HOFFMANSEGGIA GLAUCA: SURVIVAL MECHANISMS AND POSSIBLE ALLELOPATHIC EFFECTS ON THREE DICOTS

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PREFACE

The legume, Hoffmanseggia glauca was studied anatomically to clarify interpretations of its structures. Experiments were also conducted with the underground structures as a source of aqueous extracts used in determination of allelopathic effects on selected dicots. Comparisons were made experimentally in an effort to explain inhibition of cotton growth as observed in the field. The data collected during the course of this study provided a basis for those comparisons. Difficulties in obtaining viable H. glauca propagules complicated work in the study. This project was undertaken to increase information leading to potential uses of these native plants as sources of chemically active substances for use in biocontrol of other plants.

I wish to express my sincere gratitude to the individuals who assisted me in this project and during my coursework at Oklahoma State University. For his steadfast support and encouragement in the completion of the research, I wish to thank Dr. James Ownby. I am also grateful and appreciative of my other committee members, Dr. Paul Richardson and Dr. Earl Mitchell in their advisement and support during my project.

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To my wife, Connie, who believed in my efforts and abilities and to my children for their patience; I thank you with all my heart. Many thanks to my parents, George and Pat, for their belief in my goals.

Special thanks go to the people of Oklahoma and Oklahoma State University who allow returning students the oppurtunity to improve their lives and contribute to improvement of their State. Above all, Praise goes to God for giving me the strength to complete this study.

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CHAPTER I

INTRODUCTION

There are many plants which have been named, yet little is known of their role in nature nor are there any uses. Many legumes are used or have been used by native populations for medicinal and other purposes. The current trend is to categorize and identify natural plant compounds which may be beneficial. Thus many useful compounds have been found recently.

The purpose of the present study was to examine the morphology of the hog potato (Hoffmanseggia glauca (Ort.)Eifert) incorporating microtechnique in an anatomical examination of the underground structures. Experiments were conducted with the underground storage structure of the hog potato to determine if inhibition effects could be shown on the growth of specific dicots. SDS-PAGE gels were run on the aqueous extract of the propagule to determine if proteins were present.

CHAPTER II

TAXONOMICAL REVIEW OF *HOFFMANSEGGIA GLAUCA*

Overview of Fabaceae

The family Fabaceae has 16,000 to 19,000 species worldwide. There are many species of economic importance. The family is comprised of three subfamilies: Mimosoideae, Caesalpiniodeae and Papilionoideae which together contain about 750 genera. Many of the genera and species have been taxonomically reclassified at different times although the differences between subfamilies are accepted. All subfamilies have members which may be trees, shrubs, woody vines, and annual or perennial herbs. Flowers in the Fabaceae are typically 5-parted and the fruit is a pod (legume) which may be dehiscent or indehiscent (Allen and Allen,1981).

Taxonomy of *Hoffmanseggia glauca*

Hoffmanseggia glauca (Ort.)Eifert [formerly *H. densiflora* (Benth.) *ex* A. Gray, *H. falcaria* Cav., *Larrea densiflora* (Gray)Britt., and sometimes included in *Caesalpinia*] is in the subfamily Caesalpinoideae and the tribe Eucaesalpineae. Common names for *H. glauca* include Hog Potato, Indian Rushpea, Mesquite Weed, Pignut, Rush Pea, Rat Sweet Potato and "Camote

de Raton"(Allen and Allen, 1981; Ball and Robbins,1935; Correll and Johnston,1970; Johnston,1988; McCoy,1987; Stubbendieck and Conard,1989; Waterfall,1979; Weise,1982).

The hog potato inhabits arid, alkaline regions of southwestern United States and northern Mexico (Stubbendieck and Conard,1989). It is an erect to slightly decumbent plant of 2-40 cm. The spreading habit of it's blue-green foliage is due to small diameter stems (1-2mm) and the angle from which the individual stem axes arise from nodes. The herbaceous stems arise from a caudex or from the apical ends of fleshy propagules. Leaves are odd-bipinnately compound, with 3-15 pinnae and 12-22 leaflets per pinnae. Glabrous leaflets are oblong to elliptic, 1-2mm across and 2-5 mm long. Petioles are equal to or longer than the rachis. Hairs and glands are present on petioles, rachis and petiolules. The primary pulvinus and petiolar sheath at the node of each leaflet account for the ability of leaves to fold leaflets together along the axis of the rachis. The inflorescence is a terminal raceme of up to 12 cm and is orange to dark burgundy in color. Peduncle, pedicel, sepal and petal surfaces are dotted with many hairs and stipitate glands. The peduncle is 1-9 cm with relatively short pedicels (<0.5 cm). Flowers have five petals (4-7.5mm) which are clawed and a brilliant yellow with red-speckling on proximal surfaces of uppermost petals. The corolla becomes orange-red upon senescence. Lower filaments of dark-burgundy stamens adhere to the base of the pistil. Sepals are recurved at maturation.

Fruit are flat, falcate legumes 0.5-3.5 cm long. Seeds are flat, ovate and brown.

RANGE AND SOIL TYPES

The Hog Potato (H. glauca) is a herbaceous, indeterminate, nonnodulating perennial legume which grows in the semi-arid to arid regions of North America. Personal sightings in Oklahoma include Tillman, Jackson and Harmon counties. It has been reported in Greer county by personnel at the OSU Research Station in Altus, Oklahoma. Most sites tended to be previously disturbed by man with the exception of three locations in which the hog potato was found growing in close proximity to Mesquite trees (Prosopis glandulosa) (McCoy,1976) on native prairie which had not been recently cultivated. A review of Soil Surveys of Oklahoma for these counties (Bailey and Graft,1961; Lamar and Rhodes,1974) reveal the soils to be reddish-brown silty clay loam to dark brown silty loam top soil. The subsoils are reddish-brown to red clay with a blocky structure and calcareous or soft to hard calcium carbonate concretions. All soils are alkaline to some degree. Soils are classified as Inceptisols, Mollisols and Vertisols (Bailey and Graft,1961; Lamar and Rhodes,1974).

Propagation of Hoffmanseggia glauca

As a plant of semi-arid regions, H. glauca is able to grow under severe conditions. It has the ability to flourish under highly disturbed circumstances. Recorded personal observations of the hog potato in southwest Oklahoma in 1992 have shown that the plant grows very well in some fields, bar ditches; in disturbed, gravelly areas along roadsides and even through the asphalt of the driving surface of a resurfaced state highway. My observations in the field suggest that the hog potato may be a pioneer species which is able to dominate in the initial stage of disturbance. Its biomass, however, seems to diminish when it is subjected to competition with other plants (particularly monocots) under "optimum" conditions. Sites in which Johnson grass (Sorghum halpense) and Bermudagrass (Cynodon dactylon) were growing in competition with H. glauca along roadsides and bar ditches revealed very sparse populations of hog potato. Castner,et al. (1989) demonstrated that hog potato dry weight was reduced by 54% in field tests in which it was grown in full season interference with cotton(Gossypium hirsutum,cv. Paymaster 404 and 145). In contrast, Allen and Allen(1981), describe H. glauca (H. densiflora) as a climax species which acts as a drought-resistant soil binder with little other use in dry, sandy clay and limestone regions.

Stubbendieck and Conard (1989) depict in their map of the range of the hog potato that the plant is on the edge of its range in Oklahoma. Observations in the field in southwest Oklahoma revealed primary patches of the plant to be along roadsides and ditches adjacent to cultivated fields. Examination of cultivator debris from a cotton field in Tillman County, Oklahoma revealed remnants of H. glauca.

H.glauca propagates itself under adverse conditions in four ways. Primarily it establishes new plants through the growth of lateral roots initiated from the underground propagules. These lateral roots may extend for as much as six feet through the soil before sending new shoots to the surface. If the rooted-runners are separated from the parent plant, the undisturbed portions will begin shoot tip formation within a few days at the apex of the runner. The second means of propagation is a tuber-like propagule which develops after a few months of floral growth. This propagule is found in the clayey subsoil, up to 39 inches underground (Hackett and Murray,1985). The propagule has a thickened periderm and can regenerate new shoot-tips and roots if all other plants parts are removed or after a period of disturbance. At maturation the propagule periderm develops a rough, dark appearance which differs from the light color of the immature epidermis of the developing structure. After about three months of propagule development, the propagule begins to develop a gummy covering around its periderm. Soil encasing the underground periderm differs in color and texture from adjacent soil, indicating

possible chemical secretion by the plant. This species of Hoffmanseggia is not known to have a nitrogen-fixing relationship with bacteria as do many legume species (Allen and Allen,1981). The third method of regeneration is by means of the above ground stems. Normally secondary growth and shoot axillary development does not occur immediately in undisturbed plants. However, if the foliar growth is removed the plant will develop secondary growth generated from axillary nodes. Castner and Murray(1985) reported that single propagules planted in steel cylinders produced an average of eight propagules 110 days after planting. The fourth method of establishing new plants is through seed. H. glauca is not a prolific seed producer. There seems to be difficulty in pollination of the plants observed in Oklahoma. Collections of seed pods from plants at several sites, including thriving patches from the Tillman cotton field and heavy populations along State Highway 6 near the Texas border in southwest Oklahoma showed few seeds per legume. Hackett and Murray (1987) found that lack of development or predation were principle factors resulting in non-viable seed. Of the viable seeds produced they found germination was approximately 94%. Seed germination is reduced by low temperature, high pH (>7) and salinity of 200mM concentration (Hackett and Murray,1985).

The growth habit of the plants varies from prostrate to erect in highly variable field conditions with only slight variation observable in growth chambers(Allen and Allen,1981; Castner and Murray,1985). Some foliar characteristics,

including shape, size, and number of leaflets per pinnae vary from site to site, but are similar within any site. Similarities within sites suggests that the primary means of propagation in Oklahoma is asexual through foliar establishment from propagules and propagule formation rather than new plants from seed production.

CHAPTER III

CLASSIFICATION OF THE UNDERGROUND STRUCTURES OF HOFFMANSEGGIA GLAUCA

Introduction

In any attempt to understand the mechanisms by which a plant survives, reproduces or interacts with other plants, the morphological structures of the plant must be recognized. Any effort to control a plant which is an agricultural problem must begin with comprehension of the plant's characteristics and structures. This is particularly true if a plant is to be controlled by the newer generation of growth-regulator type herbicides.

H. glauca is a legume which has not only been reclassified several times, but its underground structures have been referred to by different names. Ball and Robbins(1935), Wiese(1982) and Castner, et al.(1989) have all described the underground storage propagule as a tuber or tuberlike structure. An unpublished report (Castner and Murray,1985) which compared fixed, stained upper and lower transverse sections of the propagule has suggested a transitional nature for the structure. In addition, both the propagule and the underground runners appear to have the

ability to give rise to vegetative structures from cauline buds at the proximal terminus and apical meristem, respectively (Castner and Murray,1985). Occasionally propagules may give rise to secondary growth at sites adjacent to the feeder roots. As doubt exists about the structure of these underground propagules, an anatomical study if these structures was done.

Materials and Methods

Specimens of young and mature propagule (2X4mm and 11X20mm, respectively) as well as the terminal one cm of underground runners were collected in the field from actively growing H. glauca plants. They were fixed in formalin, propionic acid, ethyl alcohol mixture (FPA),(Johansen,1940) then dehydrated/infiltrated by use of ethyl alcohol and tertiary butyl alcohol before embedding in paraffin (Johansen,1940; O'Brien and McCully,1981). Staining was carried out according to Johannsen's Safranin and Fast Green Schedule (1940) omitting clove oil. An American-Optical Rotary Microtome was used to produce serial sections at 6-13 micrometers. Photography was performed using an American-Optical microscope with a tungsten light source with camera and a Nikon Labphoto with Nikon FX35WA camera. Film was Kodak 160T speed color slide film. Field photograpy used Kodak 400 speed color print film.

Results and Discussion

In the young propagule (Fig.1), the tetarch protoxylem develops metaxylem centripetally. The phloem develops in the region between the xylem poles separated by the cambial zone. The cambium eventually forms a continous band between phloem and xylem. Both xylem and phloem regions proliferate storage parenchyma cells. As expansion of the root continues, cambial initials around the vascular elements divide forming regions of secondary growth. Phloem and parenchyma cells develop centripetally to form a pith-like region in the center of the propagule. Presence of the central tetrarch cylinder with anastamosing strands of metaxylem readily identifies the structure as of root origin. In addition, leaf scales (or scale scars) have not been observed on the periderm of young propagules around sites of secondary growth. This strongly suggests a root origin for the propagule development.

Tertiary growth occurs throughout the propagule as it matures (Fig.2). Interior to the primary cambium occasional centripetal rows of metaxylem form and parenchyma continue to proliferate. Starch grains are readily evident in the parenchyma throughout the propagule. Exterior to the primary cambium and the phloem, parenchyma continues to develop anomalous secondary cambia to form a cortical-like region of storage cells containing starch grains (Fig.3) The cortex is bounded by a periderm of phellem, phellogen and phelloderm

(Fig.2 and 3). The storage root development and organization of the hog potato described above is similar to that described by Esau (1977) and Hayward (1938) for Ipomoea batatas (Convolvulaceae). Isodiametric cells which stain red with safranin suggesting lignification is present beneath the phelloderm in a nearly continous band (Fig.2 and 3). These cells may be brachyschlerids as described in Hayward(1938) for I. batatas suggesting that the periderm of H. glauca may be derived from the pericycle. Between the periderm and the isodiametric cells are dense deposits of an anomolous substance in cells and interstitial spaces (Fig. 3). These deposits are interspersed in the cells throughtout the propagule.

Adventitious roots of a small diameter arise from the periderm of fleshy storage roots which act as propagative organs when environmental factors are suitable. The diarch protostele of the very fine roots develops into a tetrarch protostele in roots developing secondary growth (Fig.5). The small adventitious roots possess a few large xylem elements in a tetrarch pattern with minimal phloem, fibers, rows of parenchyma between axial xylem and a single layer of pericycle. The phloem which begins as a few cells between xylem poles becomes a narrow region in the vascular cylinder of maturing absorptive roots. Medicago sativa (Fabaceae) has a similar secondary root development (Esau,1977).

Sections of the mature horizontal roots (Fig. 4) were often mishapen and appeared to be the result of poor sectioning. However, comparisons of sections infiltrated with plastic by C. Richardson (personal communication) showed there to be a lacunar region between the periderm and the stele possibly derived from a lysigenous cortex. Formation of periderm progresses very slowly and in only a few adventitious roots. These more or less horizotal roots with the periderm appear to be the primary means of vegetative shoot propagation which Castner and Murray(1985) reported in their study with the spread of hog potato in the field. In this study he reported an average of 129 secondary plants per plot($2m^2$) in 1984 and 160 secondary plants per plot($3m^2$) in 1985. This large amount of propagation was derived from four propagules per plot and was achieved in an average of 68 days after planting the propagules at a depth of 10 cm. Fifteen months after the 1984 experiment, Castner(1985) reported the experimental plots to be a dense mat of H. glauca growth measured at approximately 127 plants per m^2.

Initiation of an underground shoot apex begins from a bud formed in the primary cambium of the periderm which matures to become continuous with the vascular cylinder in mature roots(Woods,1991). These buds form at the proximal terminus of the propagule or in cases in which the root runner gives rise to a bud in the apical meristem of the procambial initials of the root. The emergent shoot-tip appears with a leaf primordium recurved over the meristematic apex and

subsequent leaf primordia tightly packed about the uppermost bud primordia and shoot-tip.

Comparisons of tissue sections 1mm below (Fig.5), at (Fig.6) and 1mm above (Fig. 7)the transition zone of the root runner clearly show the rearrangement of the stele from root to stem.

Conclusion

Hoffmanseggia glauca grows in soils that are alkaline and often with calcareous deposits. Roots and propagules of the hog potato are often found in the calcareous subsoil up to 1.2m below the soil surface(Castner and Murray,1985). Research by others (Chandra, et al.,1982; Pooviah and Reddy,1991) has shown that calcium is often transported to root tips where it is found in relatively high concentrations and is an important component of the middle lamella and cellular functions. Research should be undertaken to ascertain the concentrations of calcium present in the transition zone of the root tip. This might demonstrate if the hog potato's unique ability to vegetatively propagate is due to a reverse asymmetrical concentration of calcium which might be located in the upper portion of the horizontal root tip or if a calmodulin inhibitor is present in the lower region of the root tip (Dauwalder,et al.,1986). In 1987, Pooviah, et al. published the results of two studies which

suggest that calcium ions in plant root tips may work with messenger protein to modulate gravity perception.

The possession of numerous cauline buds with a basal bud primordium within the base of these as well as the presence of many propagules attached to a single above-ground shoot, allows vegetative propagation to occur at times when conditions favor foliar growth for H. glauca. Potential for this species to be cultivated for the edible tuber(Tull,1987) as well as its persistence as a weed is demonstrated by the ease with which dispersal and proliferation are facilitated mechanically when soil distubance such as conventional cultivation occurs. Cultivation practices and the ability of the hog potato to become established by several means are apparently responsible for dispersal to a number of sites occupied by this taxon. The presence of the propagule in the clay subsoil enhances H. glauca's ability to tolerate drought, cold, shoot removal and respond with new shoot growth when conditions allow them to be formed. The thickened periderm over lacunar spaces around the horizontal root-runners probably enhances the ability of the plant to absorb moisture quickly in semi-arid conditions. The large number of vessels observed in the root sections suggests that the plant can efficiently move available water to the storage propagule and foliage.

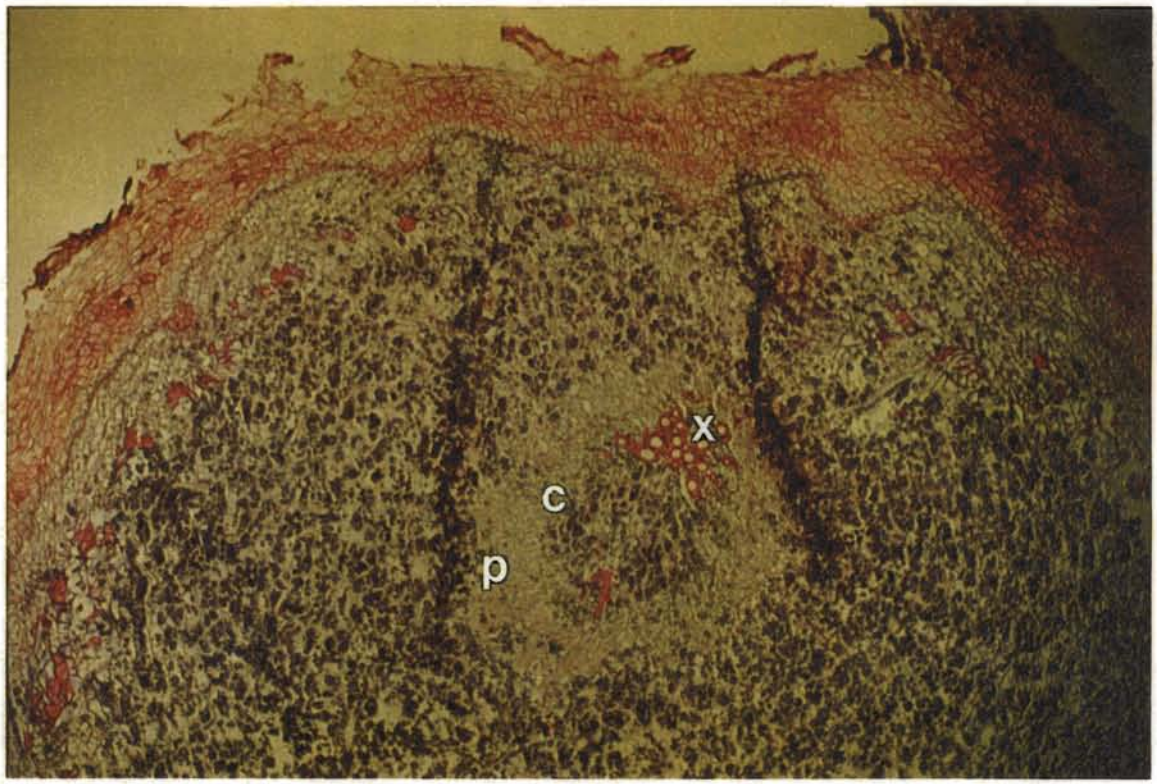

Figure 1. Transverse section of young propagule of Hoffmanseggia glauca. Stained with safranin and fast green, 40X. Cambium (C), phloem (P), metaxylem (X).

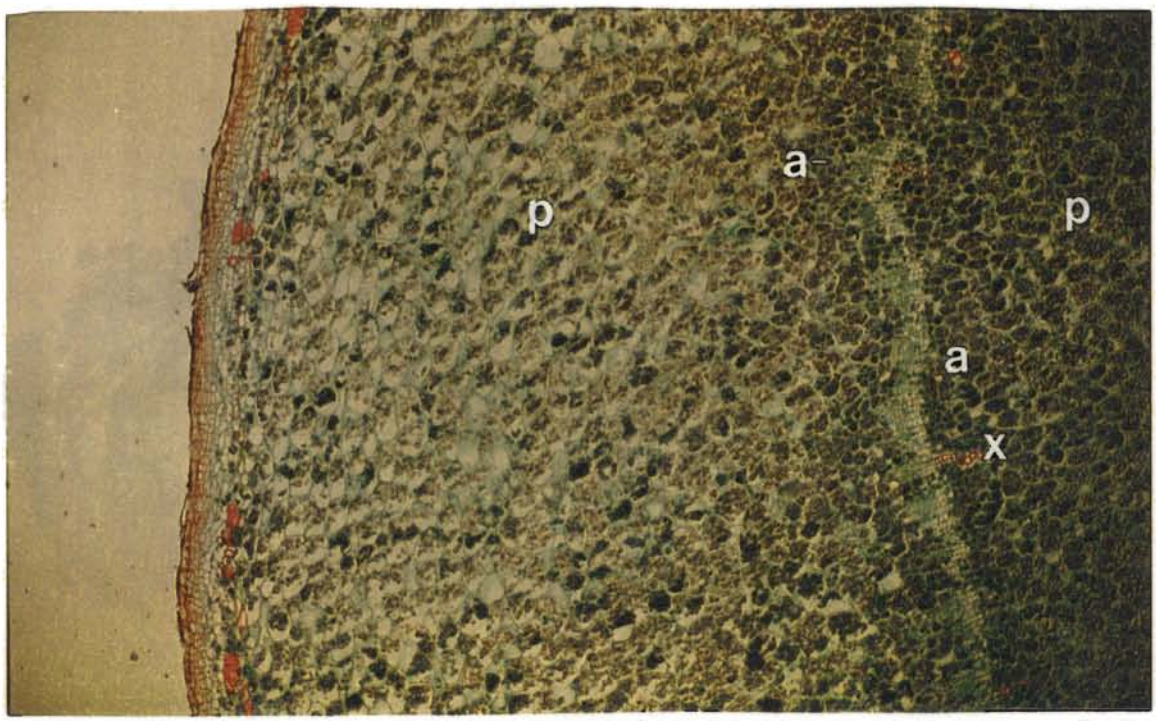

Figure 2. Transverse section of H. glauca of mature propagule showing anomolous cambium(A), secondary xylem(X) and storage parenchyma(P). Stained with safranin and fast green, 40X.

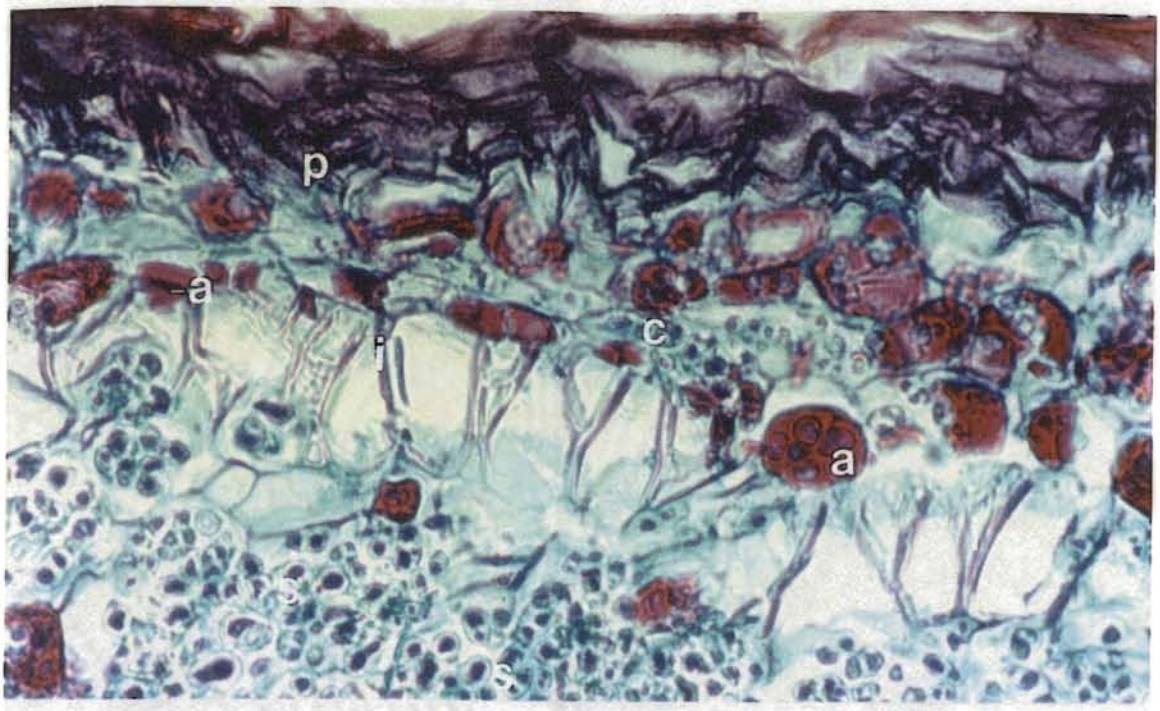

Figure 3. Transverse section of H. glauca propagule showing periderm(P) with lignified isodiametric cells(I) and anomolous bodies(A). Starch grains labeled (S) and secondary cambium (C). Stained with safranin and fast green, 400X.

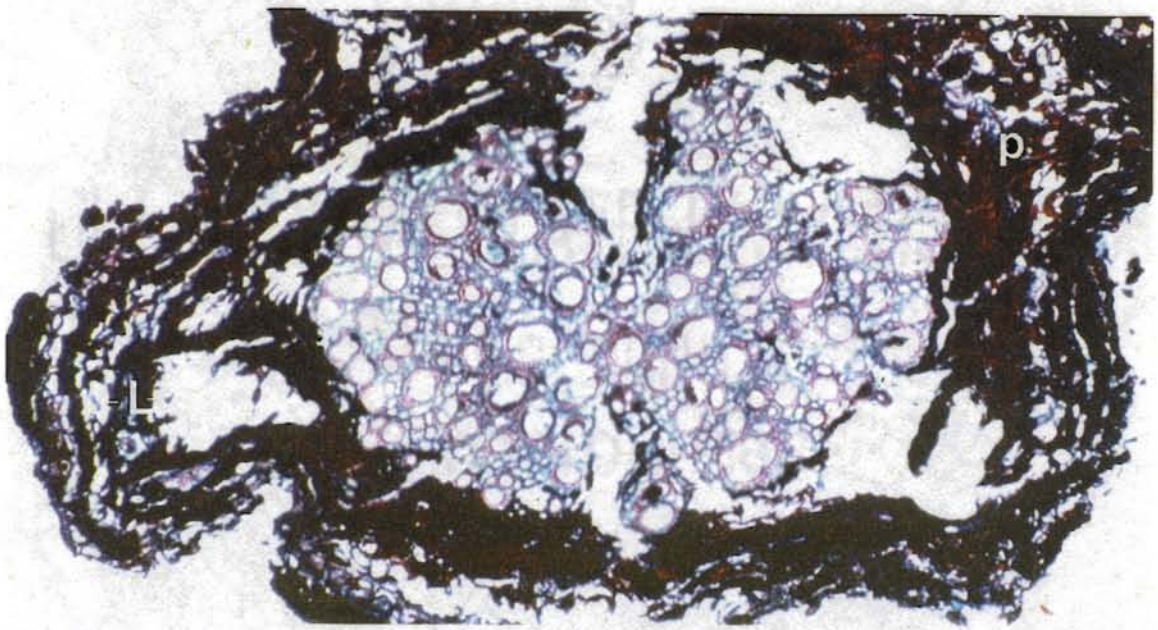

Figure 4. Transverse section of mature root-runner of H. glauca. Disfigured section shows thick periderm(p) and lacunar spaces(l) around stele. Stained with safranin and fast green, 40X.

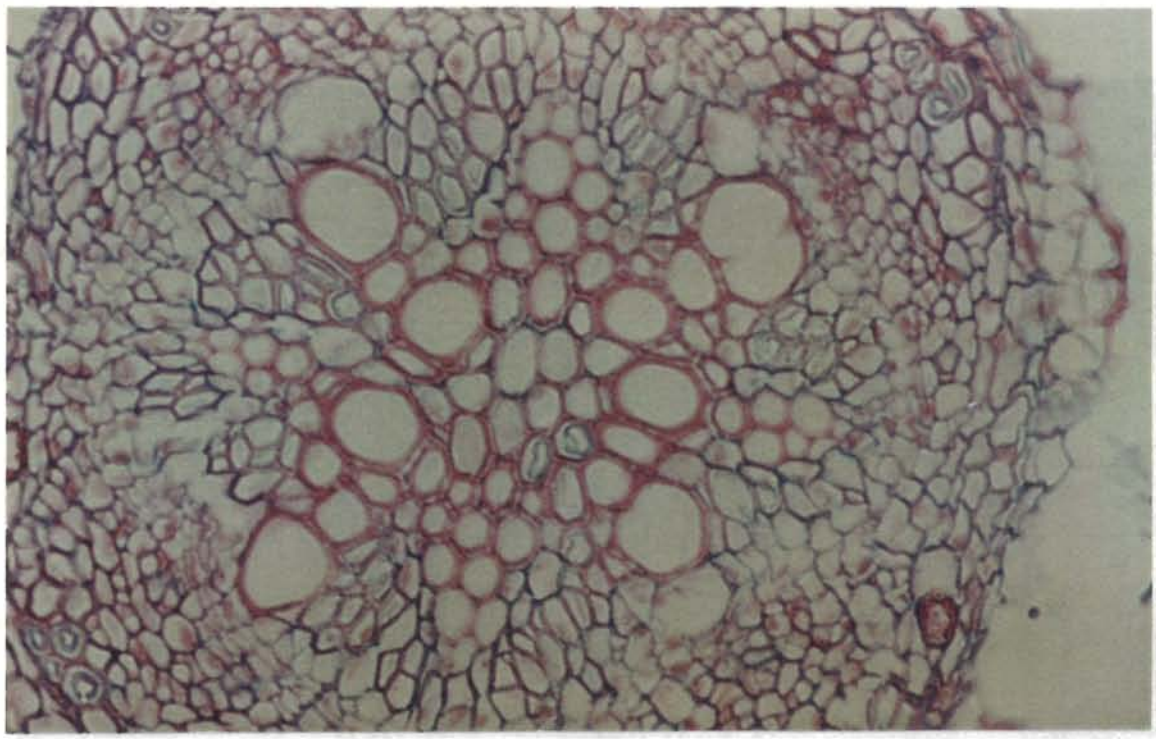

Figure 5. Stele of root-runner of hog potato 1mm below transition zone showing arrangement of vascular tissue. Transverse section stained with safranin and fast green, 400X.

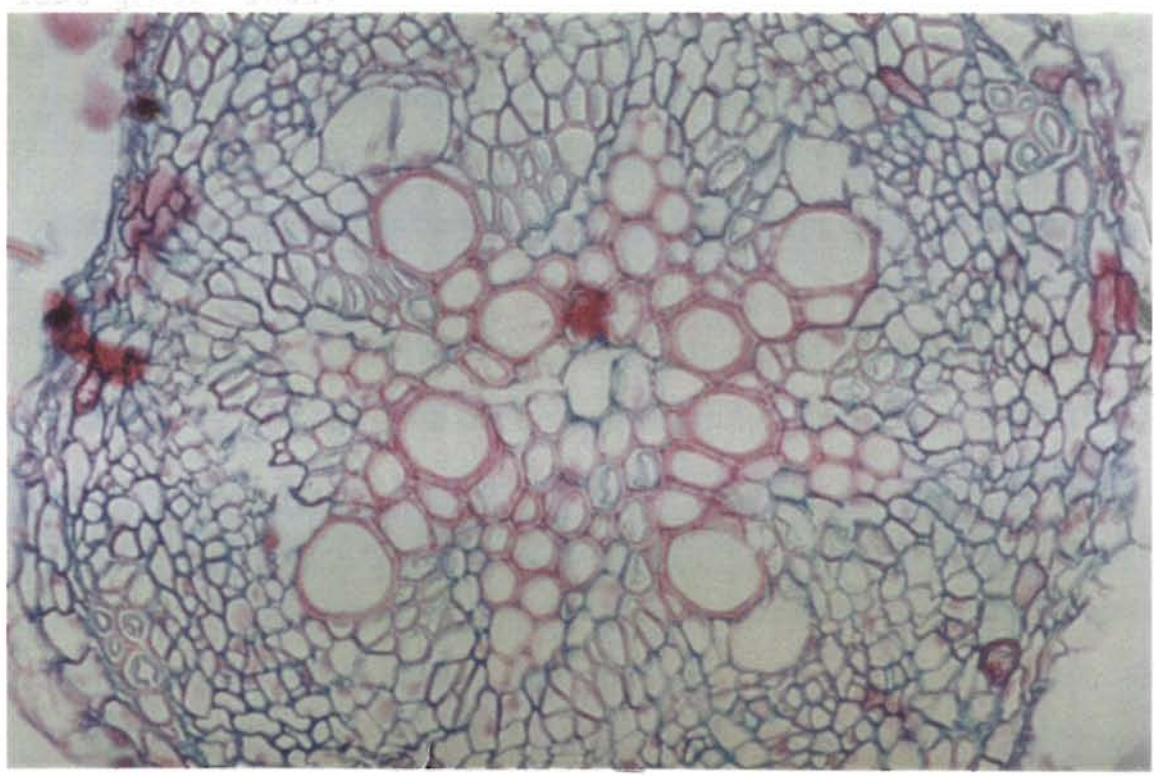

Figure 6. Stele of hog potato root-runner near the transition zone showing vascular arrangement. Transverse section stained with safranin and fast green, 400X.

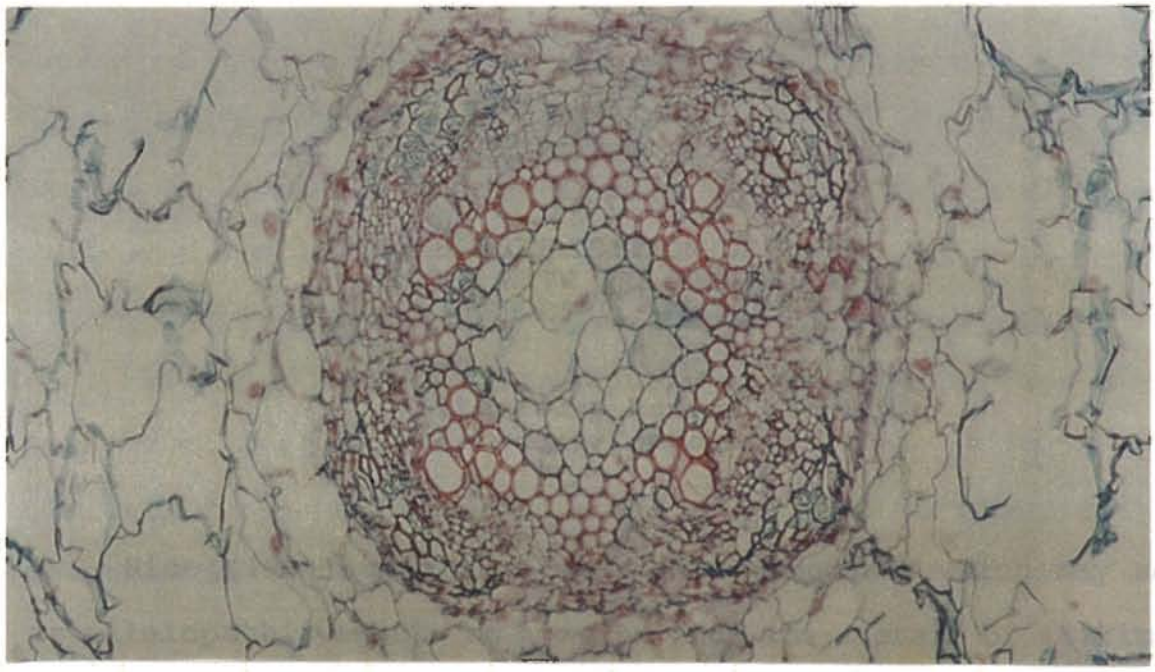

Figure 7. Transverse section of root-runner above transition zone showing vascular arrangement. Stained with safranin and fast green, 100X.

CHAPTER IV

POSSIBLE INHIBITION OF THREE DICOTS WITH THE PROPAGULE WASH OF H. GLAUCA

Literature Review of Fabaceae Compounds

Rice (1984) describes secondary compounds which may act as allelopathic agents as arising from the acetate or shikimic acid pathways. Of the fourteen categories he cites, he describes amino acids as the point of origination for phenylpropanes and alkaloids. Flavonoids are compounds whose components are derived from both the acetate and amino acid sources. In addition, phenolics and terpenoids have been described as natural growth inhibitors (Kefeli,1978; Rice,1984). Finally, it is well known that auxins, gibberellins, cytokinins, abscisic acid and ethylene are growth regulators which may activate, accelerate or inhibit plant growth in various combinations and concentrations.

Many compounds are known and extracted for use by man as medicines, chemicals and dyes (Allen and Allen,1981). Isoflavonoids have not been identified in undisputed members of Caesalpinoideae, but they are common in species of the subfamily Lotoideae (Papilionoideae, Faboideae, Papilionatae)

and two disputed members of Caesalpiniodeae. Poiretia tetraphylla (Poir.)Burk. of the Papilionoideae is a possible source of the toxin rotenone. Prosopis juliflora (Sw.)DC. of the subfamily Mimosoideae, the mesquite tree of southwestern United States, is a source of tannin from the bark which is used as a dye(Allen and Allen,1981).

The subfamily Caesalpinioideae has many species with commercial and medicinal uses. Cordeauxia edulis Hemsl. is the only taxon of Fabaceae known to yield a naphthoquinone, the magenta stain cordeauxione(Harborne,1971). Dialium L. has several species used as insecticides and folk remedies(Allen and Allen,1981). Griffonia Baill. species are used by populations of Africa as purgatives and for delousing(Allen and Allen,1981). Hymenaea courbaril L. is a source of tannin from the bark; the gum from the tree is used medicinally and for some varnishes, shellacs and polishes(Allen and Allen,1981). In the same subfamily, the bark of Peltophorum ptericarpum Backer is used as an astringent and a yellow brown dye(Allen and Allen,1981). Bark decoctions of Saraca L. species are used as astringents and uterine tonics in Asia(Allen and Allen,1981). The Sindora Miq. species yield an oil which have many commercial uses.

Alkaloids (diterpene ethanolamine esters,e.g. cassamine, bufotoxins and erythrophleine) are known in the genus Erythophleum of the subfamily Caesalpiniodeae (Mears and Mabry,1971; Allen and Allen,1981). E. suaveolens also yields

a rhamnoflavonoloside, taliflavonoloside from the leaves. In this same subfamily, Gleditsia L. has several species which contain saponins and G. triacanthos contains the alkaloid tricanthine in the leaves. The Kentucky coffee tree,Gymnocladus dioica L. of Caesalpinioideae contains the toxic alkaloid cytisine in the leaves and pulp; however the seeds are roasted as a coffee substitute(Allen and Allen,1981). In the genus Caesalpinia, C. brevifolia Baill. and C. tinctoria HBK are a source of gallic acid used for medicinal and manufacturing purposes. A glycoside, Bonducin, used in folk medicines is obtained from the seed cotyledons of C. bonduc Roxb., C. bonducella Flem., and C. crista L. Several genera of this subfamily have diterpenes in the wood. Caesalpinia bonducella has alpha-, beta- and lambda-caesalpin. In comparison with the other two subfamilies, diterpenes are prevalent in Caesalpinioideae(Allen and Allen,1981). Triterpenes are also prevalent in this subfamily (Harborne,1971). Several species of Cassia and Caesalpinia have been found to contain phytohaemagluttinins tannins and glucosides(Allen and Allen,1981).

In the subfamily Caesalpiniodeae, many plants are used as stains and dyes. Caesalpinia echinata Lam. is the source of a red dye used as a nuclear stain of biological materials (Allen and Allen,1981). Two of the three species of Haematoxylon are also sources of dyes and stains. H. brasiletto is another source of brazilein and H. campechianum is the source of the dye used in the stain hematoxylin.

Harborne(1971) classified haematoxylin and brazilin as neo-flavinoids.

Haematoxylin is a constituent of the logwood of Haematoxylon species and brazilin is derived from the logwood of the Caesalpinia species. Brazilin is a homolog of haematoxylin containing one less hydroxyl group (Clark and Kasten,1983). These products are extracted from log-wood chips with ether, evaporating, digesting with alcohol and standing with water. The alcohol is distilled off and the haematoxylin separates out in crystals (Lillie,1969). Haematoxylin and brazilin alone are not dyes and are colorless glucosides in solution until "ripened" by oxidation on exposure to the atmosphere or with mordants (Lillie,1969; Clark and Kasten, 1983). Upon oxidation, haematoxylin gives hematein (haematein) and brazilin gives brazilein. These are the dyes used in stains. Upon decomposition, these two compounds yield catechol and pyrogallol (Perkins,et al.,1926-1928; Lillie, 1969). The exact chemistry of haematoxylin is not entirely understood although a theoretical structure is proposed. Reliable tests for analysis and identification have not been devised (Lillie,1969) Lillie, et al. (1976f) reported that hot acid extractions and deoxyribonuclease digestion prevented cationic dye staining and removed Fuelgen stain while alum- and Fe-haematoxylin nuclear staining persisted long after nucleic acid ceased to stain.

Benzene ring containing compounds like haematoxylin contain chromophore radicals known as chromogens. Although a chromogen may be colored, it posesses no affinity for fibers or tissues and therefore cannot stain. For a substance to stain, it must contain a group in addition to the chromophore which will allow that substance to bond with a tissue-end group more or less firmly (Lillie,1969). With haematoxylin and brazilin, the metal salts are the mordants which allow this bonding through chelating action. Haematoxylin (hematoxylin) is a "dye" which is used in conjunction with a mordant to stain tissues. This dye is a colorless solid which complexes (chelates) with complex cations formed when salts of iron or aluminum are dissolved in water. The chelate then binds to acidic sites in the tissue so the chelate behaves like a basic dye. When an iron chelate of haematein is used on plant tissue, chromosomes and nucleoli are stained black, chromatin blue-black, cytoplasm and unlignified cell walls a delicate shade of blue (O'Brian and McCully,1981). Metal salt solutions may be used as a premordant preceding the dye, mixed with the haematoxylin to ripen it or following the dye as an afterchroming. Many metal salts may be used as mordants. Metal granules of Al, Cr, Ga, Hf, In, Fe and Zr stain dark-blue with haematoxylin solution; Be, Dy, Ho, In, Pb, Mn, Mo, Nd, Ni, Pt, Rh, Tb, U, Yb and Zn stain blue; blue-green color is obtained with Cu; purple with Bi, purple-red with Sn and Th; brownish-red with Ta; brown with Nb and Ti and blue to greenish-brown with Os. Lakes of haematoxylin and Al-salts are blue; with Fe- and Cr-salts the lakes are blue-black;

blue-green with Cu-salts; lilac with Ni; red with Sn and dark-brown with Pb. Haematoxylin may be used to demonstrate the presence of metals in tissues (Lillie,1969). Long(1961) reports haematoxylin alone may be used as an indicator, changing from red to yellow at pH 0.0 to 1.0 and from pale-yellow to violet at pH 5.0 to 6.0.

In the genus Hoffmanseggia, the root bark of H. melanosticta has astringent properties while the stems yield 25-30 percent tannin and a red dye (Allen and Allen,1981). Initial stain tests on fixed sections of hog potato propagule have revealed that ferric chloride (a mordant) cause a black stain of cellular contents without the use of the haematoxylin dye. Preliminary studies of contents stained appeared to be nucleii and some other organelles. Fresh sections of the propagule placed on polished iron or steel will permanently discolor the metal black within five minutes at the site of contact. In addition, filtered, crude wash of the hog potato propagule external periderm demonstrated staining of fresh potato (Solanum tuberosum) tuber tissue sections when applied with ferric chloride. However, the crude wash did not stain the tuber sections when the mordant was withheld.

Introduction

It has been demonstrated that the growth of cotton is inhibited in the presence of H. glauca (Castner, et al., 1989). Once established the hog potato is difficult to control. Studies by Wiese (1982) reveal the hog potato may be controlled by 2,4,5-T, a herbicide which is banned from use in the U.S. Soil sterilization chemicals (Arsenil, Spike) have been developed to control H.glauca. However, the treated soil must remain uncultivated for 4-5 years and this leads to a total loss of crop production in the field. Due to the small demand for these sterilants, they have not been commercially developed (Murray,1992).

Sites where hog potato plants were observed included roadside drainage ditches, creek beds, a lake-side hilltop, lightly asphalted roadside, irrigation canal back-walls, the perimeters of several cotton (Gossypium hirsutum) plantings and the interior of one cotton field in Tillman County, Oklahoma (Fig.8). In this location, boll reduction and reduced plant growth corresponded to that observed by Castner,et al. (1985) in previous work. Infestation was localized within affected cotton stands with individual cotton plants displaying inhibited development (Fig.9 and 10).

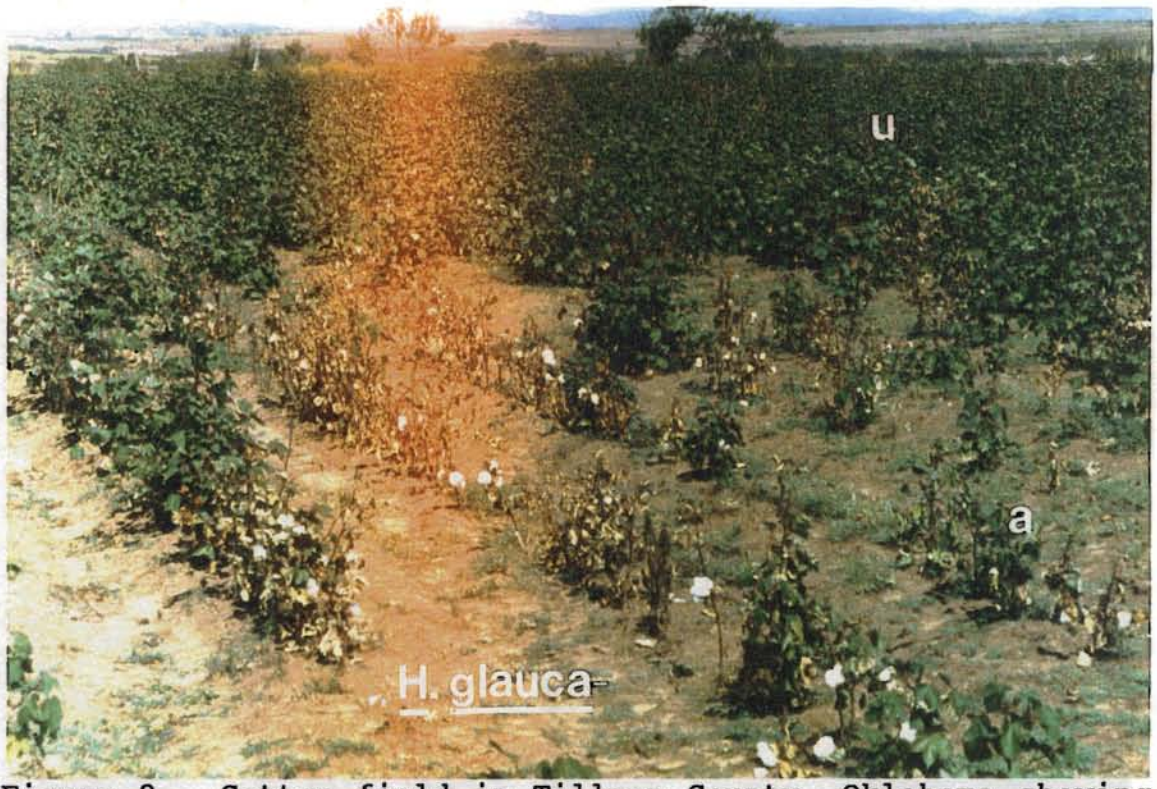

Figure 8. Cotton field in Tillman County, Oklahoma showing both affected(a) and unaffected(u) cotton plants.

Figure 9. Close up of same field with hog potato and affected(a) cotton plants. Note small size of plants in relation to unaffected(u) cotton.

Figure 10. Affected cotton removed from soil.

On July 21 and 22,1992 cotton plants which were affected by hog potato plants were examined and the following characteristics were noted:

a. Many plants showed stunting and early senescence.
b. Reddish tinge to primary shoot, petioles and leaves.
c. Supression of abscission of primary foliage on main stem and axillary vegetative growth supressed.
d. Reduced size to foliage.
e. Reduced internode length.
f. Development of fruiting branches is reduced with bolls initiating at the nodes on the main stem.
g. Tap root reduced.
h. Lateral roots grow on only upper portion of tap root are supressed and thickened.

In contrast to the previous characteristicsdescribed for the affected cotton plants observed in Tillman County; Hayward(1938), Deterling and El-Zik(1982) and Munro(1987) describe normal growth patterns for cotton which differ from those observed in the affected plants. In the axils each leaf of cotton plants with normal growth, there is an axillary bud and an extra-axillary bud. The axillary bud which is located centrally above the base of the petiole forms the vegetative branch. The extra-axillary bud is located to one side of the true axillary bud and upon development forms the reproductive branch. Normally the vegetative growth develops on the lower nodes of the main stem (nodes 3 to 4, Hayward,1938 and nodes 4 to 8,Deterling and El-Zik,1982), with the fruiting buds supressed. These vegetative branches resemble the main stem in being nearly vertical and each node containing two buds. On these branches, floral buds (squares) may develop but floral development is limited. Above the vegetative zone, a fruiting region usually develops with the reproductive branches being nearly lateral and squares forming at each node. Occasionally a transition zone occurs in the region between the vegetative and fruiting zones in which shoot behavior is irregular. Both vegetative and reproductive buds may abort, remain dormant. Either or both may develop and grow.

A normally developing cotton plant has a tap root which may grow to several feet in length(Hayward,1938; Munro,1987). The tap root may descend to a depth of nine inches by the time

of true leaf emergence(Deterling and El-Zik,1982). From the main root, many laterals will develop. The extent of penetration into the soil depends upon soil conditions and available moisture. The laterals generally occupy the first six inches of soil with a radial expansion of several feet(Hayward,1938) Two thirds of the root by weight are found in the top 30 cm of soil (Munro,1987). Secondary laterals often arise, filling the upper layer of soil. Another layer of laterals may develop on lower regions of the tap root(Hayward,1938).

The difference between the text descriptions and those observed in the field and by Castner et al. (1989) prompted the question as to why the cotton plants which had populations of H. glauca interspersed among the crop should show such a drastic reduction of vegetative growth and loss of boll production as compared to the normal growth habits. As the foliage of the plant is usually decumbent and is much shorter than the cotton it affects; it does not seem likely that the small amount of leaves which are shed could account for the reduction in growth which resembles reduction of IAA and nitrogen. Due to this reasoning and the large amount of below surface growth, this study is focusing on the underground structures as a source for the observed inhibition.

The many symptoms which were observed on the field grown cotton plants could be the result of one or more causal agents. Munro(1987) states that reddening of the leaf as a response to stress and internode shortening is one of the

first effects of water stress. Another symptom of water stress, leaf wilt, was not observed in the Tillman County cotton. Castner et al.(1989) studied soil water use with both plants in competition, each plant alone and bare soil as a control. In the early part of the season, the hog potato/cotton treatment and hog potato alone showed a greater use or water in the upper 15cm of soil compared to the cotton alone or bare soil. This was attributed to the established root system of the hog potato. Early water use between the two treatments was not statistically significant according to Table 5 of Castner's study(1989). The hog potato used water from the lower soil profile(>105cm) and cotton in the upper soil region(<90cm). Statistical analysis of late season water use showed all cropping treatments to be significantly different (Table 5, Castner et al.,1989). Late season determinations suggest that although available soil water was lower in the soil where both plants were present, competition from water alone could not account for reduction in yield observed (Castner et al.,1989). Personal comparisons in the field in Tillman County also revealed nearly a total loss of harvest for those cotton plants which were infested with H. glauca.

Experiment 1. In an attempt to determine if a chemical interference could be contributing to the biomass and boll production loss; an experiment was designed to test for the presence of a water soluble, active agent from the black, gummy soil and periderm which surround the mature hog potato propagules.

Null Hypothesis: No difference between plants tested for plant growth inhibition and untreated plants.

The experiment was designed to determine if a difference could be statistically observed between dry weights or growth habits in the control plants (Amaranth, Brassica and Cotton) and the test plants treated with the filtered wash from propagules of Hoffmanseggia glauca.

The experiment was designed to test three dicots grown in washed sand. The plants chosen to be tested for inhibition by hog potato propagule wash were Amaranthus hypochondriacus of the family Amaranthaceae, Brassica rapa, rapid cycling brassica(RCBr) of the family Brassicaeae and Gossypium hirsutum var. Paymaster HS26 of the family Malvaceae. Although from different families, these plants have some related characteristics which makes them suitable for this study. All of these plants are: a)dicots, b)related to crops or grown as crops, c)used in research and d)known to be sensitive to plant growth inhibitors from microorganisms and other plants (Christidis and Harrison, 1955; Cole, 1979;

National Academy of Sciences, 1985; Nishi,S.,1980; Snogerup,S.,1980; Wisconsin Alumni Research Foundation, 1989).

Experiment 2. A second experiment was performed to determine if the hog potato could prevent germination or hypocotyl elongation of the seeds of the test plants.

Null Hypothesis: No difference between germination rates of treated (hog potato wash) and untreated test plants.

Materials and Methods

Procurement of Propagules. The propagules of H. glauca were obtained primarily by traveling to the field in southwest Oklahoma and digging them from the ground with a shovel. The difficulty involved in excavating into the clay subsoil was the major limiting factor in the effort to aquire hog potato propagules for this study. Two main sites were used for collection: the southwest shoreline of Lake Fredrick east of Manitou, Oklahoma and a portion of the cotton field owned by Frank Maloyed. Both sites are in Tillman County, Oklahoma. In addition, Dr. Mike Anderson of Agronomy donated one tray (approximately 15 propagules). For the first replication, propagules were planted in trays of sand and clay-loam from native soil and allowed to renew vegetative growth. Later collections for replications two and three were collected and stored in plastic bags in the refrigerator.

Preparation of the propagule wash. Propagule wash was prepared in 500ml batches. For each batch, 30 propagules were counted and weighed. Total weight for the propagules ranged from 65.6g to 87.96g per batch. Initially, the propagules and the residual soil clinging to them were washed by gently rubbing them by hand while rinsing with a squeeze bottle of deionized water. Later batches were prepared in a sonic wash. Initial washing required about 350ml of water.

Upon completion of washing the propagules, the crude wash was filtered with Whatman No. 4 filter in a Buchner funnel under vacuum to remove the coarse particles and the bulk of the soil. Several filters were required to filter the 350 ml of crude wash in the first step. This was followed by filtration with Whatman No. 2 filters in the Buchner funnel under vacuum to remove the finer particles. The first three batches were filtered in this way. Later batches were prepared by first centrifuging the coarse wash at 3000rpm for 10 minutes before filtration. Upon completion of filtering, the filtrate was brought up to 500ml with deionized H_2O. Each batch was tested to determine the pH. Range of the pH was from 7.37 to 7.62. Filtrate was stored in the refrigerator at 21 C until needed. After a day, a tan precipitate settled out of the filtrate in each batch. Each preparation of filtrate was administered to the test plants within seventeen days. Prior to application to the test plants, the filtrate was stirred to resuspend the precipitate and returned to room temperature.

Experiment 1. Comparison of test plant heights, root weights and shoot weights.

Nutrient Solutions. Once a week all plants received Peter's 15-30-15 fertilizer solution (replication I) or Hoagland's #1 NO_3^- solution (replications II and III). Peter's 15-30-15 was prepared according to the package rate of 2.45ml per 3.785l(one-half teaspoon per gallon). The Hoagland's solution was mixed in 25 liter batches from stocks of nutrients which were prepared and stored in the refrigerator until required. Both nutrient solutions were prepared with deionized water.

Germination of Seedlings. For each replication, the seedlings for the experiment were pregerminated in sterile petri dishes lined with sterile paper towels prior to planting in the sand. Due to the differences in seed size and germination rate, the seeds were started on different days . The cotton seeds were started four days before the start of the experiment. The amaranth was imbibed three days before the planting date and the brassica two days before. Sterile DI H_2O was used for imbibing the seeds. Each replication required a minimum of 20 seeds. Twenty-five to thirty seeds were germinated for each species. All seedlings were germinated to the stage in which the cotyledons were exposed and the hypocotyl in extension.

Medium and Planter Preparation. All plants (all species and all replications) were grown in 10.2cm(four inch) plastic pots containing sand up to the inner lip. The pots were washed with Ivory dishwashing liquid, rinsed with the hottest water available and then disinfected with sodium hypochlorite in water (1:10 concentration for one minute). After disinfection, pots were rinsed with clear tap water. The pots were then inverted on clean paper towels and allowed to air dry.

The sand used in the experiment was sifted masonary sand which was washed three to four times until the water draining from the rinse containers was clear and no foam or debris remained on the surface. The wet sand was then autoclaved for 30 minutes and allowed to cool (covered with aluminum foil). Ten pots were marked for each treatment or control for each of the three species used. Each replication of the experiment totaled 60 pots. Seedlings were removed from the petri dishes in which they were germinated and planted to a depth slightly deeper than the transition zone. All transplants were watered to the point of run-off and removed to the growth chamber in a random order. In each replication the random order was determined by a QuatroPro computer program.

Growth Chamber Preparation. Prior to use, the growth chambers were cleaned and disinfected. In the growth chamber used in Rm 022, Life Science East, the shelf was raised to

within 30.5cm(12 inches) of the lights. Fresh 40 watt incandescent and flourscent tubes were installed where needed. Testing with a light meter showed the intensity to be 300μE at 10.2cm(4 inches) below the lights. Initial height of the plants in pot was approximately 16cm below the lights. After two weeks, the shelf was lowered to 40cm below the lights to prevent the plants from being burned by close proximity to the lights. The growth chamber, in Rm 116 Nobel Research Center, had a movable light source which could be pulled down to the plants. Light measurement at approximately 30.5cm above the deck was 520μE. The light source was adjusted for the duration of the experiment to maintain a distance of approximately 10cm above the tops of the plants. Temperature for both chambers was set at 26°C. No added humidity was used.

Initiation of Experiment. Plants were given several days to acclimate to growth chamber conditions. The experiment did not begin until all plants showed emergence of the apical portions of the first true set of leaves. During this time, any plants which died were replaced from stock in the petri dishes. Watering was with deionized water to the point of run-off until the experiment began.

Tests prior to the experiment showed the pots with sand (no plants) could be given 40 to 50ml of water before run-off ocurred. This was used as a guide for determining the amount of water or nutrient solution the plants recieved.

All plants were watered daily. The control plants of all three species recieved 40ml of DI water per pot. The test plants recieved 39ml of DI water followed by an application of 1ml of the filtrate from the hog potato propagules. This was delivered to the base of each test plant with a 1ml pipette. At one week and weekly thereafter nutrient solution was substituted for the DI water.

Due to the reduced life cycle of the RCBr, the growth time for each replication was limited to six weeks. When the Brassica went into senescence, this species was removed in each replication for evaluation. The amaranth plants were evaluated next and the cotton last. This order was chosen because the cotton is a long season crop, and research by Castner et al.(1989) suggested that the earliest response time of cotton to the interference by the hog potato would be four weeks.

Evaluation of Plants. Plants of all species were evaluated in several ways. Initial observations were made of the vegetative top growth including height and appearance. Photographs were taken of the extremes of both control and test plants. Above ground growth was then removed at sand level and the tops placed in labeled paper sacks which were sealed and dried in a herbarium oven.

Roots of the plants were removed from the pots and carefully washed to remove all sand. Roots were kept in a

labeled order, photographed and then dried in the same manner as the shoots.

Shoots and roots of all species for all three replications were weighed, recorded and analyzed statistically. Data was analyzed in a randomized block design using SAS. For all species, variables tested were shoot height, shoot dry weight, root dry weight and total dry weight. Due to the procedure used, all results are the sum of the three species.

Experiment 2. Comparison of germination rates and radicle lengths for each of the test species as compared to the water treated controls.

Preparation of Materials. Standard glass petri-dishes with tops were washed and rinsed. Two Whatman No. 1 (9 cm in diameter) filter papers were placed in the base of each petri-dish. Petri dishes were placed in groups of three and wrapped in aluminum foil. For each trial there were two replications for each species of seeds. The twelve petri-dishes were autoclaved for thirty minutes in a steam autoclave. Other equipment and tools were cleaned and disinfected prior to use. For each species tested: amaranth, brassica and cotton; two petri-dishes were used for the test of the hog potato wash and two were used for the control with sterilized water. Each petri-dish recieved ten seeds which recieved no disinfection treatments for the seeds. The dishes each recieved 10 ml of hog potato wash for the test seeds or 10 ml sterile water for

controls with sterile 10 ml pipettes. Each petri-dish was then wrapped in aluminum foil and placed in random order on a shelf in the laboratory at 25 C for 48 hours. At the end of the 48 hours, each dish was opened and the seeds examined for germination and the radicles measured.

Results and Discussion

Experiment 1.

Differences in nutrient solutions. In initial use of Peter's 15-30-15 as the nutrient solution in replication, I did not take into account the micronutrient needs of plants growing in washed sand. As a result, in the third week of the first part of the experiment, signs of nutrient deficency were observed in some of the plants. Particularly in the cotton and amaranth, chlorosis was present in the lower leaves. In the amaranth this was often exhibited as interveinal chlorosis, reddish spots on the older leaves and reduced internodal length in the apical portion of the shoot. This resulted in the formation of rossettes of leaves in the amaranth.

As nitrogen, phophorus and potassium was being supplied in the nutrient solution the symptoms suggested a lack of micronutrients. When the sand was washed with water, the run-off was a reddish, iron-oxide color. Since the sand was not acid-washed, this suggested that small amounts of iron should have been present in the sand. The symptoms listed above

suggest that the deficiency may have been due to lack of micronutrients (Taiz and Zeiger,1991).

Due to the chlorosis, lack of growth and plant loss of replication I, the last two replications utilized Hoagland's solution. Although statistically similar results were obtained in replications II and III; large differences occurred between species in heights of plants, shoot dry weights and root dry weights(Table 1). These differences could be related to the large deviations encountered in the statistical analysis.

Statistical Results. Comparisons of means in control plants vs. test plants (Table 1) by height, shoot dry weight, root dry weight and total dry weight for replications II and III show that although heights and weights are less for treated plants compared to control plants, the results are not statistically significant. In all cases, the standard deviation is greater than the difference between the treated and control plants. A trend is suggested in a review of the data for brassica shoot dry weight and brassica and cotton root dry weight. Shoot dry weights for brassica test plants are 35% less than the controls. Root dry weight for brassica test plants are 35.8% less than the controls. Cotton root dry weights for the test plants are 27% less than the controls. An exception to the observation is that amaranth test plants had greater shoot growth than the controls.

Comparisons of shoot to root percentages for all three species for replications II and III (Table 2) had similar results. Although not statistically significant at 0.05, these comparisons show all three species to have lower weights in treated plants than control plants. It would appear that the young control plants have a low root/shoot ratio. Klepper(1991) observed that young, developing plants typically have a high root/shoot ratio during early development and the ratio does not reverse until the reproductive stage begins.

Overall analysis (Tables 3 and 4) does not show any significant difference between the control and test plants for any of the three species in comparison of treatments versus controls (Treatments) or for interaction between species and treatments (Sp*Trt). However as one might expect, there was shown to be a difference between replications and between species. Castner et al.(1989) observed reduction of lint yield due to interference by H. glauca during the first 7 weeks of growth. Results of this experiment do not support personal observations of affected cotton plants or Castner's results. The Brassica rapa used for this experiment had a six week life cycle. Each experiment was terminated when the RCBr went into senescence. Due to the six week time limit on these experiments, long term results and production results could not be determined.

The difference between these results and previous observations could be due to several factors. The most

obvious is that the propagule and/or root-runners of the hog potato may not be the sole determining feature in H. glauca's affect on cotton. Another factor may be that the concentration of solution to which the treated plants were tested may have been too dilute to cause interference with plant development. In the field, cotton and hog potato grow in a high clay soil which may prevent leaching of any active compound. In contrast, the experimental plants often experienced run-off after several weeks of growth. This may have precluded the possibility of the build-up of substanes from the hog potato wash from increasing concentrations in the test pots. As plants were grown in relatively clean sand, there is also the possibility that populations of microorganisms which could convert exudate from the hog potato to an active substance may not have been present. Surface algal growth was noted in some of the pots in later stages of each experiment. Also, the propagule wash was not sterile and could have been degraded by any microorganisms that might have been present. Lovett(1986) summarizes work(Rovira,1965) that the interaction between rhizosphere microorganisms and the rhizosphere can affect all aspects of a plant's physiology, nutritional processes and root/shoot ratio.

Table I. Comparison of means of treatment to control. Heights, and dry weights for amaranth, brassica and cotton, replications II and III.

	amaranth		brassica		cotton	
	ctrl	trt	ctrl	trt	ctrl	trt
Ht.(cm)	23.99	24.56	18.62	15.32	11.4	10.3
StDev.	(6.91)	(5.28)	(7.03)	(6.26)	(2.71)	(3.23)
ShtDWt(g)	4.33	4.25	1.22	0.79	2.40	2.29
StDev.	(2.05)	(1.73)	(0.44)	(0.30)	(0.92)	(0.97)
RtDWt(g)	2.56	2.20	0.92	0.59	1.29	0.94
StDev.	(1.37)	(0.99)	(0.79)	(0.48)	(0.64)	(0.46)
TtlDWt(g)	6.89	6.44	2.15	1.39	3.69	3.23
StDev.	(3.31)	(2.68)	(1.16)	(0.73)	(1.48)	(1.39)

Table II. Percentages of Root and Shoot for Replications II and III for amaranth, brassica and cotton.

		Shoot	Root
amaranth	ctrl	63.9a*	36.09a
	trt	66.29a	33.70a
brassica	ctrl	61.5a	38.5a
	trt	62.6a	37.3a
cotton	crtl	65.76a	34.24a
	trt	71.04a	28.96a

* numbers with the same letter are not significantly different

Table III. F and p-values from Randomized Block Analysis of three replications for height and total dry weight.

		Height		Total Dry Weight	
	df	F	p-value	F	p-value
Replication	2	8.12	0.008	7.19	0.0116
Species	2	71.00	0.0001	11.12	0.0029
Treatment	1	2.63	0.1361	0.18	0.6793
Sp * Trt@	2	1.28	0.3195	0.05	0.9508
Error*	10	1.17		30.35	

@ Species * Treatment, * Type III MS with Replication* Species* Treatment as MSE.

Table IV. F and p-values from Randomized Block Analysis for three replications for shoot dry weight and root dry weight.

		Shoot Dry Weight		Root Dry Weight	
	df	F	p-value	F	p-value
Replication	2	5.61	0.0232	8.77	0.0063
Species	2	9.63	0.0047	11.47	0.0026
Treatment	1	0.08	0.7832	0.40	0.5388
Sp * Trt	2	0.04	0.9606	0.15	0.8624
Error	10	32.19		14.75	

Experiment 2.

Measurement of Radicles. A metric ruler was used to measure the length of the radicle for each seed. Measurement was from the base of the seed where the root emerged from the seed coat to the root-tip. The twenty values for each test and control sample were averaged. Results are listed in Table V.

Table V. Average radicle length comparison for seeds using hog potato wash and sterile water.

Species Tested		Average (in mm)	Std. Dev.	% Diff
amaranth	ctrl	3.45	2.65	83.3
	trt	0.57	1.50	
brassica	ctrl	4.50	3.79	37.8
	trt	2.80	2.40	
cotton	crtl	3.48	3.60	15.2
	trt	2.95	3.56	

Conclusion

Experiment 1 was analyzed statistically using a random block design by ANOVA with a SAS program. Means comparisons of control plants to test plants by height, shoot dry weight, root dry weight and total dry weight are not statistically significant. In most cases however, height and dry weights were less for test plants than for the controls. A trend is suggested in the data for brassica shoot and root dry weights, being 35% and 35.8% less than the controls, respectively. Cotton root dry weights for the test plants are 27% less than the controls. An exception to observations is that the height of the amaranth test plants were greater than the controls. For comparisons of root to shoot dry weight percentages although not statistically significant at 0.05, Table 2 shows all three species to have lower weights in treated plants than control plants. It should be noted that weights for the control plant roots are less than should have been expected. Overall analysis does not show any significant difference between the control and test plants for any of the three species in comparison of treatments versus controls or for interaction between species and treatments. There was a significant difference between replications and between species. This is due to the use of two separate growth chambers, probable variability in the propagule washes and the choice of using plants from three different families. This experiment did not achieve results of inhibition similar to

those reported by Castner et al. (1989). The six week time limit on these experiments may not have been long enough for long season crop like cotton to demonstrate the reduced growth as observed in the field.

Experiment 2 revealed unexpected results. Since cotton has been shown to be inhibited by H. glauca in the field and in test plots; tentative expectations were to see some results with the seed germination test. Although there was an initial lag in the emergence of the radicle of the cotton seeds treated with wash after 24 hours, measurement of the radicle after 48 hours revealed only a 15% difference between the lengths of test and control cotton radicles. These results supported the null hypothesis of no difference between test and control cotton seeds regarding radicle growth. The initial lag in cotton radicle emergence could most likely be explained by the longer imbibing time required for the larger seed and the lesser amount of liquid per seed as compared to the smaller seeds of amaranth and brassica. In the initial trial of this experiment, cotton radicle emergence for treated seeds was approximately 90% delayed (data not shown) as compared to the controls after 48 hours, but the presence of fungal mycelium rendered the first trial void. The test cotton in the first trial showed radicle emergence when checked at 72 hours. The amaranth seeds in the second trial showed results similar to the first. Imbibing with resulting seed swell occurred within 24 hours. In the results listed for the second trial the results reveal an 83% difference

between the trial and control seeds. Many of the seeds did not germinate at all and only two seeds developed radicles longer than 5mm. At the end of 72 hours, those seeds which had not germinated remained in that condition. The brassica seed was also imbibed after 24 hours. As can be seen from Table V, the treated brassica seed differed from the control by 38%. Limited radicle growth was also observed for treated brassica seed after 24 hours, but radicle emergence was ongoing in all seed after 48 hours. Six brassica seeds out of 20 reached a length of greater than 5mm after 48 hours. Only six seeds did not have radicle emergence compared with four for the control seeds.

Although two trials of germination inhibition cannot be considered definitive in considering that the hog potato wash contains an active chemical agent which inhibits seed germination, the results of the trial do suggest that the presence of the wash seems to have a temporary effect on the emergence of brassica and amaranth radicles.

As with experiment one, the possibility is likely that the wash which was prepared may not have been at a concentration which exhibited persuasive results. Another method of improving the experiment could have been made by testing the germination effect of a very sensitive plant, such as Grand Rapids lettuce.

Although the literature describes many chemically active compounds found in plants of the subfamily Caesalpinioideae; present research does not show conclusive evidence for the presence of an active, water-soluble substance which inhibits the growth of the tested dicots. However, many other factors could be tested to determine the cause of the observed effect of H. glauca on G. hirusutum. Among factors which were not examined are presence of microorganisms contributing secondary compounds from the breakdown of the black coating on the periderm of the propagule, deprivation of essential elements and the production of volatile compounds from living, adjacent foliage of the hog potato.

Currently agricultural research has focused strongly on chemicals which may be derived from plants for use as growth regulators and inhibitors(Putnam and Tang,1986; Rice,1986). Many of the previously used agricultural chemicals designed to control weeds and regulate crop responses have been banned or are under review. Chemicals are needed which: a)provide a specific agricultural response, b)are derived from natural sources, and c)break down to biologically safe compounds after use. Research into the use of naturally occurring plants which provide these natural chemicals could benefit both humanity and the environment. As such, further investigation into the unknown properties by which the hog potato inhibits the growth of cotton is warranted. If H. glauca does contain a water soluble substance which depresses the development of cotton, other uses could easily be devised.

CHAPTER V

TESTING THE PROPAGULE WASH OF *HOFFMANSEGGIA GLAUCA* FOR THE PRESENCE OF PROTEIN

Introduction

In the study of interference or possible allelopathy between higher plants, factors which can affect the success of the experiment are as manifold as the sum of the processes by which a plant lives. There are conditions in the rhizosphere which affect every phase of plant development. These include morphological development of underground structures, their interactions with the rhizoplane microorganisms and the uptake of essential nutrients which allow the plant to survive(Lovett,1986; Rice,1984). External factors such as light intensity, day length, availability of nutrients and water stress can affect the production of inhibitory compounds by the producing plant and their interaction with neighboring plants(Rice,1984). Many compounds produced by plants may have either subtle or pronounced effects upon the mechanisms and metabolic reactions which occur in plants at all stages of growth(Einhellig,1986). In addition, it is seldom that any one chemical is responsible for an allelopathic action on a plant(Putnam and Tang,1986;

Rice,1984). More often the effect is the result of combined environmental conditions and the combined or additive effects of plant compounds(Putnam and Tang,1986;Rice,1984).

In the rhizosphere, compounds are exuded by plant roots as sugars, amino acids, organic acids, nucleotides and enzymes(Raven, et al., 1986). In turn, secondary reactions occur with the soil particles and micoroorganisms which produce other compounds (Einhellig,1986; Rice,1984; Tang,1986). Rice(1984) describes the origination of known and suspected inhibitors as arising from the acetate and shikimic acid pathways. Among the many compounds described as inhibitory by Rice(1984) are hydrolyzable and condensed tannins, flavonoids, terpenoids, amino acids and alkaloids.

Rice(1984) describes most chemical inhibitors of plants as being secondary in nature, being derived from the primary biosynthetic pathways. Acetogenins, alkaloids, phenylpropanes, steroids and terpenoids are described by Whittaker and Feeny(1971) as being the primary groups of secondary compounds.

It was mentioned in the previous chapter that wash from the propagule caused staining of a white potato tuber with a mordant and that fresh sections of the propagule would stain an iron blade black where it lay in contact. In order to begin understanding the nature of the solution, samples of the filtrate were tested to see if they contained protein. The

method used was 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Morrissey (1981).

Methods and Materials

Samples tested were the filtered wash used in the experiment, 0.45μm filtered extract and a two week-old sample of the filtrate which was concentrated with ethanol. For each sample two replications were used in each gel. Stains used were Coomassie Blue and Silver Nitrate for which two gels each were run.

Preparation of mini-gels. For a single batch of 12% acrylamide gel the components were: 3.0ml 40% acryl-bis, 2.5ml 1.5M Tris HCl(pH8.8), 100μl 10% SDS, 4.85ml D H_2O, 5.0μl TEMED and 50μl 1% APS (prepared fresh). Acryl-bis, Tris-HCl and SDS were combined and degassed for 15 minutes. The TEMED and 1% APS were added and mixed. Prepared gel plates were then immediately filled with the solution using a pipette. Plates were filled to the line and D water added to flatten the surface while the gel set for approximately 45 minutes.

The 4% stacking gel was prepared from the following: 600μ 40% acryl-bis, 1.5ml 0.5M Tris-HCl(pH6.8), 60μ 10% SDS, 3.8ml D H_2O, 6.0μ TEMED, 30.0μ 1.0% APS (prepared fresh). Stacking gel was prepared in the same manner as the plate gel described above. Prepared solution was then added with a pipette to the top of the gel plate from which the water had

been removed. The well card to form the wells was then inserted and the gel allowed to set.

The following samples were used in the gels: supernatant of filtrate before mixing, mixed filtrate used on the plants, supernatant of .45μ filtrate before mixing, mixture of filtrate filtered with .45μ filter, ETOH concentrated protein with .45μ filtration and a mixture of ETOH protein pellet with out .45μ filtration. The ethanol precipitated protein was prepared by first centrifugation of the filtrate/ETOH soluiton at 5000rpm for 10 minutes. The protein pellet was then dried with ETOH and further dried in a vacuum. The pellet was then redissolved in 100μl of D water. 45μl of the samples which were described above were mixed with 15μl of buffer in micro centifuge tubes. Tubes were then capped, mixed and placed in boiling water for two minutes.

Samples were placed in the wells with a small syringe to the top of the well without mixing samples. The samples were placed in the gel from left to right in the following order: standard, ETOH mixture with no secondary filtering, ETOH supernatant filtered at .45μ, supernatant of .45μ filtrate, mixed solution filtered at .45μ, plant sample supernatant, sample miture, and concentrated sample. Running buffer was prepared as described in silver nitrate staining (Morrissey,1981). Electrophoresis equipment was then connected and the gels were run at 50v for 20 minutes and then 70v for 45.

After electrophoresis, the gels were removed from the plates and fixed in the following manner before staining. Gel was prefixed in methanol and 10% acetic acid for 30 minutes. This was followed by a bath of 5% methanol and 7% acetic acid for 30 minutes. The gel was then fixed for 30 minutes in 10% glutaraldhyde (E.M. Sciences, biological grade). After the 30 minutes the gel was rinsed in distilled water. Gel was soaked overnight in a large volume of distilled water and the next day rinsed briefly in D water. Gel was then soaked in 5μg/ml dithiothreitol (DTT), enough to cover, for 30 minutes. The DTT was poured off and without rinsing 150ml of 0.2% silver nitrate was added and the gels treated for 30 minutes. After the time elapsed, the gel was rinsed once rapidly in a small amount of deionized water and then twice rapidly with a small amount of developer. The gel was then soaked in approximately 300ml of developer (50μl of 37% formaldehyde in 100ml of 3% sodium carbonate) until the desired level of staining is attained. Staining process is stopped by adding 5ml 2.3M citric acid directly to developer and agitating for 10 minutes. The solution of developer and citric acid was then discarded and the gel washed several times in deionized water over a 30 minute period. Gels were stored in zip-lock bags with a small amount of D-water.

For the Coomassie Blue staining, the gels were prefixed as described above and then the prepared stain allowed to remain on the gel overnight. Gels were rinsed with deionized water several times the next day.

Results and Discussion

Both gels stained in Coomassie Blue and silver nitrate showed the same results. All gels and replications showed a strong band of proteins at approximately 35 kilo Daltons (Fig.11). Of the different preparation methods employed in purifying and concentrating the sample, only the mixture of ETOH precipitate with no 45μm filtering showed a strong tendency to be unusable on the gel. The other six runs as described above revealed primarily a singular band of protein. Most encouraging was the presence of a single band from the crude sample, concentrated sample and the sample supernatant. While no determination can be made regarding any chemical activity relating the presence of the protein to growth inhibition; the presence of a single band of protein from a crude extract is unusual.

No further work was done due to time and financial limitations. While the identification of this protein is not essential or necessary, the presence of the protein from an easily grown plant would seem to indicate that it be identified to see if it has any significance.

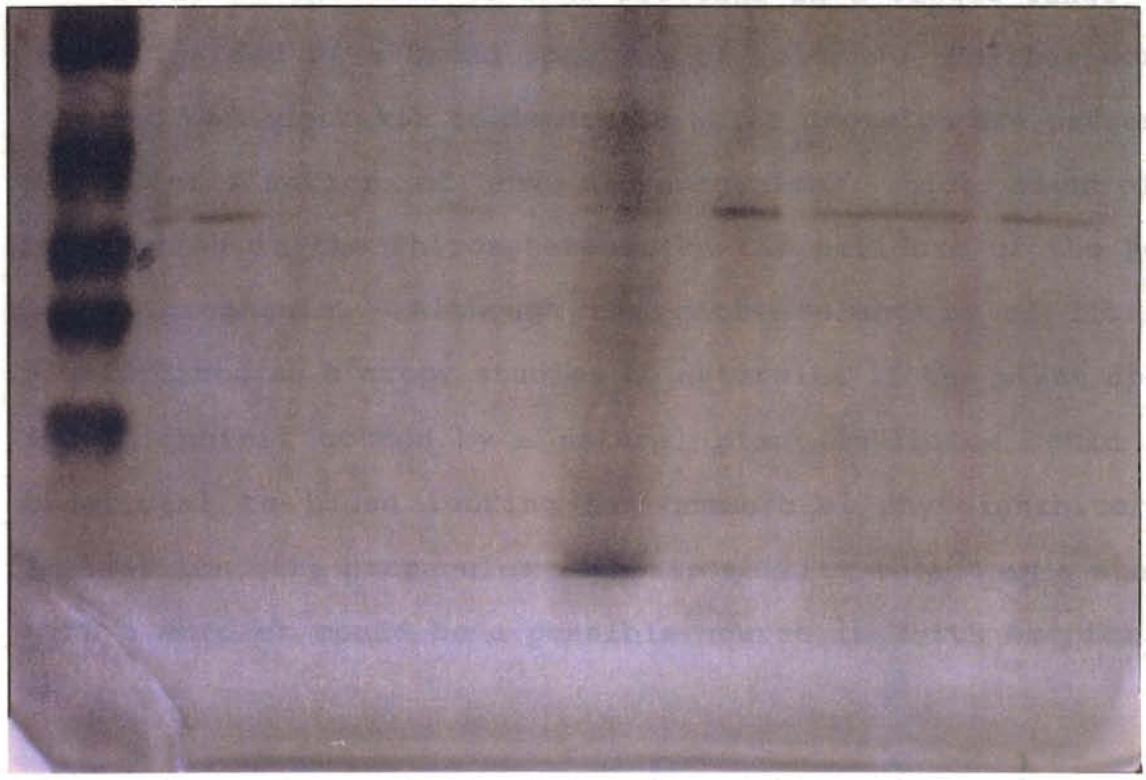

Figure 11. SDS-PAGE gel stained with silver nitrate.

Conclusion

Presence of water soluble proteins exuding from the exterior of an underground storage structure is not unusual as one would expect such a structure to secrete various enzymes and amino acids for the procurement of nutrients from the soil. In addition, microorganisms which might be present in the rhizosphere of a non-nitrogen fixing legume would be expected to interact in some degree to the presence of exudates from the plant.

It is of interest to find proteins in a single range of weight instead of a broad spectrum of weights. Further work needs to be undertaken to determine which proteins are present and identification of any microorganisms which might be interacting in the rhizosphere or on the periderm of the hog potato propagule. Although the native plant is of little significance as a crop, studies to determine if the plant does indeed inhibit cotton by a natural plant inhibitor could be beneficial to those looking for commercial phytoinhibitors. In addition, the propagules wash/sap ability to act as a stain with a mordant could be a possible source in North America.

Chapter VI

SUMMARY AND CONCLUSIONS

Summary

The current studies have been focused on eliminating some of the confusing names regarding morphological origin of the H. glauca propagules. While this is useful, the major thrust of this research has been an effort to determine what part of the hog potato causes a reduction in growth and loss of production in the cotton plant. The focus of this study has been on the testing of the underground structures to determine if an active chemical agent is derived from the periderm of the propagule and the associated root structures. While an effort was made to elucidate some of the factors relating to the probable source of the active component; no definitive results were obtained due to the complexity of the variables associated with the propagule. Some of these factors are the composition of the periderm, micro-organisms that might have been associated with the propagule in its natural environment, the composition and pH of the soil and the morphological differences in the structure of the periderm of the propagule due to age.

As the propagule matures, its periderm changes color from a light tan to a dark, reddish-black covering which is very hard and brittle. In addition, the soil surrounding the propagule takes on a dark, gummy texture for several millimeters from the periderm of the propagule as it matures(Fig.3). The horizontal roots (Fig.4) also appear to develop a thickened covering as they mature. The hog potato grows in alkaline soils which often have a high iron-oxide content. The possibility exists that the propagule of H. glauca may exude substances which could have chromogen or siderophoric properties which may bind to metal ions in the soil. As seen in Figures 1-3; anomolous bodies are present in the region of the outer cortical cells and especially in the cells adjacent to the periderm. Haslom(1981) describes tannins as polyphenols which may precipitate alkaloids and proteins, form complexes with organic matter or minerals and may exert growth inhibitory function by inhibiting growth induced by gibberillins. While the study did not address the reddish substance in the cells of the propagule as being polyphenolic; clearly a substance does accumulate on the exterior of the periderm as the propagule matures. Earlier descriptions in the text of the subfamily Caesalpinoideae describe genera related to Hoffmanseggia as containing tannins as well as glucosides and neoflavonoids. The neoflavonoids are the red dyes of the genera Caesalpinia and Haematoxylon which bind to metal ions to produce commercial biological stains. The ability of the cytosol of Hoffmanseggia glauca to form an insoluble black stain in the presence of iron suggests

that the hog potato may have compounds with similar attributes. Hahlbrock(1981) describes all flavonoids as being derived from acetate and phenylalanine. He further defines neoflavonoids as being equivalent to 4-phenylcoumarins. As the coumarins are glucosides, then it is possible that the subfamily Caesalpinioideae may contain genera other than those listed in the literature which contain these compounds. Brown(1981) and Bearden(1980) in their summaries of large numbers of studies; state that coumarins, flavonoids and the cinnamic acids can act as inhibitors of germination and root development. The phenolic compounds may also suppress mitochondrial metabolism and interact with IAA oxidase resulting in the reduction of IAA. The reduction in growth and vigor in cotton, due to the interaction with the hog potato, shows symptoms which could be associated with the interference in growth regulation.

In all fairness, it must be noted that various derivatives of all the phenolic compounds produced by plants or their synthetic analogs may have either a stimulatory or inhibitory affect on growth hormones or enzymes. However, the inhibitory effects on growth regulation of plants has been well documented.

The hog potato sends out roots in the soil which contact a great amount of soil. Although the root mass is not thickly fibrous, the horizontal runners spread quickly through the clay soil canvassing the upper surface and delving to over a meter deep. This ability to survive a harsh environment as well as the chemical compounds which have been identified in

other members of the Fabaceae would seem to suggest the hog potato interferes with cotton by hormonal or enzyme regulation. The evidence presented should at least warrant the identification of the active principle in the propagule which causes that interference.

Conclusion

Previous studies in the literature have focused on H. glauca as a weed of little value with a great deal of confusion over the scientific name of the plant and doubt about the proper designation of its anatomical structures. Most of the recent research has looked at the hog potato in the light of the loss of cotton boll production it causes to some cotton fields. A start has been made in an effort to identify the active principle that causes the loss of cotton plants and their crop. Due to the limited impact on cotton farmers, the chances are very unlikely that suchresearch will be continued. Chemicals such as Arsenil and Spike have been developed which provide some control of the hog potato, but the limited sites for which it is a problem will most likely prevent any marketing of the control chemical. In an era when research funds are becoming increasingly limited, only those plants which show a marketable return are likely to be studied.

The present study has been initiated in an effort to renew interest in one of the few remaining native plants which

seems to have some useful qualities that have not been identified. The hog potato is a native food source which is highly resilent in its ability to flourish on poor soil. Many of the family members related to H. glauca in the subfamily Caesalpiniodeae are used to provide chemicals for various human needs. As the interest in biological agents and searches for wild genomes continues, it is most likely only a matter of time before an enterprising individual finds useful properties of the hog potato.

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