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SOME WEED SPECIES AFFECTING SOYBEAN NODULATION AND NODULE FUNCTION

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ABSTRACT

Experiments aimed at examining the effect of aqueous extracts of three weed species on nodulation and nodule function of soybean cv. Melrose have been carried out at the Laboratory of Plant Physiology and Biotechnology, Department of Agronomy and Soil Science, University of New England, Australia. Aqueous extracts of fresh weed material (*Amaranthus powellii*, *Cyperus rotundus* and *Paspalum dilatatum*) at the concentration of 10% (w/v) were added to a minus-nitrogen Hoagland's nutrient solution in which the soybean plants were grown with 14 hours day length, day and night temperatures of 28 and 20°C, respectively, light intensity of 790 μmol/m^2/s, and the relative humidity of 65%. The plants were kept for three weeks prior to the measurement of activity of nitrogenase enzyme and ammonium content of the root nodules. All weed extracts tested resulted in impairment of soybean nodulation and nodule function as indicated by reduced activity of nitrogenase enzyme activity (acetylene reduction assay - ARA). Although amaranth extract was most inhibitory to the nitrogenase enzyme activity, it was less inhibitory than nutgrass extract in reducing the total ammonium content of the soybean root nodules.

Keywords: allelopathy, soybean, nodulation, nutgrass, amaranth, paspalum

INTRODUCTION

Similar to other crops, weed interference in soybean cultivation is common and causes serious problems. Coble *et al.* (1981) reported that weeds competed directly with soybean for light, nutrients, and moisture, and might interfere indirectly through the production and release of allelochemicals that inhibited crop growth. Allelochemicals refer to secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals)' (Narwal, 1999). In addition, weeds often serve as hosts for insects and plant pathogens that attack soybean, and the physical presence of weeds in the crop may interfere with other pest control. These constraints make weeds more significant than diseases and insects to soybean farmers (Eyherabide, 2002).

Some stress conditions decreased nodule activity in soybeans such as temperature and light-dark period (Schweitzer and Harper, 1980), salt stress (Serraj *et al.*, 1998), water stress (Müller *et al.*, 1996), and heavy metals (Balestrasse *et al.*, 2003). Allelochemicals, causing abiotic stress, may affect leguminous nitrogen fixation through different mechanisms such as interference with legume hosts, the microsymbionts, and the nodulation process. For example, 10% (w/v) aqueous extracts of phalaris (*Phalaris aquatica*) reduced 60% nodule formation in subclover (*Trifolium subterraneum*) and lotus (*Lotus pedunculatus*) (Halsall *et al.*, 1995). However, the authors argued that reduced nodulation was a secondary effect of the allelochemicals following the reduced growth in roots that retarded root hair development. Similarly, redroot pigweed extract at 20 mg/mL or lower severely inhibited growth, and completely suppressed nodulation in soybean (Mallik and Watson, 1998), demonstrating the consistent effect of allelochemicals on nodulation even at low concentrations. It was speculated that these results were an indication of water solubility of the allelochemicals in the weed residues.

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Zimdahl (1980) reported that a 10% loss of agricultural production could be attributed to the competitive effects of weeds, in spite of intensive weed control in most agricultural systems. Soybean yield losses of 50 to 90% are common for soybean grown in natural weed populations (Coble *et al.*, 1981), and this yield loss has become serious due to the slow growth of the crop at early stages (Andrade, 1995), highlighting the significance of weed-free competition in soybean.

Little is known about nutgrass (*Cyperus rotundus*) allelopathy on soybean nodulation and nitrogenase enzyme activities. Similarly, effects of Powell's amaranth (*Amaranthus powellii*) and paspalum (*Paspalum dilatatum*) extracts on soybean nodulation remain unclear. Therefore, there is a need to study the allelopathic effects of amaranth, nutgrass and paspalum extracts on soybean nodulation to better understand the weed extracts mode of action on soybean growth reduction. Research reported here was aimed at studying the effect of those weed aqueous extract on nodulation and nodule function of soybean cv. Melrose.

MATERIALS AND METHODS

The experiments were conducted at the Laboratory of Plant Physiology and Biotechnology, Department of Agronomy and Soil Science, University of New England, Australia from January to April 2004. A completely randomised block design with 5 replicates was used to study the effects of weed extract on soybean growth and nodulation. The 4 experimental treatments included 10% (v/v of solution) of amaranth, nutgrass or paspalum extracts, and the control group containing no weed extract. Soybean cv. Melrose seeds were surface sterilised using NaOCl and ethanol solution before rinsed with sterile distilled water. The seeds were then inoculated with Nitrogerin 100, (No. CB 1809) at a rate of 0.3 g of inoculant per 100 soybean seeds for 30 minutes at room temperature. The seeds were germinated in sterile sand, and were kept for 7 days in a glasshouse with an average temperature range of 25 and 32°C under the ambient light regime.

One seven-day-old soybean seedling was transferred into an individual 1 L jar filled with modified, minus-nitrogen Hoagland's nutrient solution (Schweitzer and Harper, 1980). Cotton wool was wrapped around the hypocotyl before insertion into the centre of the jar lid to hold the seedling in place. To prevent the effect of light to the roots, the jar was wrapped with aluminium foil. The solution was aerated with a continuous flow of air bubbles using a fish-tank pump. Fresh nutrient solution plus the weed extracts, according to treatments, was added to the jars as needed, and was renewed every week during the study period to ensure that sufficient ions and unoxidised irons were available. The plants were kept in a growth cabinet (Thermoline Plant Growth Cabinet™) which was set for a day length of 14 hours, and day and night temperatures were 28 and 20°C, respectively. The light intensity was 790 $\mu mol/m^2/s$ and the relative humidity was 65%.

Three weeks after transfer to the nutrient solution, the acetylene reduction assay (ARA) was carried out to measure nitrogenase enzyme activity (Hardy *et al.*, 1973). Plants were removed from the nutrient solution and nodulated roots were blotted dry with paper towel followed by detaching the roots from shoots at the cotyledonary node. The roots from each plant were then quickly enclosed in a 1 L jar followed by an injection of 10% acetylene through a rubber septum assembled on the lid. Prior to acetylene injection, 10% of the air volume was removed from the jar and replaced by the same amount of acetylene. The roots were incubated at room temperature for 30 minutes. The jar was gently shaken intermittently every 10 minutes to allow good contact between the root nodules and the acetylene. After incubation, 500 μL samples of gas were withdrawn from each jar using a hypodermic syringe and were injected into a gas chromatography (GC) to measure the amount of ethylene released by the root nodules. A gas chromatography with a hydrogen flame-ionization detector, using Helium as carrier gas, was used in this assay. The GC column was 30 m long and 320 μm wide. The column temperature was 50°C. The injector and detector temperatures were 220 and 300°C, respectively. The nitrogenase activity is expressed as μmoles C_2H_4 produced/plant/hour. At the end of the nitrogenase assay, the nodules were detached from roots and were dried in an air-forced oven at 70°C for 48 hours.

For measuring the nodule function through measuring ammonium content, the following procedures were implemented. Root nodules were

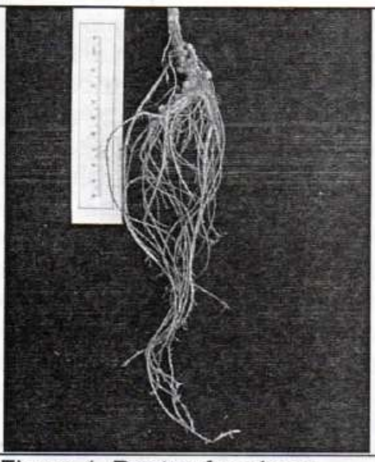

Figure 4. Roots of soybean cv. Melrose grown in modified Hoagland's solution with 10% (w/v) nutgrass extract. (Ruler is 10 cm)

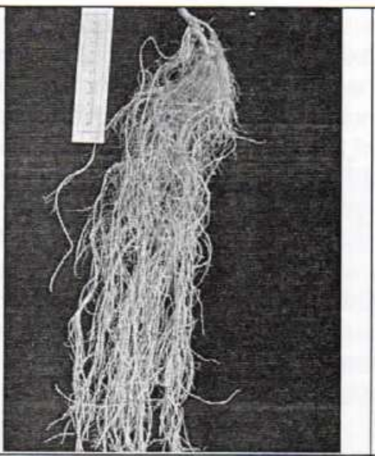

Figure 5. Roots of soybean cv. Melrose grown modified Hoagland's solution lacking the weed extracts (Ruler is 10 cm)

Figure 6. A closer look at the roots of soybean cv. Melrose grown in modified Hoagland's solution (Note the smaller nodules along the roots systems that were not observed in the other treatments)

Figure 4 shows the adverse effects of nutgrass extract on soybean root growth. Roots from this group were less developed and had a very small proportion of root hairs, suggesting root growth inhibition was accompanied by changes in root appearance. In contrast, soybean roots from the control group developed well (Figure 5) and produced smaller nodules along the lateral roots that did not occur at the weed-extract treatment groups (Figure 6).

Weed extracts markedly increased total ammonium in the root nodules of soybean cv. Melrose ($P<0.001$), and nutgrass extract resulted in the greatest increase ($P<0.001$) in total ammonium (Figure 7). Amaranth and paspalum extracts increased total ammonium but the increase was not highly significant compared to the control group ($P=0.02$ and 0.04, respectively), indicating that interference in ammonium accumulation in root nodules is one of the allelopathic mechanisms of the weed extracts in soybean nodulation.

The present experiment confirmed that all weed extracts reduced both nodule function (nitrogenase activity, ARA) (Figure 1) and nodulation (Figure 2 and Figure 3). However, the mechanism through which the weed extracts inhibited soybean nitrogen fixation remains unclear. The nitrogenase enzyme system consists of two component proteins, the iron (Fe-) protein and the molybdenum-iron (MoFe-) protein. Both are responsible for the ATP dependent reduction of N_2 from the atmosphere (Van Kammen, 1995). Since the reduction of N_2 requires large amounts of energy which is generated by oxidative phosphorylation, there is a high demand for O_2 in nodules. Respiration involves the oxidation of NADH, coupled to the phosphorylation of ADP which then generates ATP. Therefore, there is a strong link between respiration and nitrogenase enzyme activity. The overall reaction of biological nitrogen fixation (Van Kammen, 1995) from which we can see that nitrogenase enzyme activity requires large amounts of energy (ATP) is described below.

$$N_2 + 8H^+ + 8e^- + 16\,MgATP \xrightarrow{\text{nitrogenase}} 2NH_3 + H_2 + 16MgADP + 16P_i$$

Source: Van Kammen (1995)

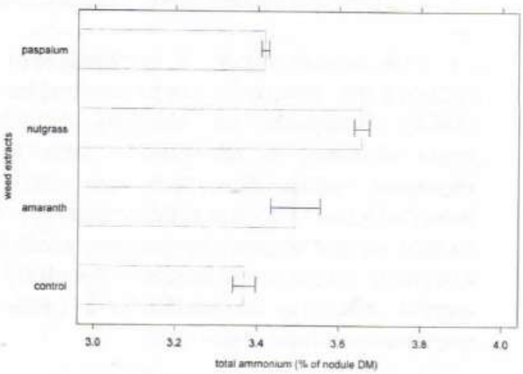

Figure 7. Total ammonium (% of nodule dry matter) from root nodules of soybean cv. Melrose at different aqueous weed extracts with concentration of 10% (w/v).

Allelochemical effects on respiratory activity have been reported in corn (Koeppe, 1972; Abrahim *et al.*, 2000; Abrahim *et al.*, 2003a), soybean (Penuelas *et al.*, 1996; Jose and Gillespie, 1998; Sert *et al.*, 1998; Abrahim *et al.*, 2003b), onion root tips (Kupidlowska *et al.*, 1994), mycorrhizal fungi (Boufalis and Pellissier, 1994), and yeast mitochondria (Einhellig, 1995 and papers therein). Chaniago (2004) demonstrated that amaranth, nutgrass or paspalum extracts reduced respiratory activity of germinating soybean. Reduced respiration reflected growth reduction due to less respiration-generated energy, which may indirectly lead to reduced nitrogenase enzyme activity.

Minchin *et al.* (1981) proposed that N_2 fixation and its conversion to organic N compounds is an energy intensive process which may require as much as 25% of legume's net photosynthate. An adequate supply of energy by photosynthesis is required for efficient nodule initiation and development (Fransisco Jr and Harper, 1995; Schultze and Kondorosi, 1998). Therefore, reduced soybean biomass may lead to less photosynthate supply to the nodules (Walsh, 1995). Amaranth and nutgrass extracts significantly lowered the net assimilation rate (NAR) of soybean (Chaniago, 2004), reflecting the decreased capacity of shoots to supply photosynthate to the nodules. Therefore, it is suggested that weed extract effects on nitrogenase enzyme is a secondary effect.

Weed extracts may enhance the mechanism through which soybean nodule number is regulated. One of the mechanisms is known as autoregulation or feedback inhibition (Fransisco Jr and Harper, 1995) through which the inhibition of further nodule formation is regulated by existing or developing nodules. This is in accordance with Figure 3 that shows nodule number in the control group was significantly higher than that of the weed-extract-treated groups. Soybean from the control group had, in addition to the large nodules at the crown region of the root, large amount of medium and small size nodules (≤ 3 mm in diameter) in the lateral roots (Figure 6). These nodules did not appear to be regulated by the feedback inhibition mechanism.

The autoregulation controlling root nodule numbers acts systematically and the autoregulatory signals originate from the shoot (Kosslak and Bohlool, 1984). However, in addition to autoregulation, reduced nodule number was perhaps a combined effect between allelochemicals and soybean preference to form the N-fixing symbiosis.

Reduced root growth and nodulation may result from the indirect effect of weed extracts through changes in plant internal nutrient status, which resulted from modified growth environment. Soybeans were grown in nutrient solution supplemented with weed extracts. Roots that were exposed to the nutgrass extract were poorly developed and had fewer lateral roots with significantly fewer root hairs (Figure 4). It is possible that nodulation inhibition was a reflection of this effect. This finding agrees with work reported by Halsall *et al.* (1995), that residue extracts of phalaris retarded root hair development in subterranean clover as well as root length and numbers. Allelochemicals may interfere with root meristematic processes and result in impaired cell division (Vaughan and Ord, 1990), which may reduce root growth.

The negative allelopathic effects of weed extracts, especially amaranth and nutgrass, were more pronounced in root biomass. Roots and the rhizosphere is the primary site where allelochemicals can continuously be supplied by donor plant roots, where crucial chemical and biological conversion take place, and where allelochemicals enter the target plant (Tang *et al.*, 1989). Although the system used in this study differed to field condition, soybean roots were directly in contact with weed extracts and therefore directly absorbed the extracts.

Weed extracts affected soybean nodulation through reduced nitrogenase activity and ammonium assimilation. Amaranth extract resulted in the lowest nitrogenase activity, although its effect on ammonium was less pronounced than that of nutgrass extract, reflecting the complex mechanisms involved in nitrogen fixation. Ammonium is toxic to cells and must be rapidly assimilated. This is achieved by the concerted action of two highly regulated pathways, glutamine synthetase (GS) and glutamate synthase (GOGAT). Both pathways result in the synthesis of glutamine which is the donor for the biosynthesis of major nitrogen-containing compounds including amino acids, nucleotides, and chlorophylls (Loulakakis *et al.*, 1994; Coruzzi and Last, 2000; Lancien *et al.*, 2000; and Nkoa *et al.*, 2003). Therefore, any interference in the metabolic pathways of ammonium assimilation may interfere with other physiological processes. Reduced chlorophyll content and plant biomass reflected the indirect effects of weed extracts on photosynthesis through chlorophyll synthesis reduction, due possibly to a decrease in glutamine assimilated from ammonium.

High ammonium content in root nodules reflected the imbalance of inorganic nitrogen. Fixation and assimilation of inorganic nitrogen, in the form of ammonia, into carbon skeletons to produce amino acids is one of the most important biochemical processes in plants (Lancien *et al.*, 2000 and Balestrasse *et al.*, 2003). For the GS/GOGAT cycle to work, N metabolism must interact with C metabolism, since GS activity requires energy in the form of ATP, and the GOGAT uses C skeletons and reductant in the form of 2-oxoglutarate and reduced ferredoxin or NADH, respectively (Lancien *et al.*, 2000).

However, how weed extracts inhibited ammonium assimilation was not studied further in this research. Interference in ammonium assimilation may be explained by analysing enzymes involved in the process such as glutamate dehydrogenase, glutamine synthetase, and aspartate aminotransferase. An increase in nodule total ammonium from the weed-extract-treated groups may be one of the allelopathic mechanisms of the weed extracts on soybean nodulation.

CONCLUSIONS AND SUGGETIONS

Amaranth, nutgrass, and paspalum extracts reduced the nodulation and nitrogenase activities (ARA) of soybean cv. Melrose. Amaranth was most inhibitory to nitrogenase enzyme activity. However, amaranth extract was less inhibitory than nutgrass extract in total ammonium. Since N_2 fixation is a complex mechanism involving many enzymes, future work requires the study of weed extract influence on GS/GOGAT cycle and the enzymes involved in the cycle.

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