ▲—▲ heavy loam.

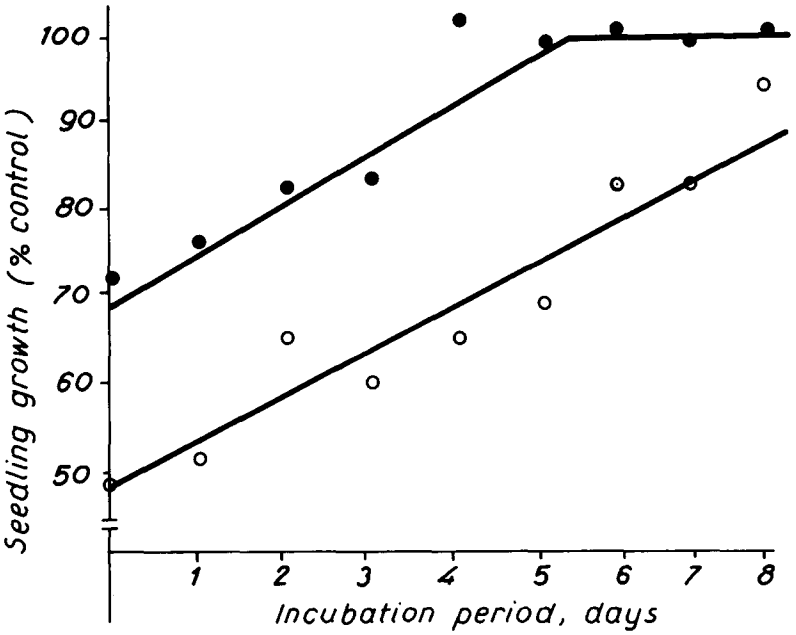

Fig. 3. Wheat seedling growth in response to alfalfa roots incubated in coarse sand. Symbols: o—o seedling roots, ●—● seedling shoots.

incubation whereas extracts from coarse sand after 6 days and from loamy sand after 3 days were completely not toxic. Water extracts from heavy loam even at the beginning (*i.e.* very soon after alfalfa roots were mixed in) caused no inhibition of fungus growth. It is worth noticing that water extracts from loamy sand at the beginning of incubation inhibited fungus growth to a lesser extent than similar extracts from lighter soils. The incubation of alfalfa roots also had influence on wheat seedling growth on coarse sandy soil (Fig. 3). A linear correlation between time of incubation and seedling shoots ($r = 0.89$) and roots ($r = 0.92$) length was found. In this experiment, loss of toxicity was very fast: seedling shoots did not suffer after 4 days, roots after 7 days of incubation.

Alfalfa root saponins, like alfalfa roots, lost toxicity when incubated in water extract from this soil (Table 5). After 2 days of incubation no difference was found between seedlings grown in 0.1% cholesterol precipitable saponin solution and those grown in incubated saponin. However after 4 days seedling growth inhibition dropped sharply, from 51% to 20% for shoots and from 80% to 45% for roots.

This loss of allelopathic potential by saponins incubated in soil water extract is closely related to qualitative changes occurring in the saponin fraction as a result of incubation (Fig. 4). After two days a new com-

Table 5. The effect of incubation in soil water extract on saponin toxicity to wheat seedlings

Days of incubation	Wheat seedling growth inhibition (%)	
	Shoot	Root
0	51	81
2	59	80
4	20	45

pound, earlier not present in the saponin fraction $R_f = 0.59$ appeared. This had the same green colour under UV light after development with Liebermann-Burchard reagent as did most of the spots on TLC. At the same time, the intensity of the spot with $R_f = 0.75$ and that of the spot $R_f = 0.54$ slightly decreased. The intensity of spots with R_f ranging between 0.15–0.42 also slightly lessened. After another 2 days of incubation the TLC picture of the fraction changed very dramatically. All spots with $R_f = 0.15$–0.42 completely disappeared, leaving only spots having higher R_f values. During a similar incubation period red clover root saponins and alfalfa cholesterol non-precipitable saponins were not effected as proved by TLC test.

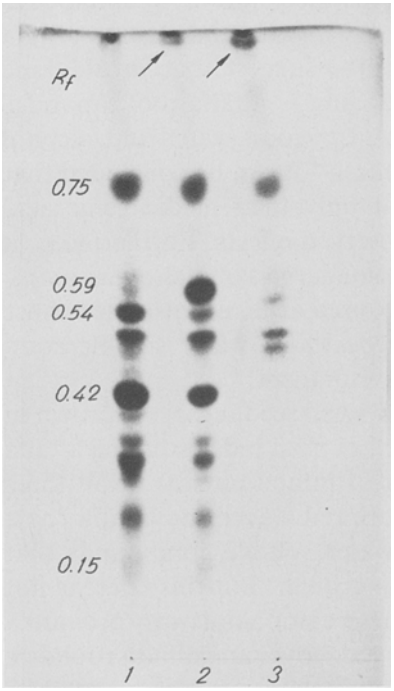

Fig. 4. Thin layer chromatography picture of medicagenic acid glycosides fraction before (**1**), after two (**2**) and after four (**3**) days of incubation in soil water extracts. *Arrows* show the medicagenic acid spots.

Saponins after 0, 2 and 4 days of incubation were also tested for aglycone presence by the TLC method. The TLC revealed the presence of a small amount of medicagenic acid in the sample after 2 days of incubation and a considerable amount of 4 days (spots at the front of solvent system marked with arrows, Fig. 4). Its identity was confirmed by chromatography and co-chromatography with an authentic medicagenic acid sample. Both R_f values of 0.18 (in the A_1 developing system) and 0.,1 (A_2) as well as observed green colour under UV light were identical with results for medicagenic acid and were in a good agreement with data published elsewhere[18].

Cholesterol precipitable alfalfa root saponins were sorbed in the soil sorption complex. The amount of saponins sorbed in loose sand, coarse sand, loamy sand and heavy loam was respectively 0; 6; 24 and 78 mg per 100 g of the soil.

Discussion

A comparison of wheat germination, seedling and *T. viride* growth in the first experiment allowed the designation of three treatments for conditions where inhibitory effects were the most severe. Simultaneous retardation of fungus and seedling growth by water and alcohol alfalfa root extracts as well as by crude saponins suggest that in all these treatments the allelopathic agents are the same — alfalfa root saponins. But considering the allelopathic agent's very good water and alcohol solubility and the results of previous research[18] it can be concluded that not the whole alfalfa root saponins but only their medicagenic acid glycoside fraction was responsible for observed effects. Furthermore, it can be assumed that in experiments requring large samples of saponins, finely powdered roots can be successfully used as a substitute for those saponins, because medicagenic acid glycosides as water soluble compounds can readily be released to the environment.

Some growth retardation was observed when seedlings were grown in water extracts from red clover roots as well as from red clover and alfalfa roots after alcohol extraction. The level of inhibition was in all three cases almost the same, suggesting that both red clover and alfalfa roots contain, besides saponins, some other water soluble compounds that may have an inhibitory effect on seedling growth. But this effect is not as severe as that of alfalfa saponins. These compounds can provide a kind of background in experiments where red clover and alfalfa roots are used for soil amendment.

This background was observed when red clover or alfalfa and red clover roots after ethanol extraction were mixed into silica sand in the rate study experiment. In these treatments there was no correlation

between the amount of roots in the soil and seedling growth. But a correlation was very clear when alfalfa roots were incorporated. Wheat seedling roots suffered severe damage at alfalfa root concentrations as low as 0.25%. This is approximately the concentration that would occur in field conditions if alfalfa root dry matter were uniformly distributed in the whole soil volume. But in ploughed alfalfa stands, like in the other stands, uniform distribution does not exist[22], and high local concentrations due to uneven root distribution during ploughing are highly probable. For this reason in some of the presented experiments 1% root additions were applied.

Soil texture can seriously modify the expression of allelopathic effects due to the presence of medicagenic acid glycosides. The highest inhibitory effect appeared when alfalfa roots were mixed into loose sand. Effects were lower when heavier soils were tested. On clay loamy soils with alfalfa roots mixed in, wheat emergence and growth was comparable to that under treatments with red clover or with no roots incorporated. This finding fully supports previous literature data concerning soil texture effects[2,3,24].

An exception from this general trend was found in our study of treatments of loam sandy soil. In this soil, admixture of alfalfa roots caused the lowest emergence and seedling growth rates of any soil. An explanation of this phenomenon lies in the structure of this particular soil. As a result of surface watering, this soil forms a very cohesive, hard surface layer, impermeable to oxygen. This caused oxygen deficiency that together with alfalfa root saponins has a synergistic effect both on emergence and wheat seedling growth. This observation greatly supports a view of some authors[24] that a combination of different allelochemicals, or of allelochemicals and the environment can often result in synergistic action. In some unfavorable growing conditions an expression of allelopathy may be much stronger than in experiments where growth environment parameters are usually set at optimum.

The modification of saponin activity in differently textured soils was closely related to the ability of particular soils to bind medicagenic acid glycosides to its sorption complex. It was clearly shown that there was no sorption in loose sand and that sorption increases in heavier soils. The heavy loam soil sorbed as much as 78 mg of saponins in 100 g sample. This level of sorption may explain the small expression of allelopathy in this soil. Considering that the alfalfa root medicagenic acid glycoside content is around 6% of dry matter[18], it is easy to calculate that introducing 1% (w/w) of alfalfa roots into the soil we really introduce 60 mg of glycosides, a slightly less saponins than heavy loam soils can bind. Naturally admixing 1% of alfalfa roots, a wide spectrum of water soluble organic compounds are introduced to the soil, which can be competitive

with saponins in occupying soil-sorption centers. In this way some saponins stay unbound and are available to the receiver plants and thus some inhibitory effects were observed.

This finding may suggest a more general conclusion, that medicagenic acid glycosides express their allelopathic activity to winter wheat as long as they stay in soil solution in an unbound form, and the sorption is one of the processes of their deactivation. Thus it becomes very important to trace the fate of unbound saponins in soil. The incubation of alfalfa roots in different textured soils caused a rapid decrease of saponin toxicity both to *T. viride* and to wheat seedling growth. This is in good agreement with results that have been published elsewhere[10]. But the velocity of decrease was higher in heavier soils than in loose sand. For heavy clay soil no toxicity to *T. viride* was observed even without incubation. This finding gives further support to the conclusion that all saponins are being sorbed in this soil.

The decrease of activity during incubation was tightly connected to soil microorganism, mainly fungus (unpublished data) activity. The mechanism of detoxification depends on gradual hydrolysis of longer sugar chain glycosides that are readily soluble in water, into simple more hydrophobic glycosides and finally into pure medicagenic acid that is not soluble in water. This process was in most cases quite fast but was much slower or completely arrested if higher saponin concentrations were used in the experiment (unpublished data). This is a second process, next to the sorption, in which soils can modify the allelopathic effect of alfalfa root saponins.

At the same time red clover root saponins and cholesterol non-precipitable alfalfa root saponin fractions that show no activity to *T. viride* remained untouched when added to soil water extracts.

The presented manner of glycoside detoxification by microorganisms fully corresponds to literature data on detoxification of steroidal alkaloids, compounds related to triterpene saponins. *Septoria licopersici*, the leaf-infecting fungal parasite of tomatoes, was found to detoxify tomatine by hydrolyzing one glucose unit from the tomatine molecule[1]. Another tomato parasite *Fusarium oxysporum F. sp. Lycopersici* is able to overcome the toxicity of tomatine by producing an inducible extracellular enzyme which cleaves the glycoalkaloid into tetrasaccharide lycortetraose and tomatidine[4]. Thus, for some fungi sensitive to the presence of allelochemicals, it becomes necessary to induce mechanisms to detoxify those compounds. Our findings on medicagenic acid glycoside detoxification give more evidence to support this view. This also provides a possible explanation of why cholesterol non-precipitable alfalfa root saponins and red clover root saponins remained untouched *in vitro* experiments. They were less soluble than medicagenic acid gly-

cosides in water and/or were not toxic to microorganisms, and thus were not immediately attacked.

These findings give some evidence that in optimal growing conditions the expression of allelopathy or phytotoxicity in the soil environment can be attributed to the presence of active substance in unbound form, but that these substances are very quickly attacked by soil microorganisms. The chemicals bound in soil particles or organic matter seem to be little or not active unless they are unavailable to the plant receiver. In this light, the thesis presented by some authors[15], that in soils with alfalfa monocultures, older alfalfa stands accumulate more saponins thus causing higher expression of phytotoxicity than in younger stands is very doubtful. It seems more probable that this higher toxicity is due to the fact that often bigger amounts of alfalfa roots are left in the soil by older alfalfa stands. During decomposition these roots release large amounts of saponins that can effect following crop seedling growth. In some soil areas, the concentration of saponins may be extremely high. The rate of decomposition at these concentrations can be slow and following crop seedling can suffer severe damage, as observed in field conditions[13].

The fate of bound saponins however remain unclear and needs more research.

The presented data partially explain the reason for detrimental effect of alfalfa stands on following crops and for the very specific role of alfalfa root medicagenic acid glycosides. However, these results can not be fully extrapolated to field conditions where the environment can modify the mechanisms established in laboratory tests.

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