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Noncompetitive Effects of Morning Glory

on Growth of Soybeans (TITLE)

ΒY

D.R. La Bonte

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY CHARLESTON, ILLINOIS



I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING THIS PART OF THE GRADUATE DEGREE CITED ABOVE



NONCOMPETITIVE EFFECTS OF MORNING GLORY ON GROWTH OF SOYBEANS

D.R. La Bonte and R.L. Darding Eastern Illinois Universiy

ABSTRACT

Competitive effects of weed infestations in soybean fields can severely reduce yields. Noncompetitive factors (allelopathy) may also be involved. Soybeans (<u>Glycine max</u> (L.) Merrill, Asgrow .A 3127 mid group 3) show a marked reduction in growth due to root exudation, leaf leachate and total plant extracts from white-flowered morning glory (<u>Ipomoea lacunosa L.</u>).

INTRODUCTION

Morning glory (<u>Ipomoea hederacea</u> (L.) Jacq. and <u>I purpurea</u> (L.) Roth.) infestations of soybean (<u>Glycine max</u> (L.) Merrill) fields can reduce growth and development significantly (10,22). Field experiments involving weed infestations typically illustrate the total effects a weed has on a crop. This reduction is commonly attributed to competition for water, sunlight, and minerals. In certain situations, noncompetitive (allelopathic) factors should be considered to more fully explain the ecophysiological situation. Allelopathy is a term describing a plant which exudes phytotoxic chemicals into the environment which can inhibit the growth and development of other species (6).

Early investigations dealt with ecological situations in which certain succession patterns could be more fully explained in light of allelopathy (3,7,11,15,23). Agricultural situations also exhibit allelopathy between various crop and weed species (2,8,9,16,18,19,20).

These phytotoxins belong to a group of secondary plant compounds that include simple phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, cyanogenic glycosides, and glucosinolates (14). The phytotoxins can either be volatile or water soluble. Volatile phytotoxins from certain shrubs have been shown to have an effect on annual grasses and forbes in drier habitats (7), while water soluble-phytotoxins are more prominent in wetter habitats (4).

The various sources of water soluble phytotoxins include leachates from leaves (1,3,15,20,23), exudates of roots (1,2,3,11,15,17,23), and accumulated organic matter (1,2,8,13,15,19,23).

The nature of this investigation was to examine the possible' effects of water soluble phytotoxins contained in morning glories on soybean growth.

Literature Review

Plants carry on metabolic processes which produce toxic wastes like all other forms of life. They avoid autotoxicity either by compartmentalization of compounds that could be acted upon by enzymes to produce toxins or by converting toxins to inert compounds. A substantial amount of toxin is released into the environment by leaching, root exudations, or volatilization (6).

Leaching is a passive process involving predominately the leaves. All common inorganic and organic nutrients, amino acids, organic acids, simple sugars, and various carbohydrates are found in leachates (5). Young leaves tend not to leach as much as older leaves, because of their more hydrophobic nature. This aids in preventing nutrient deficiencies. Leaching varies depending on the degree of pubescence, injury, temperature, and nature of the cuticle.

Leaching nutrients might appear as a wasteful process. large trees, though, can act as miners in obtaining nutrients from deep in the soil that are inaccessible for most plants and later leaching these nutrients to the topsoil. Calcium is a nutrient, that once transported and deposited, does not move basipetally out of the leaves. Mature leaves leach calcium, making it available for root transport to younger leaves of the same plant or other plants (20).

Roots also exude a wide variety of compounds: sugars, peptides, enzymes, amino acids, vitamins, organic acids, and miscellaneous compounds. The specific mechanism has not been thoroughly investigated (17).

Higher plants which exude phytotoxic chemicals into the environment can inhibit the growth and development of other species and, thus, would have a definite selective advantage. But this is a secondary factor. The primary one is to avoid autotoxicity (6). Allelopathy refers to this inhibition by a higher plant of one species on another species (14).

Phytotoxic chemicals belong to a group of secondary plant compounds that include simple phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, cyanogenic glycosides, and glucosinolates (14). The phytotoxins can be volatile or water soluble (7).

Volatile phytotoxins are characteristic of drier habitats.

<u>Salvia leucophylla</u> Greene, white-leaved sage, and <u>Salvia melifera</u> Greene, black sage, release several volatile compounds, cineole and camphor, which inhibit the growth of annual grasses and forbes. The compounds are thought to enter through the cuticular layer of the mesophyll cells and then enter the protoplasm by plasmodesmata to inhibit some enzyme mechanism (7).

• Water soluble phytotoxins are characteristic of wetter habitats(4). In stands of Sporobolus pyramidatus (Lam.) Hitch., dropseed grass, elimination or reduction of growth of several associated species <u>Cynodon dactylon</u> (L.) Pers. and <u>Buchloe dactyloides</u> (Nutt.) Engelm. was noticed. Experiments showed that <u>S</u>. <u>pyramidatus</u> supplemented any competitive advantage it might have by foliage leaf leachates, root exudations, and decaying material. Eventually, <u>S</u>. <u>pyramidatus</u> eliminates itself by a build-up of its own toxins in the soil (15).

A higher plant may indirectly inhibit another higher plant, due to the intervention of an intermediate microorganism which produces the toxin.

Root residues from peach trees, when acted on by microorganisms, produce a compound, amygdalin, which is toxic to peach trees replanted in the same ground (13).

Farms that are subsurface tilled have an increase in accumulated organic matter and coupling this with above average rainfall, provides an ecologically favorable environment for growth of microorganisms. <u>Penicillium urticae</u> Bainer is such a microorganism and produces a phytotoxin, patulin, which causes reduced yields and abnormal growth of wheat (8).

Allelopathic Weeds

Soybean fields are invaded by a number of weedy species that adversely affect crop yields by competing for light, nutrients, and water. Allelopathy should also be considered, since many of the problem weeds exhibit allelopathy towards various species. Setaria faberii Herrm, giant foxtail, was allelopathic to Zda mays L. (Wf9 x M14), corn, by its root exudations and incorporated debris. The incorporated debris showed a delayed effect; possibly caused by the time necessary for microorganisms to break down the tissue to release the toxin or the microorganisms may alter the innocuous leachate into a more toxic form. Giant foxtail was also tested against <u>Glycine max</u> (S.) Merrill var <u>adelphi</u>, but no adverse affects were noted. Allelopathy, as the soybean experiment shows, is species specific (2).

Root debris experiments with giant foxtail supported the previous findings, but <u>Setaria glauca</u> (L.) Beauv, yellow foxtail, and <u>Digitaria sanguinalis</u> (L.) Scop., crabgrass, showed only slight effects against corn (19).

Giant foxtail was seeded in stands along with <u>Setaria viridis</u> var <u>robusta alba</u> Schreiber, robust white foxtail; <u>Setaria viridis</u> var.<u>major</u> (Gaud.) Posp., giant green foxtail; and <u>Setaria lutescens</u> (Weigel) Hubb., Yellow foxtail. Giant green foxtail, along with a small amount of yellow foxtail, dominated the stand in 4 years. The dominance occurred irrespective of the greater seedling vigor, more rapid growth rates and production of more seeds from robust purple and robust white (18).

Tuber extracts from <u>Cyperus esculentus</u> Ten. var <u>richt</u>, yellow nutsedge, inhibits oat coleoptile sections and germination of <u>Beta</u> <u>vulgaris L., Lotus corniculatus L., Lolium perenne L., Pisium sati-</u> <u>vum L., Trifolium repens L., Lactuca sativa L., and Lycopersicum</u> <u>esculentum Ludwig. P-hydroxybenzoic acid, vanillic acid, syringic</u> acid, ferulic acid, and p-coumaric acid were isolated and found inhibitory to oat coleoptiles at high concentrations, but are without effect at lower concentrations (20).

Hot water extracts of roots, stems, and rhizomes of <u>Agropyron</u> <u>repens</u> (L.) Beauv., quackgrass, reduced seedling lenghts of alfalfa by 65-80% as compared to controls. Germination was reduced 30-50% by leaf extracts, but only 5-15% for all others. Older leaf extracts had no greater toxic effects than 2-4 week old seedlings. Soil extracts

were also deleterious. Tests determined that leaves and rhizomes may have a common active compound, but the leaves may contain an additional one (9).

Extracts from roots and foliage of <u>Cirsium arvense</u> (L.) Scop., California thistle, inhibited germination of its own seeds and those of <u>Trifolium subterraneum</u> L. Extracts of <u>C. arvense</u> inhibited growth of its own seedlings, <u>Hordeum distichon</u> L., <u>T. subterranean</u>, <u>Lolium perenne</u> L., and <u>three annual thistles</u> <u>Silybum marianum</u> (L.) Gaertn., <u>Cirsium vulgare</u> (Savil) Ten., and <u>Carduus pycnocephalus</u> L.

The findings complement field phytosociological observations. Annual thistles are noteably inhibited, and to a proportionally greater extent, as the age of the California thistle fields increases. This suggests a build-up of phytotoxic metabolites in the soil Thus, the establishment of perennial ryegrass/subterranean clover pastures may be difficult in California thistle areas. Autotoxicity of California thistle is accentuated during dry, hot summers. Generally, biochemical inhibition is most likely to occur in dry situations (3).

<u>Sorgum halpense</u> L., Johnson grass, is inhibitory to many early invaders of abandoned fields. Seed germination and seedling growth are inhibited by leaf or rhizome extracts and decaying leaves or rhizomes. Johnson grass is also inhibitory to its own seeds and seedlings, but only slightly to <u>Aristida olignatha</u> Michx., which explains the eventual dominance of <u>A</u>. <u>olignatha</u> in the second stage. Chlorogenic acid, P-coumaric acid, and P-hydroxybenzaldehyde were the main plant inhibitors present in leaf and rhizome extracts (1).

<u>Helianthus annuus</u> L., common sunflower, is dominant in the first stage of succession in certain old fields. Its' phytotoxins are thought to differ for the various extracts of decaying leaves, poot exudations, leaf leachates, and soil collected from around <u>H</u>. <u>annuus</u> and associated species. An additive effect with each group of toxins is likely. Again, <u>Aristida olignatha</u> Michx. is not inhibited and eventually replaces <u>H</u>. <u>annuus</u> (23).

Digitaria sanguinalis (L.) Scop., crabgrass, is predominent in the first stage of certain old-field successions. Crabgrass is inhibitory to seed germination and seedling growth of associated species. Crabgrass is eventually eliminated by its own toxins and ones from <u>Sorghum halpense</u> L., <u>Helianthus annuus</u> L. and species of <u>Euphorbia Spp.</u>. Crabgrass is inhibitory to <u>Aristida olignatha</u> Michx. Seedling growth, but the eventual disappearance of crabgrass permits an invasion of <u>A</u>. <u>olignatha</u>. Inhibitory compounds identified from root exudations are chlorogenic acid, isochlorogenic acid, and sulfosalic acid (11).

Aquaeous extracts of <u>Asclepias</u> <u>syrica</u> L., common milkweed, inhibited growth of grain sorghum seedlings. A proportional reduction in dry weight occurred with continually greater extract concentrations. A proportional reduction in seed germination of grain sorghum and radishes occurred with greater concentrations of two unidentified phenolic compounds (16).

Soybean-Morning Glory Competition

<u>Ipomoea purpurea</u> (L.) Roth., tall morning glory, is competitive against <u>Glycine max</u> (L.) Merrill., soybeans, once it obtains enough size and/or leaf area to intercept light and absorb water and nutrients at a rate found equal to that of soybeans. Soybeans are more competitive until about 6-8 weeks after emergence of morning glory, then morning glory competition reduces soybean growth and development (10).

Morning glory infestations of soybeans can reduce yields by 12% with 1 morning glory for every 2 feet of row to 44% with 8 morning glory plants per foot row.

Growth of morning glory at various distances from soybeans showed no difference between a 12" band removed in rows and a 24" band removed between rows. Height, yield, harvest difficulties, and severe lodging occurred. Removal of morning glory every 2 weeks or after 8 weeks from date of planting permitted maximum soybean yields. When morning glory is allowed to grow longer than 8 weeks, soybeans never recover. Removal of morning glory before 6-8 weeks from date of planting permitted growth of new morning glory which reduced height, yield, and increased lodging.

No significant difference in effects resulted from either <u>Ipomoea</u>
<u>hederacea</u> (L.) Jacq., ivy-leaved morning glory, or <u>Ipomoea</u> <u>purpurea</u>
(L.) Roth. in all experiments (22).

Few experiments of species allelopathic against soybeans have been done, but various allelopathic chemicals: caffeic acid, chlorogenic acid, t-cinnamic acid, P- hydroxybenzaldehyde, 5-sulfosalicylic acid, vanillic and vanillin have been tested on effects of growth, photosynthesis, water relations, and chlorophyll content of 3 week old soybeans, <u>Glycine max</u> (L) Merrill 'Tracy'. Concentrations of 10⁻³M of caffeic, t-cinnamic, P-coumaric, ferulic, gallic, and vanillic acids significantly reduced dry matter production, leaf expantion, height, and leaf production. Also, a significant reduction occurred in net photosynthetic rate, chlorophyll content, and stomatal conductance of single, fully-expanded leaves (12).

SEED GERMINATION EXPERIMENT

Materials and Methods

Morning glory plants were grown under greenhouse conditions in 15 cm diameter plastic pots filled with clay loam soil. The photoperiod was extended for the morning glories to 13 hours with a 40 watt Duro-lite, Vita-lite fluorescent light. Average daily temperatures recorded with a hygro-thermograph ranged from highs of 76C to lows of 58C.

Seed Germination Experiment

A bioassay was performed to determine if bacterial action on morning glory extract significantly reduced toxins. The extract was prepared by taking upper plant parts (all material 1 cm above uppermost root) and roots of 6 week old whole, fresh morning glory plants and pulverizing each portion in a Waring blender at a -concentration of 30 grams/600ml of deionized water for 90 seconds. The respective extracts were then stirred for 4 hours before filtering with Whatman's No. 1 filter paper. One 25 ml portion of both extracts was immediately tested on separate petri plates lined with paper towels and each containing 55 soybean seeds. In conjunction, a control was set similarly except using deionized water. Half of the remaining portions were allowed to stand for 1 week in dilution tubes at 25C. The 1 week old extracts were then tested as above with a control. All soybean seeds were germinated in an incubator set at 25C for 72 hours before weighing radicles. pH readings were taken on all extracts.

SEED GERMINATION EXPERIMENT

Results

The results of a seed germination experiment for residual effects of morning glory toxins are presented in Figure 1. The data are presented as a percentage of the control. Results from seeds subjected to fresh morning glory root extract demonstrates a 11% decrease in germination and a 13% decrease in radicle weight. An 11% decrease in germination was also obtained from the upper plant part extract along with a 17% decrease in radicle weight. The effects of toxins on soybean seeds after allowing the extracts to stand for 1 week were not significantly different from the control.



TREATMENT

Figure 1 - Allelopathic influence of <u>Ipomoea</u> <u>lacunosa</u> root and upper plant part extracts on <u>Glycine max</u> germination (white bars) and radicle weight (black bars). Bars represent percentage of control after 72 hours of growth. (A) fresh morning glory root extract; (B) fresh morning glory upper plant part extract; (C) one week old extract; (D) one week old upper plant part extract. Significant difference at the .050 level.

TOTAL PLANT EXTRACT EXPERIMENT

Materials and Methods

The experiment was carried out under greenhouse conditions. Morning glory plants were grown in 15 cm diameter plastic pots filled with clay loam soil. Soybeans were grown in 8 cm plastic pots. The photoperiod was extended for the morning glories to 13 hours with a 40 watt Duro-lite, Vita-lite fluorescent light. Average daily temperatures recorded with a hygro-thermograph ranged from highs of 76C to lows of 58C. Average drying temperatures ranged from 78C to 58C.

Total Plant Extract Experiment

To substantiate any allelopathic effects, soybeans were treated with a morning glory extract. Pots containing 14 - 2 week old morning glory plants were grown concurrently with newly germinated soybeans for a 5 week test period. Entire morning glory plants were removed from the soil and placed in a Waring blender at a concentration of 30 grams/600ml of deionized water and blended for 90 seconds. The extract was slowly stirred using a magnetic stirrer for 6 hours at 230. This extract was then filtered using glass cloth. Twentyfive randomly selected pots, containing 1 soybean plant/pot, were each treated with 40ml portions of extract when the soil was visually dry (about every 3 days). The control was watered similarly, except with deionized water. Soybean plants were removed from the soil and allowed to dry under greenhouse conditions for 3 days before weighing roots and upper plant parts (all material 1 cm above uppermost root). Root nodules were also weighed.

TOTAL PLANT EXTRACT EXPERIMENT

Results

The results of the total plant extract experiment are presented in Figure 2. The data are presented as a percentage of the control. Results of soybean plants subjected to morning glory extract shows a 38% decrease in root weight, a 43% decrease in nodule weight, and no significant difference was noted for upper plant part weight. Soybeans involved in the test series showed no amount of drought stress.



TREATMENT

Figure 2 - Allelopathic influence of <u>Ipomoea lacunosa</u> total plant extract on <u>Glycine max</u>. Bars represent percentage of control growth after 5 weeks of treatment. (A) dry weight of roots; (B) dry weight of root nodules; (C) dry weight of upper plant parts (1 cm above highest root). *Significant difference from the control at the .050 level. **Significant difference from the control at the .010 level.

LEAF LEACHATE EXPERIMENT

Materials and Methods

The experiment was carried out under greenhouse conditions. Morning glory plants were grown in 15 cm diameter plastic pots filled with clay loam soil. Soybeans were grown in 8 cm plastic pots. The photoperiod was extended for the morning glories to 13 hours with a 40 watt Duro-lite, Vita-lite fluorescent light. Average temperatures recorded with a hygro-thermograph ranged from highs of 76C to lows of 58C. Average drying temperatures ranged from 78C to 58C.

Leaf Leachate Experiment

Three week old morning glory plants in 2 sets (6 pots/set, 10 plants/pot, and each plant about 30 cm tall) were grown concurrently with 25 randomly-selected, 1 week old soybean plants (1 plant/pot). The upper parts of 1 set of morning glory plants were submerged in 1 liter of deionized water for 1 hour/pot. The water was stirred during this time using a Corning magnetic stirrer. Each soybean plant was then treated with 40ml of the leachate when the soil was visually dry (about every 3 days) for a period of 5 weeks. The 2 sets of morning glory plants were alternated every other watering period. A control was similarly established using deionized water only. The soybean plants were removed from the soil and allowed to dry under greenhouse conditions for 3 days. The roots were then cut-off and weighed. Similarly. the upper plant parts were weighed (this constituted all plant . material 1 cm above uppermost root). Root nodules were also weighed.

LEAF LEACHATE EXPERIMENT

<u>Results</u>

The results of the leaf leachate experiment are presented in Figure 3. The data are presented as a percentage of the control. Results of soybean plants subjected to morning glory leachate show a 40% decrease in root weight, a 32% decrease in nodule weight, and no significant difference was noted for upper plant part weights.



TREATMENT

Figure 3 - Allelopathic influence of <u>Ipomoea lacunosa</u> leaf leachate on <u>Glycine max</u>. Bars represent percentage of control growth after 5 weeks of treatment. (A) dry weight of roots; (B) dry weight of root nodules; (C) dry weight of upper plant parts (1 cm above highest root). *Significant difference from the control at the .050 level. **Significant difference from the control at the .001 level.

ROOT EXUDATION EXPERIMENT

Materials and Methods

The experiment was carried out under greenhouse conditions. Plants were grown in 15 cm diameter plastic pots filled with clay loam soil. Average daily temperatures recorded with a hygro-thermograph ranged from highs of 102C to lows of 72C. Average drying temperatures ranged from 96C to 70C.

Doot Exudation Experiment

A drainage type watering arrangement of plants was employed to determine noncompetitive inhibitory effects of morning glory root exudations on soybeans. A pot containing morning glory plants was waterd until enough excess water drained out to saturate the soil of a soybean plant. Twenty-five pots containing 2 week old morning glory plants were thinned from 6-8 plants/pots to 2 plants/pot in conjunction with 25 pots of 1 week old soybean plants that were thinned from 2 plants/pot to 1 plant/pot. The test series consisted of 5 groups. Each group consisted of 5 morning glory pots and 5 soybean pots. Watering occurred when the soil was visually dry (about every 3 days). The testing period lasted for 5 weeks. The soybean plants were removed from the soil and allowed to dry under greenhouse conditions for 3 days. The roots of each series were then cut-off and weighed. Similarly, the upper plant parts were weighed (this constituted all plant material 1 cm above uppermost root). Root nodules were also weighed.

ROOT EXUDATION EXPERIMENT

Results

The results of a root exudation experiment for inhibitory effects of morning glory root exudations on soybeans are presented in Figure 4. The data are presented as a percentage of the control. Results show that the effects of morning glory produced a 22% decrease in root weight, a 31% decrease in the weight of root nodules, and a 14% decrease in upper plant part weight.



Figure 4 - Allelopathic influence of <u>Ipomoea lacunosa</u> root exudations on <u>Glycine max</u>. Bars represent percentage of control growth after 5 weeks of treatment. (A) dry weight of roots; (B) dry weight of root nodules; (C) dry weight of upper plant parts (1 cm above highest root). *Significant difference from the control at the .010 level. **Significant difference from the control at the .001 level.

DISCUSSION

Studies involving the adverse effects of weed infestations in a crop typically do not describe the nature of this plant-plant interaction (10,22). Although competitive factors are predominant in most situations, noncompetitive factors in some instances may contribute to reductions in growth and development of a crop. In order to better understand these interactions, the crop species must be grown separately from the weed to eliminate any competitive effects.

The data from the seed germination and the total extract experiment were used as preliminary investigative techniques to determine if any phytotoxins are present, the source of any phytotoxins and possible degradation of these phytotoxins.

The data from the seed germination experiment (Figure 1) show an 11% decrease in germination for both upper plant part and root extracts, and a greater than 10% decrease in radicle weight for seeds subjected to both extracts. The data suggests that phytotoxins are present in both upper plant parts and roots in essentially the same concentrations. Of interset is that no significant difference in germination and radicle weight existed in comparison to the control when these extracts were allowed to stand for 1 week. This suggests a possibility of auto-decomposition or that bacteria or fungi degrade the phytotoxin. Bacterial and fungal action on plant material are known to actually produce toxins (8,13).

The technique in this experiment minimizes error introduced into the data by physiological drought. The week old extract, minus the phytotoxin, did not decrease germination or radicle weight. pH was also monitored and no significant difference occurred.

The data from the total plant extract experiment (Figure 2) show a 38% decrease in root weight and a 43% decrease in nodule. weight. No difference was noted in upper plant part weight. This was belived to result from less than optimal sunlight conditions during the testing period.

This data suggests taht phytotoxins are operative in at least two of the parameters tested. No symptoms of physiological drought were observed.

The previous experiments do lend support that phytotoxins contained in morning glory inhibit growth and development of soybeans. The next two experiments were designed to more closely approximate an actual field situation.

Morning glories are a climbing vine that grows among soybean plants. Phytotoxins contained in leaves of morning glory could be leached and taken up directly by the above ground parts and/or the leachate is taken up through the root. The later is more likely. The data (Figure 3) show a 40% decrease in root weight and a 32% decrease in nodule weight. No difference was noted in upper plant part weight. This was belived to result from less than optimal sunlight conditions during the testing period. This data suggest that phytotoxins contained in the leachate and taken up by the roots are operative in at least two parameters.

The drainage type watering apparatus is a useful technique to examine phytotoxic exudates from roots (2). The data (Figure 4) show a 22% decrease in root weight, a 31% decrease in nodule weight, and a 14% decrease in upper plant part weight. All parameters suggest phytotoxins are exuded from roots of morning glory.

These greenhouse experiments reveal that phytotoxins from leaf leachate and root exudations do have inhibitory effects on soybeans and these noncompetitive influences may carry over into field situations. One important variable is that these phytotoxins may adversely effect the <u>Rhizobium</u> in the root nodules which in a secondary fashion may show up as inhibitory-like effects on soybean plants.

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