ALLELOPATHY IN CUCUMBER (CUCUMIS SATIVUS L.)

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ABSTRACT

ALLELOPATHY IN CUCUMBER (*CUCUMIS SATIVUS* L.)

BY

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Selected cucumber accessions were compared with the cv 'Pioneer' for growth interference of several weed indicator species. Plant introduction (PI) 169391 suppressed proso millet (*Panicum miliaceum* L.) populations 10-57% in three of five field evaluations, whereas 'Pioneer' had no suppressive effect on germination. Reduced weed suppression by cucumber coincided with increases in rainfall and soil organic matter content. Inhibition of weed germination decreased as the distance from cucumber to weed seed increased. Inhibition of germination was attributed to allelopathy rather than competitive interactions.

Growth analyses during the time period associated with biochemical interactions indicated that the allelopathic PI 169391 is not quantitatively superior to 'Pioneer' in net assimilation or relative growth rate. Leaf area ratios indicated that PI 169391 may have a greater competitive shading advantage than 'Pioneer'. Allelopathy appears to be the more important component of interference during the early growth stages of PI 169391.

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Seed tissue and extract bioassays demonstrated that PI 169391 seed testa contain a water-soluble germination inhibitor which is both intra- and interspecific. Fermentation, leaching, or the addition of the adsorbant activated charcoal eliminated the growth inhibition. Similar toxicity was obtained with PI 169391 fruit juice which may indicate the source of the seed coat toxin. Cucumber leaf and root extracts inhibited proso millet, but not cucumber growth. Application of leaf, root and juice extracts to soils of increasing adsorptive capacity decreased their toxicity. Applications of PI 169391 leaf, root and fruit tissue as a soil amendment could reduce weed populations under certain edaphic and environmental conditions.

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By

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INTRODUCTION

Plants growing in both natural and agronomic ecosystems continuously interact to influence their growth and subsequent survival. Some plants are segregated into exclusive associations due to similar adaptability and response to specific environments. Many species are predominant due to a morphological advantage, whereas, others may gain advantage by tolerance to unfavorable growth conditions. Undoubtedly, the composition of many plant communities is influenced by competitive parameters. However, all growth interference cannot be attributed to plant competition.

It is well established that many lower organisms influence each other biochemically. Penicillium notatum suppresses the growth of many bacteria. The importance of the utility of antibiotics has resulted in a rapid accumulation of specific information on microbial biochemical interactions. Although postulation that certain plants release phytotoxins is not new, specific knowledge involving allelopathic interactions among higher plants is limited.

Toxic plant mechanisms are often obscure due to the similar effect of many competitive and biochemical growth parameters. Detailed investigations are necessary to determine the components of plant interactions. The objective of this study was to identify and measure the components of plant growth interference in cucumber. The utility of allelopathic traits in crop plants for suppression of nondesirable plant species indicates potential as a supplemental means of weed control. Manipulation of plant interference may provide several effective and inexpensive techniques for integrated pest management.

CHAPTER 1

LITERATURE REVIEW

Kolines in higher plants. Kolines is the term used to describe phytotoxins produced by higher plants. Knowledge of the utilization of natural plant products in pest management has existed for centuries. Democritus (5th century B. C.) advised soaking seeds in the juice of leek flowers to control blight and Theophrastus advocated the use of olive juice to suppress tree root growth (86).

DeCandolle (24) in 1832 was one of the first scientists to suggest that one plant can produce and release a substance toxic to another plant. Liebig (56) originally supported DeCandolle's theory but later as a result of his depletion of soil minerals theory, discounted toxic plant substances as being important in regulating plant growth. Until recently, plant growth interference has been almost unanimously interpreted as resulting from competition. According to Loehwing (58), the 19th century literature did not provide evidence for an important role of any toxic substance in normally aerated soils.

The classical report of Stickney and Hoy (88) in 1881 on the injurious effect of black walnut (*Juglans nigra* L.) to surrounding vegetation was not accepted as a biochemical interaction until the middle of the century. Pickering (71) in 1903 suggested that an inhibitory effect of grass on the growth of apple trees was due to the release of toxic substances. Nutrient competition and oxygen exclusion was ruled out by experimentation and soil poisoning was concluded to be the causal factor. The toxic theory was further emphasized by Schreiner et al. (83) when water cultures from monoculture soils were found to be toxic to wheat seedlings. Toxic chemicals were isolated and identified as picolinic acid, salicylaldehyde, vanillin and dihydroxystearic acid.

Davis (23) provided evidence for the existence of toxic interactions among plants with the identification of juglone as the substance associated with the injurious effect of black walnut. In 1905, Livingston (57) proposed that plants fail to grow in peat bogs because of toxic chemical substances. Cowles (18) suggested in 1911 that plant toxins were important as causative agents in plant succession. Annual plants rarely occur in the Rosmarino-Ericion in France (25). Field soil from this shrub association contained toxic compounds that inhibited the growth of numerous annuals. Parks of black locust (Robinia pseudoacacia L.) are nearly devoid of all other vegetation, and bark and wood of black locust contain compounds toxic to barley (Hordeum vulgare L.) (94). Went (96) reported that certain organic compounds endogenously produced by plants stimulate or retard the growth of other plants.

In some cases, the liberation of organic substances from higher plants has been associated with the 'soil sickness' phenomenon. The difficulty of re-establishing peach (Prunus Persica (L.) Batsch.) trees in old peach orchards has been recognized for years. Proebsting and Gilmore (72) showed that soil incorporated peach roots are toxic to peach seedlings. The effect was duplicated by alcohol extracts of peach roots with the alcohol-insoluble residue being non-toxic to peach trees. It was further demonstrated that the peach roots contain a growth inhibitor but it was not established that the substance leaches into the soil or is active under field conditions. Benedict (6) reported a condition known as 'sodbinding' in which old bromegrass (*Bromus inermis* L.) stands thin out. Leachates from old plants growing in sand culture were toxic to seedlings. Toxicity was further demonstrated by incorporating dried bromegrass roots into the media where seedlings were grown. However, it was not shown that toxic substances from bromegrass roots accumulate in the soil of old stands. Here again, the presence of a toxic substance in plant parts was established but inhibition of plant growth under field conditions could not be directly attributed to the substance.

Recent discoveries of natural occurring plant growth regulators have renewed interest in the biochemical influence of higher plants on each other. Molisch (63) coined the term allelopathy in 1937 to refer to any biochemical interaction among plants and microorganisms. The suggestion that allelopathy should cover both detrimental and beneficial biochemical interactions is erroneous since the term is derived from two Greek words meaning mutual harm.

Grummer (41) in 1955 suggested a system of naming natural occurring inhibitory plant compounds on the basis of the type of plant that produced the toxin and the type of plant affected. Antibiotic was suggested to describe a chemical inhibitor produced by a microorganism that is effective against another microorganism. Phytoncide was proposed as a term for an inhibitor produced by a higher plant that is effective against another microorganism. Furthermore, the term marasmims was suggested for compounds produced by microorganisms that are harmful to higher plants and koline was defined as a chemical produced by higher plants which is toxic to other plants.

The mutual interactions of microorganisms and higher plants are now grouped into individual areas, with allelopathy being used to refer to the effect of a higher plant on another. The currently accepted definition of allelopathy as proposed by Rice (76) in 1974 refers to any direct or indirect harmful effect by one plant on another through the production of chemical compounds released into the environment. The occurrence of allelopathy is dependent upon a chemical compound being added to the environment. Therefore, allelopathy can be differentiated from competition which involves the reduction or removal of some growth factor from the environment that is required by other organisms sharing the same habitat. Muller (65) has suggested the term plant interference for situations where both allelopathy and competitive parameters are evident and inseparable.

Many plant growth responses have been directly associated with allelopathy. Bonner and Galston (10) observed that the edge rows in guayule (Parthenium argentatum L.) plantings had much larger plants than the center rows and that the differences could not be eliminated by heavy watering and/or mineral application. Additionally, roots of adjacent plants did not intermingle but grew in separate areas and the guayule seedlings never grew under large guayule plants. Conversely, the seedlings were commonly found to grow under other shrubs. Experiments with nutrient solutions and distilled water leachates from guayule roots enabled Bonner (9) to identify the principle compound as trans-cinnamic acid, a substance highly toxic to guayule seedlings with growth reduction resulting from as little as 1.0 mg/l of culture solution. The toxic substances produced and released by guayule are rapidly destroyed under cultivation as evidenced by toxicity being associated only with the outside rows in cultivated guayule plantings.

Curtis and Cottam (21) reported that the fairy-ring pattern of the prairie sunflower (Helianthus rigidus L.) is due to an autotoxic inhibitor. A large reduction in plant numbers and flowers was evident in the center of the clone and additional water and nutrients did not alleviate the toxicity. However, when prairie sunflower plants were grown in soil from which all its roots and rhizomes were removed, the plants grew normally and flowered. It was concluded that inhibition resulted from the degradation of plant parts.

Substances capable of inhibiting the germination of tobacco seeds and the respiration and growth of seedlings have been isolated from residues of timothy (*Phleum pratense* L.) and rye (*Secale cereale* L.) (70). Mergen (62) in 1959 reported that natural succession appears to be slow in areas containing tree-of-heaven (*Ailanthus altissima* L.). Alcohol extracts of the rachis, leaflets, and stems of this plant caused rapid wilting of similar plants when applied to the cut surface of a stem. Toxicity also occurred when these extracts were applied to 35 gymnosperm and 11 angiosperm species. Approach grafting of *Ailanthus* with several species gave results similar to those with extracts.

Flax (*Linum usitatissimum* L.) stands are reduced when a small percentage of flaxweed (*Camelina alyssum* L.) is growing among them (43). Plants in close proximity to flaxweed plants produced 40% less dry matter than control plants in which the same amount of water was applied directly to the soil instead of allowing it to fall on the leaves and drip to the soil. Twenty species of native trees, shrubs, and grasses in northern Arizona inhibit the growth of wheat (*Triticum aestivum* L.) radicles (49). Schlatterer et al.(80) suggests that arid regions are abundant with allelopathic compounds due to the lower leaching effects caused by sparse rainfall.

There are numerous examples of weeds suppressing the growth of other weeds or crop plants, but there is limited information on the economic potential of crop plants being biochemically injurious to weeds. Putnam and Duke (73) in 1974 evaluated the world collection of cucumber (Cucumis sativus L.) and identified several accessions toxic to proso millet (Panicum miliaceum L.) and white mustard (Brassica hirta Moench.) in sand culture under controlled environmental conditions. One accession inhibited indicator plant growth by 87% and 25 inhibited growth by 50% or more. The transfer of toxic leachates from pots containing inhibitory cucumbers to indicator plants germinated in separate containers confirmed allelopathy.

Several plant organs as well as different plant species have been identified as being allelopathic. Leaf secretions appear to play a major role in nature as plants interfere for space. According to Rice (76), leaves are the most consistent source of growth inhibitors. Volatile secretions from the burning bush of Moses (Dictamnus alba L.) are so strong that the air surrounding the shrub can be burned during calm weather (1).

Seedling growth and seed germination of fennel (Foeniculum vulgare Mill.) is inhibited by incorporating the leaves of wormwood (Artemisia absinthium L.) into the soil (1). Encelia farinosa L. produces 3-acetyl-6-methoxybenzaldehyde which is toxic to the growth of many plants, but not Encelia (38). The inhibitor produced and released by the leaves is non-volatile, heat stable, and appears to be a neutral compound. The active material was isolated from the leaves by successive fractionations with benzene, water, benzene, and petroleum ether. Recrystallization from ether yielded 0.5 mg of inhibitor per original gram of leaf tissue. Ten grams of *Encelia* leaves per tomato plant caused severe growth suppression and a 2X rate was lethal under field conditions. Chrysanthemum (*Chrysanthemum morifolium* L.) produces a phytotoxin that leaches from the leaves and is inhibitory to the growth and development of other chrysanthemums (54).

The question of active or passive release of growth inhibitors from plants is still obscure. Oxalic acid is reported to be released by living root tissue of wood sorrel (*Oxalis stricta* L.) (34) and viable legume roots are thought to release large quantities of toxic amino acids (92,93). Recently, it has been demonstrated that numerous kinds of organic compounds can be exuded from the roots of donor plants and absorbed by adjacent plants (32,39,48,79). According to Rice (76), it is very difficult to determine if compounds on the outside of the roots are exuded or result from the sloughing off of dead outer cells. Eberhardt and Martin (27) with the aid of the florescence microscope observed the secretion of scopoletin (7-hydroxy-6-methoxy-coumarin) from oat (*Avena sativa* L.) root cells growing in distilled water. This seems to be the only case in which secretions from living cells have been experimentally demonstrated. Furthermore, they found that secretion was greater under unfavorable growth conditions.

There are very few cases where inhibitory root exudates of crop plants have been implicated. Schreiner and Reed (81) and Schreiner and Sullivan (84) have reported toxic root exudates for wheat, oats, and cowpeas (Vigna unguiculata (L.) Walp.). Conversely, there are numerous suggestions of toxic root exudations for noncrop species (2,5,8,12,68,69,74,75, 78,99).

Fruits and seed possess and secrete growth suppressing substances. Many allelopathic effects may be a secondary consequence of self-imposed dormancy, resistance to seed decay and inherent anti-infection mechanisms. The release of endogenous protective toxins into the environment may well be a common strategy for survival. The occurrence of substances inhibiting germination is a common phenomenon and has been found in more than a hundred species (1). Cox et al. (19) reported the presence of water-soluble seed germination inhibitors in the testa of cabbage (Brassica oleracea L. var. capitata). Germinating beet (Beta vulgaris L.) seeds liberate ammonia which prevents the germination of corn cockle (Agrostemma githago L.) (29). These effects are similar to the natural function of antibiotics excreted by soil fungi and bacteria which inhibit or destroy other organisms.

Many germination inhibitors appear to be nonspecific (29). Seed leachates of red clover (Trifolium pratense L.) and the fruits of table beets inhibited the germination of seeds in 28 species belonging to 14 families (35,36). Kuhn et al. (55) reported that mountain ash (Sorbus aucuparia L.) produces parasorbic acid which inhibits germination of field cress (Lepedium campestre L.) seeds in a dilution of 1:1000 and allows 10-80% germination at 1:10,000. Ahshapanek (3) found that seed extracts of buffalobur (Solanum rostratum Dunal) are inhibitory to buffalobur seedlings.

Many fruit juices also contain high concentrations of growth inhibitors. Tomato (Lycopersicon esculentum Mill.) juice inhibits the germination of wheat and oats (53). The fruit juice of Solanum coagulans inhibits the germination of wheat until diluted to 1:64 (29).

Classes of kolines. Growth inhibitors have been found in most families of the plant kingdom. Their presence seems to be wide spread and not restricted to phanerogamous plants. Many of the chemical inhibitors have been termed secondary compounds because they occur sporadically and do not appear to play a role in basic metabolism (33,97). According to Rice (76), there are many thousand secondary compounds, but only a limited number have been implicated in allelopathy. Many of the growth inhibitors that have been identified are phenolics or derivatives of phenolic compounds (15). Aamisepp et al. (1) states that flavones, unsaturated lactones, and phenols constitute the majority of known inhibitors.

Rice (76) proposes that most growth inhibitors arise through the acetate or the shikimic acid pathway. Amino acids, nucleosidic and proteinaceous compounds originate via the acetate pathway and inhibitors originating from amino acids are formed through the shikimic acid cycle. Whittaker and Feeny (97) indicate that most toxic compounds are classified as phenylpropanes, acetagenins, terpenoids, steroids, and alkaloids. They further pointed out that phenylpropanes and alkaloids originate from a small number of amino acids and the rest originate from acetate. The flavonoid compounds are considered hybrids because one ring arises from phenylalanime and the other forms from acetate.

Simple water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes and ketones have been identified as natural plant growth inhibitors. Acetaldehydes, propionic aldehyde, acetone, methanol, and ethanol are released as volatile growth inhibitors by beet, tomato and common morninglory (Ipomoea purpurea (L.) Roth.) leaves and by carrot (Daucus carota L.) roots in a closed environment (22). Patrick et al. (70) reported that acetic and butyric acids are among the toxins produced by decomposing rye. The natural occurring unsaturated lactone, patulin, is toxic to many higher plants (67). Penicillum expansum produces patulin during decomposition of apple root and leaf residues (11). It is further stated that this may be implicated in the apple replant problem.

Juglone (5-hydroxy-α-naphthaquinone) is the only quinone that has been identified as a natural growth inhibitor (76). Robinson (77) reports that higher plants produce a variety of terpenoids but only a small number have been associated with plant toxicity. The monoterpenoids are the major components of the essential oils of plants and also represent the largest group of terpenoid inhibitors that have been identified as toxic to higher plants. Muller et al. (66) identified camphene, camphor, cineole, dipentene, α-pinene, and β-pinene as the volatile inhibitors produced by three species of dessert sage. Wormwood produces three sesquiterpene inhibitors, β-carophyllene, bisabolene, and chamazulene (42).

The cinnamic acid derivatives have been commonly identified as higher plant toxins. Vanillic and p-hydroxybenzoic acid are the most common benzoic acid derivatives that have been identified as allelopathic agents (76). Guenzi and McCalla (44) reported occurrence of toxic cinnamic acid derivatives in wheat, sorghum (Sorgum vulgare Pers.), and oats, whereas, Schreiner and Reed (82) demonstrated that cinnamic acid, o-coumaric and o-hydrocoumaric acid are produced by higher plants and are toxic to seedling growth of many plants. Caffeic, ferulic and chlorgenic acids have also been implicated as allelopathic agents (29).

Coumarins are widely distributed in the plant kingdom and several are involved in allelopathic interactions. Scopoletin and scopolin have been reported as inhibitors in oat roots (30,37,60). Van Sumere and Massart (91) list several coumarins as inhibitors of seed germination in many species from several families of plants.

Flavonoid compounds are widespread in seed plants (45). Aglycones and glycosides are extremely potent toxins to seed germination (76). Kohlmuenzer (52) identified the flavonoid, diosmetin trioside as one of the growth inhibitors in bedstraw (<u>Galium</u> <u>mollugo</u> L.). Quercitrin has been identified as an additional inhibitor in the leaves of wormwood (42).

Tannins are widespread in dicotyledonous plants but only a few have been reported as possible allelopathic compounds. Corcoran et al. (16) reported that β-1-O-galloyl-D-glucose inhibits hypocotyl growth induced by gibberellic acid in cucumber seedlings. Plant residues that contain hydrolyzable tannins often contain gallic or ellagic acid which are potent inhibitors of lower plant life (76). Tannins in the grains of certain sorghum hybrids inhibit preharvest seed germination (46).

Although Evenari (29) emphasized the importance of alkaloids as seed germination inhibitors, Rice (76) indicated that there is no recent evidence of alkaloids playing a role in toxic plant interactions.

No sulfur compounds have been identified as allelopathic. Robinson (77) indicated that there is no conclusive evidence for the production of di- or polysulfides in plants and that they probably arise through secondary transformations initiated by plant enzymes such as the production of allicum when garlic (<u>Allium</u> <u>sativum</u> L.) is crushed. The only purine nucleoside that has been identified in allelopathic reactions is caffeine (76). Evenari (29) includes caffeine as one of the most potent inhibitors of seed germination.

Miscellaneous compounds such as ethylene (63), abscisic acid (4) and agropyrene (29) which is produced by quackgrass

(Agropyron repens (L.) Beauv.) have been implicated as possible allelopathic agents. Abscisic acid and 6-methoxy-2-benzoxazolinone have been identified as growth inhibiting compounds in the primary roots of corn (98). Gross (40) suggests that auxins, cytokinins and gibberellins may be involved in allelopathic plant interactions.

Mechanisms of action of kolines. There is a limited amount of information on the mode of action of natural occurring plant growth inhibitors, however, they apparently affect a variety of plant processes. A saturated aqueous solution of coumarin blocked mitosis in onion (Allium cepa L.) roots within 2 to 3 hours (17). Jensen and Welbourne (50) found a decrease in pea (Pisum sativum L.) root cell numbers 4 to 8 hours after treatment with an aqueous extract of black walnut hulls and trans-cinnamic acid. Volatile terpenes from sage leaf tissue inhibited mitosis in roots of cucumber seedling (64). Toxins from perennial sowthistle (Sonchus arvensis L.), common lambsquarters (Chenopodium album L.), and Canada thistle (Cirsium arvense L.) reduced cell division in wheat and rye (13).

Croak (20) reported that ferulic acid regulates the uptake of four macronutrients and three micronutrients. Scopoletin inhibits photosynthesis of tobacco and redroot pigweed (Amaranthus retroflexus L.) (28). The flavonoids, naringenin and 2',4,4'-trihydroxychalcone inhibits oxidative-phosphorylation in higher plants (87). Turner (90) reported that victorin reduces the transpiration rate of oats.

Some growth inhibitor effects are highly specific. Hydrolyzable tannins inactivate peroxidase and catalase, while a condensed waffle tannin inhibits the activity of polygalacturonase (7). It is evident that some allelopathic agents inhibit pectolytic enzyme processes. Schwimmer (85) found that chlorogenic acid, caffeic acid and catechol inhibit phosphorylase activity in potato. Cysteine and glutathoine have a pronounced effect in deactivating parasorbic acid in higher plants (47).

Factors influencing koline production. Many factors affect the quantity of growth inhibitors produced by higher plants. Ionizing radiation increases phenolic inhibitors in tobacco and sunflower (31,51). Lott (59) increased the chlorogenic content of tobacco (Nicotiana tabacum L. var. Mont Calme brun) 550% in the greenhouse by supplemental uv radiation. Long days increase phenolic acids and terpenes in plants regardless of the photo-inductive cycle needed for flowering (14,89,100). Watanabe et al. (95) discovered a 20-fold increase in scopoletin content of tobacco plants growing in a boron-free solution for 38 days. Subjection of sunflower plants to water stress with a NaCl culture solution resulted in a 16-fold increase in isochlorogenic acids (26). Martin et al. (61) found that 7 times as much scopoletin exuded from roots of oat plants in 72 hours at 30 C than at 135 hours at 19 C. Exudation is no indication of the amount that is actually produced by a plant, but may represent the potential intensity of an allelopathic mechanism. Koeppe et al. (51) reported that scopolin and chlorogenic acid content decreased with age of tobacco leaves.

The occurrence of biochemical interactions among plants is no longer obscure. Natural plant toxins may be directly or indirectly responsible for the regulation of species distribution and density in natural plant communities, and furthermore, may influence weed and crop growth in agroecosystems.

CHAPTER 2

FIELD EVALUATION OF ALLELOPATHIC CUCUMBERS

ABSTRACT

Cucumber (Cucumis sativus L.) accessions which had demonstrated allelopathy under controlled environmental conditions were evaluated against indicator weeds in several field tests over three seasons. Plant introduction (PI) 169391 gave the greatest reduction in populations of proso millet (Panicum miliaceum L.) with a range of 43 to 90% of control in three of five evaluations. The reduction in interference by cucumbers coincided with increases in rainfall and soil organic matter content. Plant introduction 285605 was approximately half as effective as PI 169391 in suppressing weed growth. Toxicity of PI 169391 to proso millet and redroot pigweed (Amaranthus retroflexus L.) decreased as the distance from cucumber to weed seed was increased. Early reduction of weed numbers was attributed to allelopathy rather than competition. Although weed control with allelopathic cucumbers was not consistent in the field, these tests demonstrated that excellent activity could be obtained under certain edaphic and environmental conditions.

INTRODUCTION

Higher plant species influence one another in both natural and agronomic plant communities. This can be partially attributed to their differential ability to compete for abiotic growth factors, however, suppression of growth cannot always be explained by competition. Although knowledge of toxic biochemical interactions among higher plants is still limited, allelopathy has been observed in many ecosystems (16).

The term allelopathy was coined by Molisch in 1937 to refer to any biochemical interaction among plants, including microorganisms (10). Although Molisch's definition includes both detrimental and beneficial interactions, Rice (16) recently proposed that allelopathy should be defined as any direct or indirect harmful effect by one plant on another through the production of chemical compounds released into the environment. By accepting Rice's definition, competition can definitively be separated from allelopathy. Plant competition occurs through a reduction or removal of a growth factor needed by both plants, whereas, allelopathy occurs by the addition of a toxic factor to the environment.

Until recently, allelopathy was commonly considered a part of competition or completely ignored by scientists.

Only in the last few years has there been an increasing awareness of the importance of separating these two concepts. Even so, there are inherent limitations in identifying an allelopathic response since many competitive and allelopathic effects are difficult to separate experimentally.

Cultivars within species with superior competitiveness have been reported and may often be characterized as vigorous plants with large root systems or leaf canopies. In addition, there are numerous examples of growth suppression that can be directly associated with allelopathy. Inhibition of germination and acute root inhibition among seeds and seedlings during the early stage of growth and development are indicative of allelopathy. Growth inhibition by leachates introduced from plants grown in separate containers is excellent evidence for allelopathy (15).

Competition may become a crucial factor in growth suppression during late season in the field, especially if one plant gains advantage by biochemically suppressing another during the initial stages of growth. Total growth suppression of a plant at maturity is the result of the combined effect of allelopathy and competition. In view of this, plant interference better describes overall deleterious effects of one plant on another, encompassing both allelopathy and competition (11). Interference should probably be substituted for the term competition in many previously published papers on weed-crop interactions.

De Candolle in 1835 was one of the first scientists to postulate that plants may excrete compounds injurious to other plants (3). Since that time there have been numerous classical examples of interference among higher plants in which allelopathy is implicated (2,4,6,7,9,17,18).

Patrick et al. (14) demonstrated that toxic products from decaying plant residues are produced under field conditions. Water extracts of large crabgrass (_Digitaria sanguinalis_ (L.) Scop.) have also been shown to inhibit the germination of coronilla (_Coronilla varia_ L.) (1). Neustruyeva and Dobersova reported in 1974 that wheat (_Triticum aestivum_ L.), oats (_Avena fatua_ L.), peas (_Pisum sativum_ L.), and buckwheat (_Fagopyrum esculentum_ Moench.) suppress common lambsquarters (_Chenopodium album_ L.) through biochemical activity (12). Recently, Rice (16) has compiled a thorough review of the literature on allelopathy which indicates common occurrence of this phenomenon among higher plants.

In agronomic cases, most of the allelopathic evidence has been associated with the effect of weeds on crops and crops on crops, however, an important economical potential of allelopathy may be the ability of crops to suppress weeds. Disease and insect resistance as well as stress adaptations have been genetically incorporated into commercial cultivars from wild types. Therefore, the possibility exists that weed suppressing traits may be derived from prototypes possessing an allelopathic mechanism which favors their establishment in natural plant communities. Incorporation of an allelopathic trait into commercial cultivars may enable crop plants to gain an advantage over other species through biochemical activity and subsequent competitiveness.

In 1974, Putnam and Duke screened the world collection of cucumber (_Cucumis sativus_ L.) against proso millet (_Panicum miliaceum_ L.) and white mustard (_Brassica hirta_ Moench.) (15). Of the plant introductions (PI) tested in a controlled environment, one accession inhibited indicator plant growth 87% and 25 inhibited growth by 50% or more. Leachates transferred from several toxic PIs to proso millet grown in separate containers inhibited emergence and plant growth. It was demonstrated that within the world collection of cucumber there are accessions capable of biochemically inhibiting the growth of certain species in relatively sterile media.

To demonstrate the economic potential for weed suppression by a crop it was necessary to determine if the allelopathic cucumber accessions were active in the field. The objective was to determine if selected cucumber accessions could inhibit the growth of several economically important weeds. It is hypothesized that with cucumber, inhibition of germination and early stage weed suppression would be sufficient to allow a vigorous vining crop to gain an advantage over weeds in a field situation. Additionally, high density cucumber plant populations would favor weed suppression in the field.

MATERIALS AND METHODS

Selection for field evaluation. Fifty-one accessions of cucumber and the commercial hybrid cv 'Pioneer' were evaluated against proso millet in 10.2 Styrofoam pots containing sterilized Spinks loamy sand (1.47% organic matter). Twenty indicator seed were planted in a 5 cm diameter circle around each cucumber seed with controls maintained in the absence of cucumber.

Plants were grown in a randomized complete block design with ten blocks. A 16 hr day-length was maintained using supplemental cool white fluorescent light (160 μ E m^{-2} sec^{-1}), and the approximate day and night temperature was 32 and 21 C, respectively. Treatments received 50 ml of half-strength Hoagland's solution at 24 hr intervals after initial saturation. Suppressive growth effects were recorded as inhibition of germination and suppression of shoot fresh weight 28 days after planting.

Allelopathy was verified as being partially responsible for growth interference in the initial selection screen by applying cucumber leachate to indicator plants grown in the absence of cucumber plants. Three accessions (PI 285605, 169391, and 175694) that had previously been shown to interfere with the growth of proso millet, and the non-allelopathic cv 'Pioneer' were grown in 30.5 cm Styrofoam pots containing sterilized quartz sand. Four cucumber seeds were placed in each pot and leached every 12 hr for 15 days with 300 ml of distilled water. A 16 hr day-length was maintained in the greenhouse using supplemental cool white fluorescent light and the approximate day and night temperature was 29 and 18 C, respectively.

Cucumber leachate was collected in aluminum pans and applied daily to 10 replications of 20 proso millet seed in 10.2 cm diameter Styrofoam pots. Growth reduction was recorded as inhibition of germination and shoot fresh weight 15 days after planting.

Primary field evaluation. Plant introduction 169391 and the commercial cv 'Pioneer' were field tested during August 1974 against proso millet and redroot pigweed (Amaranthus retroflexus L.) at the Horticultural Research Farm in East Lansing. Twenty indicator weed seed were planted in a circle at diameter spacings of 0, 1.27, 2.54, and 5.08 cm around each cucumber seed with controls maintained in the absence of cucumber.

Plants were grown in a randomized complete block design with five blocks. Spinks loamy sand containing 1.47% organic matter was fumigated with methyl bromide in early June to eliminate the existing weed population. Two months prior to planting, 66 kg/ha N, K_2O and P_2O_5 was broadcast over the experimental area. Rainfall was supplemented with sprinkler irrigation to maintain a minimum of 1.27 ha cm per 72 hr. Inhibition of germination and suppression of shoot fresh weight were recorded 28 days after planting.

Secondary field evaluations. Cucumber accessions PI 169391, 285605, and the commercial cv 'Pioneer' were evaluated at high density populations against a broad spectrum weed population in 1975 and 1976. The field evaluations included tests on the same soil previously used and an additional test the second year on a Miami silt loam with 3.0% organic matter. Four weeks prior to planting, 55 kg/ha of N, K_2O and P_2O_5 fertilizer was broadcast over the experimental area supplemented with 60 kg/ha of N 4 weeks after cucumber emergence. Four grasses, large crabgrass, proso millet, barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), yellow foxtail (*Setaria glauca* (L.) Beauv.) and four broadleaves, prostrate pigweed (*Amaranthus graecizans* L.), redroot pigweed, common ragweed (*Ambrosia artemisiifolia* L.), and common lambsquarters were overseeded prior to planting.

Twenty-five cucumber seeds were planted on the square, 23 cm apart in 1.3 m^2 plots, and weeded controls were maintained in the absence of cucumbers. Within blocks a comparison of hand weeding and no weeding effects on the growth and development of cucumber was made in 1975. Weeded plots were rogued twice a week to eliminate any effects from other plant species on growth of cucumber. The conditions of the 1976 tests were similar to the previous year with the following exceptions: a) hand-weeded plots were eliminated, and b) PI 285605 was not included. Plants were grown in a randomized complete block design with four blocks. Rainfall was supplemented with sprinkler irrigation to maintain a minimum of 1.27 ha cm per 72 hr.

The weed population in all tests was recorded by individual species 2 weeks after planting and at cucumber maturity. Average fresh weight for individual weed species, cucumber vine fresh weight and total plant population were recorded at harvest in 1975. Harvest date was determined by fruit maturity associated with once-over mechanical harvesting of commercial pickling cucumbers.

RESULTS AND DISCUSSION

Selection for field evaluation. From the fifty-one accessions evaluated for growth interference in sand culture, PI's 285605, 169391, and 175694 suppressed fresh weight and germination of proso millet 78, 70, and 70%, respectively (Table 1). The preliminary investigation by Putnam and Duke showed that fifteen accessions of the world collection of cucumbers inhibited growth of indicator species by more than 75%. The results of the field experiments compared favorably to the earlier experiments in sand culture.

Fresh weight inhibition by the cucumber accessions after 28 days must be attributed to the combined influences of competition and allelopathy. Inhibition by 'Pioneer' was attributed primarily to competition since leachates transferred to indicator plants showed no suppression of fresh weight or germination. Inhibition of germination is logically attributed to allelopathy.

Leachates from the cucumber accessions applied to proso millet in separate containers inhibited emergence and plant growth while leachates from the control and 'Pioneer' had no suppressive effects (Table 2). These data support the previous report that 'Pioneer' is non-allelopathic (15). PI 169391 was the most effective, suppressing fresh weight

Table 1. Growth interference of proso millet in association with selected cucumbers.

Accession or cultivar	Inhibition[a] Fresh weight (%)	Inhibition[a] Germination (%)
PI 285605	78 b	66 b
PI 169391	70 b	68 b
PI 175694	70 b	66 b
'Pioneer'	27 a	0 a

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

Table 2. Suppression of proso millet by leachates from selected cucumbers.

Accession or cultivar	Fresh weight[a] (mg)	Inhibition[b] Growth (%)	Inhibition[b] Germination (%)
No cucumber	42 c	-	-
'Pioneer'	44 c	-	-
PI 285605	27 b	39 a	21 a
PI 169391	20 a	55 b	42 b
PI 175694	29 b	34 a	26 a

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

[b] Inhibition was calculated by using 'Pioneer' as a control.

and germination 55 and 42%, respectively.

Selection of accessions for field evaluation was based on inhibition of germination, suppression of fresh weight and the consistency within a given accession. The initial investigations with allelopathic cucumbers demonstrated that accessions and cultivars differed greatly in their ability to alter plant growth in sand culture (15). These tests in soil also indicated a wide range of variation within a given accession as well as among accessions. PI 169391 was selected for the primary field evaluation since it provided the most consistent growth inhibition and germination and growth was more uniform than either 175694 or 285605.

Primary field evaluations. There were no significant differences between the fresh weight of proso millet and redroot pigweed grown in the presence of 'Pioneer' or in the absence of cucumber (Figure 1). However, PI 169391 significantly inhibited the fresh weight of both indicator species at all spacings. There was no difference in cucumber plant weights among spacings indicating the absence of a dilution effect. Maximum growth suppression of redroot pigweed and proso millet was respectively 73 and 90% at the closest spacing to the cucumber plants.

There was no significant difference in indicator emergence between the control and 'Pioneer' treatments (Figure 2). The allelopathic plant introduction suppressed indicator emergence at all spacings except 5.08 cm for redroot pigweed.

Figure 1. The fresh weight of indicator plants grown in the presence and absence of an allelopathic and non-allelopathic cucumber plant. The allelopathic accession inhibited proso millet and redroot pigweed at all spacings at the 1% probability level and the F value for the interaction of spacing times accession was not significant.

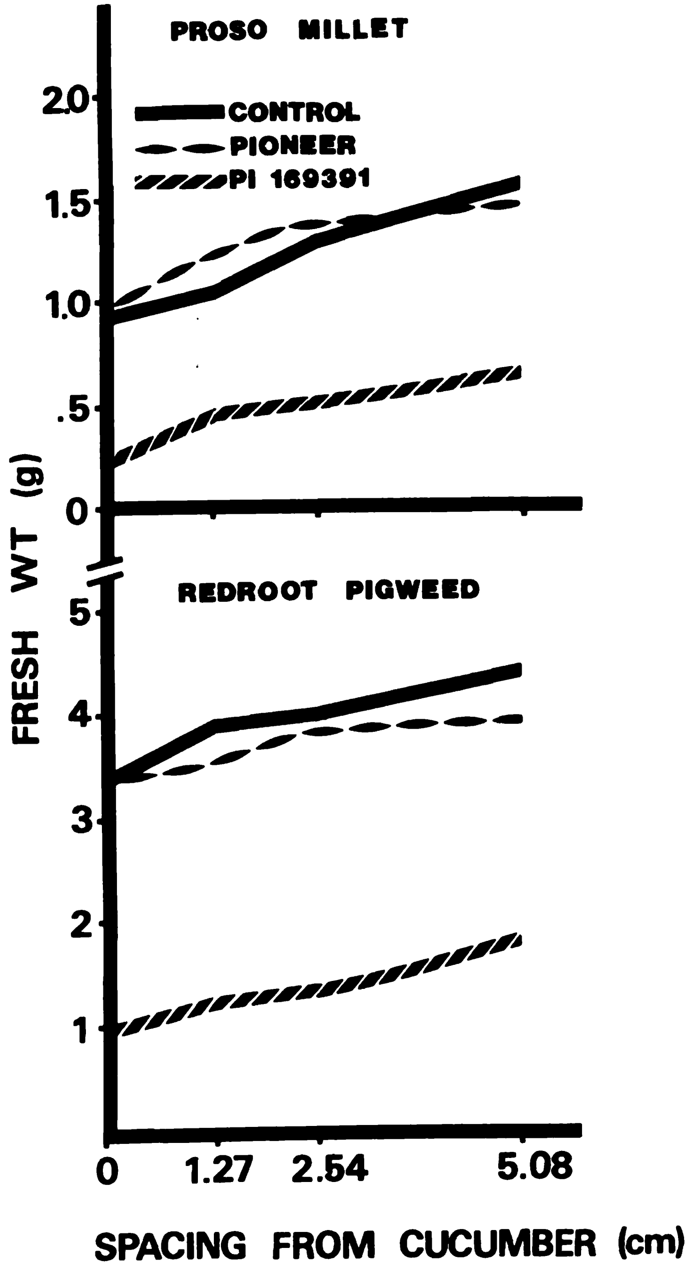
PROSO MILLET
CONTROL
PIONEER
PI 169391
2.0
1.5
1.0
.5
0
FRESH WT (g)
REDROOT PIGWEED
5
4
3
2
1
0
1.27
2.54
5.08
SPACING FROM CUCUMBER (cm)

Figure 2. The emergence of indicator plants grown in the presence and absence of an allelopathic and non-allelopathic cucumber plant. The allelopathic accession inhibited emergence of proso millet at all spacings and redroot pigweed at 0, 1.27, and 2.54 cm at the 5% probability level.

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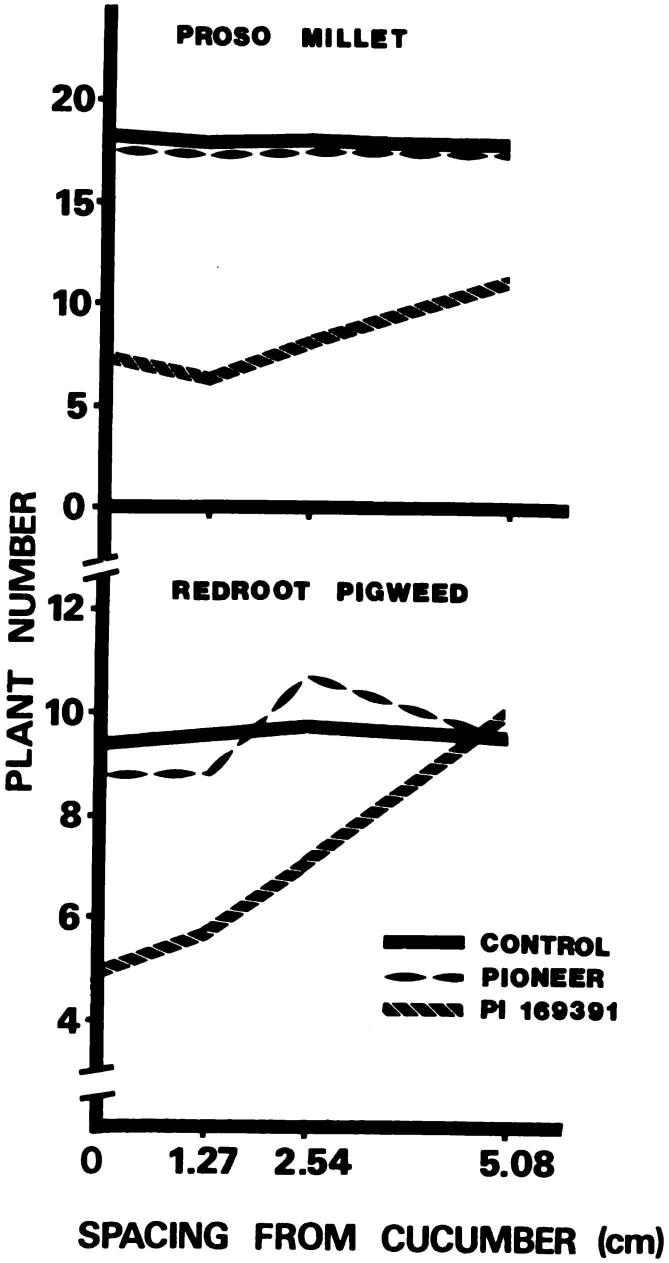

Toxicity of PI 169391 to emergence of proso millet and redroot pigweed decreased as the distance from cucumber seed to weed seed increased. The difference between cucumber lines for the spacing extremes was greater for redroot pigweed than proso millet. This may be explained on the basis of differential susceptibility among proso millet and redroot pigweed or the presence of a toxic gradient. Additionally, the root system of proso millet may have had a greater lateral distribution than redroot pigweed resulting in more contact near the cucumber seed.

The growth and development of a plant is often modified by the proximity of other plants. In a field situation it is often very difficult to separate the influence of competition and allelopathy on suppression of plant weight. However, suppression of germination during the early stage of growth and development can best be attributed to allelopathy.

Secondary field evaluations. Emergence of proso millet and barnyardgrass was suppressed 10 days after planting by PI 169391 and 285605 in 1975 (Table 3). This suppression demonstrates that allelopathy occurs early in the growth and development of field grown cucumbers. The similarity in the control and 'Pioneer' weed population supports previous evidence indicating that the commercial cultivar is non-allelopathic. Plant introduction 285605 was approximately half as effective in suppressing proso millet and

Table 3. Weeds per plot and percent inhibition 10 days after planting in the presence and absence of selected cucumbers (1975).

Accession or Cultivar	Species[a]					
	Proso millet		Barnyardgrass		Redroot pigweed	
	(No.)	(%)[b]	(No.)	(%)	(No.)	(%)
No cucumber	25 c	-	31 c	-	58 c	-
'Pioneer'	21 c	-	26 c	-	39 ab	-
PI 169391	4 a	81	7 a	73	23 a	41
PI 285605	11 b	48	17 b	35	41 ab	0

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

[b] Percent inhibition calculated by using 'Pioneer' as a control.

barnyardgrass emergence as PI 169391 and non-effective on redroot pigweed. This indicated differential quantitative and/or qualitative toxicity between cucumber accessions and resulted in the elimination of PI 285605 for future field evaluation.

Plant introduction 169391 had the greatest suppressive effect on proso millet and barnyardgrass 10 days after planting with inhibition of 81 and 73% respectively in 1975 (Table 3) and 43 and 33% respectively in 1976 (Table 4). During the three year evaluation involving five experiments, PI 169391 suppressed weed growth in three of the five tests. Suppression of redroot pigweed emergence by PI 169391 which occurred in 1975 was not evident in 1976. Population density and fresh weight was not suppressed by any treatment 10 days after planting or at cucumber maturity on the Miami silt loam. Rainfall occurred 12 days after planting the first year as compared to 2.29 ha cm 3 days after planting the following year. The decreased suppression of shoot fresh weight and weed emergence the second year on Spinks loamy sand may have resulted from leaching. Evidence which indicates a high degree of water solubility and early release of the toxic constituents suggests that leaching may remove the chemical from the germination zone. The lack of inhibition on Miami silt loam may have resulted from a combined effect of leaching and/or adsorption by soil.

Population densities of barnyardgrass and redroot pigweed indicated no difference between the control and 'Pioneer'

Table 4. Weeds per plot and percent inhibition 10 days after planting in the presence and absence of selected cucumbers (1976).

Accession or cultivar	Species[a]					
	Proso millet		Barnyardgrass		Redroot pigweed	
	(No.)	(%)[b]	(No.)	(%)	(No.)	(%)
No cucumber	15 b	-	18 b	-	32 a	-
'Pioneer'	14 b	-	16 b	-	28 a	-
PI 169391	8 a	43	12 a	33	30 a	0

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

[b] Percent inhibition calculated by using 'Pioneer' as the control.

10 days after planting (Table 3), with a subsequent significant difference at harvest (Table 5). Three factors that may interact to regulate population density are: a) germination, which initially determines the number of plants per unit area; b) plasticity, which affects the size and weight of survivors; and c) mortality which determines the number of survivors (5,8,13). Monitoring plant populations during the later stages of crop growth and development is important but may be misleading if mortality is not separated from initial germination data. Both germination and mortality must be considered when reporting effects on plant density since the physiological superiority of an older plant on a germinating seedling could result in mortality. It is also conceivable that inhibition of germination may result from reduction of light intensity by a plant that is morphologically superior. In view of this, stand densities should be monitored during the early and late stages of plant growth and development. Early inhibition of weed germination by crops can be directly associated with a biochemical effect whereas later reductions in populations may also be related to competitive parameters.

Suppression of weed populations by PI's 169391 and 285605 remained relatively constant between 10 days after planting and harvest in 1975. The reduction in proso millet and barnyardgrass populations by PI 169391 evident 10 days after planting in 1976 was not present at cucumber harvest. The possibility of early leaching in conjunction with a

Table 5. Weeds per plot and percent inhibition at harvest in the presence and absence of selected cucumbers (1975).

Accession or cultivar	Species[a] Proso millet (No.)	(%)[b]	Barnyardgrass (No.)	(%)	Redroot pigweed (No.)	(%)
No cucumber	28 b	-	38 c	-	54 c	-
'Pioneer'	20 b	-	21 b	-	40 b	-
PI 169391	3 a	85	7 a	67	21 a	47
PI 285605	8 a	60	11 a	48	39 b	0

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

[b] Percent inhibition calculated by using 'Pioneer' as a control.

dilution effect apparently allowed the weed seeds to germinate later and regain normal population density. This suggests that timing and persistence is just as critical for an allelopathic effect as it is in regulating efficacy of synthetic herbicides.

Weed fresh weight at harvest indicated no difference between the control and 'Pioneer' except for a reduction in fresh weight of barnyardgrass (Table 6). PI 169391 reduced the fresh weight of proso millet, barnyardgrass and redroot pigweed 54, 39 and 37%, respectively. PI 285605 inhibited all three indicator species at approximately half the efficiency of PI 169391.

Total weed fresh weight at harvest was reduced by all cucumbers (Table 7). PI 169391 inhibited weed biomass 84%. 'Pioneer' suppressed fresh weight 53% indicating its competitive ability. Strong competitive ability was expected for all lines as the cucumber plants vined and formed a canopy over the soil.

There was no difference in the total weed numbers between the control, 'Pioneer' and PI 285605. Although PI 285605 reduced the number of a few overseeded species, the total weed number was not reduced. Total plant population was reduced approximately 50% when grown in association with PI 169391. It is postulated that the combined effect of allelopathy and competition resulted in the long-term interference of PI 169391 on both weed number and biomass.

PI 169391 produced larger plants than either 'Pioneer'

Table 6. Average fresh weight per plant and percent inhibition at harvest in the presence and absence of selected cucumbers (1975).

	Species[a]					
Accession or	Proso millet		Barnyardgrass		Redroot pigweed	
cultivar	(g)	(%)[b]	(g)	(%)	(g)	(%)
No cucumber	77 c	-	77 d	-	33 bc	-
'Pioneer'	69 c	-	70 c	-	35 c	-
PI 169391	32 a	54	43 a	39	22 a	37
PI 285605	51 b	26	60 b	14	29 b	17

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

[b] Percent inhibition calculated by using 'Pioneer' as a control.

Table 7. Total weed growth per plot in the presence and absence of selected cucumbers (1975).

Accession or cultivar	Fresh weight[a] (kg)	Overseeded and volunteer weeds (No.)
No cucumber	10.4 c	398 b
'Pioneer'	4.9 b	365 b
PI 169391	1.7 a	182 a
PI 285605	3.6 b	386 b

[a] Means within a column followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

or PI 285605 when grown with weeds, although their weights did not differ in weed-free plots (Table 8). This indicates that the superior interference ability of PI 169391 allows it to gain advantage over weeds and make growth superior to other cucumbers. The fact that it is not a larger, more vigorous plant under weed-free conditions lends support to the idea that it gains advantage more by biochemical rather than competitive factors.

These data demonstrate that selected cucumber accessions can inhibit the growth of several weed species under certain field conditions. Early and late growth observations indicate that allelopathy was the primary factor that reduced population densities and total fresh weight. Although some of the results are encouraging, the specific causes for the variation in the field must be identified. Investigations of the effect of moisture levels and soil type interactions are needed. Undoubtedly, their influence on natural products will follow the trends already demonstrated for synthetic compounds.

There are at least two important implications for allelopathic crops. Isolation and identification of the toxic natural products could result in synthesis of them or their analogs for use as herbicides. Additionally, incorporation of the toxic mechanism into cultivars by genetic manipulation may provide at least partial weed resistance. This would provide another strategy for integrated control of weed problems in agricultural ecosystems.

Table 8. Average fresh weight per plant of cucumber vines grown in the presence and absence of weeds (1975).

Accession or cultivar	Weeded[a] (g)	Non-weeded[a] (g)
'Pioneer'	361 c	234 a
PI 169391	358 c	274 b
PI 285605	378 c	214 a

[a] Means within and between columns followed by the same letter are not significantly different at the 5% probability level with Duncan's Multiple Range test.

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CHAPTER 3

MECHANISMS FOR DIFFERENTIAL GROWTH INTERFERENCE BY CUCUMBER

ABSTRACT

Competitive and non-competitive growth parameters were compared in cucumbers (*Cucumis sativus* L.) showing strong and weak growth interference ability. Leachates collected from 0 to 9 days after imbibition from PI 169391 suppressed dry weight and percent germination of proso millet (*Panicum miliaceum* L.) 40 and 46%, respectively. Leachates collected from PI 169391 10 days after emergence to maturity as well as all 'Pioneer' leachates were non-toxic. Growth analyses conducted during the growth period associated with biochemical interactions indicated that the allelopathic PI 169391 was not quantitatively superior to 'Pioneer' in net assimilation or relative growth rate. Leaf area ratios indicated that PI 169391 may have a greater competitive shading advantage than 'Pioneer'. Although slight competitive advantages were evident, there was no indication that potential interspecific competitiveness during seedling emergence would be great enough to suppress germination or early seedling growth. Allelopathy appears to be more important than competition during the early growth stages of PI 169391.

INTRODUCTION

The growth and development of plants is often modified by proximity to other plants and may be influenced at various stages by both chemical and physical processes. Recently, the term allelopathy has been adopted to refer to the deleterious effect of one plant on another through the production of chemical compounds released into the environment (7,8,11). Although allelopathic plants suppress the growth of other plants through biochemical activity, competition may become a crucial factor in suppressing growth and development in the later stages of maturation. At some point after seedling emergence, competitive parameters of one plant may encroach upon another. Therefore, a total growth effect may be the result of the combined effect of allelopathy and one or more competitive parameters.

Competition is purely a physical process as plants compete for water, nutrients, light and space. According to Deschenes (3), competition occurs when the immediate supply of a particular factor is below the combined demand of two or more organisms growing closely together. The result may be a reduction in the rate and total amount of growth or survival of the competing organism. Unfortunately, causal factors of abnormal plant growth have often been too hastily implicated as competition. Very few research publications purporting to demonstrate some aspect of competition have eliminated allelopathy as a possible causal factor. Many scientists have either considered allelopathy a part of competition or completely ignored the phenomenon. Muller (8) suggested the term interference for deleterious effects of one plant on another by the combined interaction of allelopathy and competition.

The potential use of strongly interfering crop plants as a supplemental means of weed control has increased the importance of identifying and separating allelopathic and competitive factors. Putnam and Duke (10) suggest that heritable allelopathic traits may be used to produce a weed resistant commercial cultivar as has been accomplished by plant breeding for insect and disease resistance.

Although bioassays of exudates can provide excellent evidence for allelopathy, it is important to evaluate the role of competitive and non-competitive parameters when measuring plant interference. Allelopathic and competitive growth inhibition can appear similar and a total growth suppressive effect may result from the interaction of several factors. Therefore, a total growth analysis may be more appropriate to access biochemical and physical suppressive factors. One major danger of basing conclusions on the observation of one growth parameter is exemplified by the fact that two different plant responses may be inversely related at various stages of growth and development.

Plant growth may be assessed by a determination of plant height, fresh and dry weight. A total growth analysis offers a more precise means of identifying and measuring allelopathic and competitive growth effects. Blackman (2) developed the first ideas of a total growth analysis with the "compound interest law of plants". Growth analysis as proposed by Evans (4) is one of the best available procedures for comparing the relative growth rates between treatments. The objective was to evaluate the competitive and non-competitive growth inhibition of a cucumber accession that had previously been shown to biochemically suppress the growth of certain weeds during the early stage of growth and development.

MATERIALS AND METHODS

Cucumber exudate bioassay. Plant introduction 169391 and the commercial hybrid cv 'Pioneer' were grown separately in 30.5 cm Styrofoam pots containing 5.62 kg of sterilized quartz sand. Four cucumber seeds were planted per pot with controls maintained in the absence of cucumber. Each pot was fitted with a tygon tube and stopcock to facilitate leachate collection. Plants were grown in a randomized complete block design with three blocks. A 16 hr day-length was maintained in the greenhouse using supplemental cool white fluorescent light (50 μ E m^{-2} sec^{-1}) with approximate day and night temperatures of 30 and 23 C.

Cucumber seeds were initially saturated with 1600 ml of distilled water supplemented with 900 ml of half-strength Hoagland's (5) solution at 48 hr intervals until maturation. Leachates were collected after each supplemental application recycled twice through the media and lyophilized. Freeze dried material was combined into composite treatment samples representing 5 sequential 10 day intervals after the onset of cucumber imbibition. Composite samples were then hydrated to original volume and applied to a proso millet seed bioassay to evaluate biochemical activity.

Twenty proso millet seeds were utilized as a bioassay, and were planted in a 5 cm diameter circle in 10.2 cm Styrofoam pots containing sterilized quartz sand. Plants were grown in a randomized complete block design with six blocks. Leachate samples from PI 169391, 'Pioneer' and control treatments were surface applied to the indicator seeds in 50 ml fractions every 24 hr for 10 days. Plant responses recorded were inhibition of germination and suppression of dry weight.

Growth analyses. Growth parameters of PI 169391 and the nonallelopathic cv 'Pioneer' were monitored in the growth chamber for 10 days after the onset of imbibition. Plants were grown individually in 10.2 cm Styrofoam pots containing sterilized quartz sand. A completely randomized design was utilized with two nutrient levels and five harvest dates. Plants were redistributed within the growth chamber every 24 hr and the two nutrient treatments (distilled water and half-strength Hoagland's solution) were surface applied in 50 ml fractions at 24 hr intervals after initial saturation. A 16 hr day-length was maintained using cool white fluorescent light (175 μ E m^{-2} sec^{-1}) and approximate day and night temperature was 31 and 21 C, respectively.

Plants were harvested at 2 day intervals after the onset of imbibition and oven dried at 45 C. Parameters recorded were leaf area (cm^2), shoot and total dry weight. Growth analyses (4) consisted of leaf area ratio, relative growth and net assimilation rate. The net assimilation rate (NAR) is the increase in plant weight per unit of leaf area over a given time interval where W = total weight per plant (mg), T = time (days), and L = leaf area (cm^2). It is calculated as follows:

$$\mathrm{NAR} = \frac{W_2 - W_1}{T_2 - T_1} \text{ times } \frac{\mathrm{Log}_e\, L_2 - \mathrm{Log}_3\, L_1}{L_2 - L_1}$$

The relative growth rate (RGR) is the increase in plant weight per unit of original weight over a given time interval obtained by the equation:

$$\mathrm{RGR} = \frac{\mathrm{Log}_e\, W_2 - \mathrm{Log}_e\, W_1}{T_2 - T_1}$$

Leaf area ratio is the ratio of leaf area to dry weight of leaves over a given time interval:

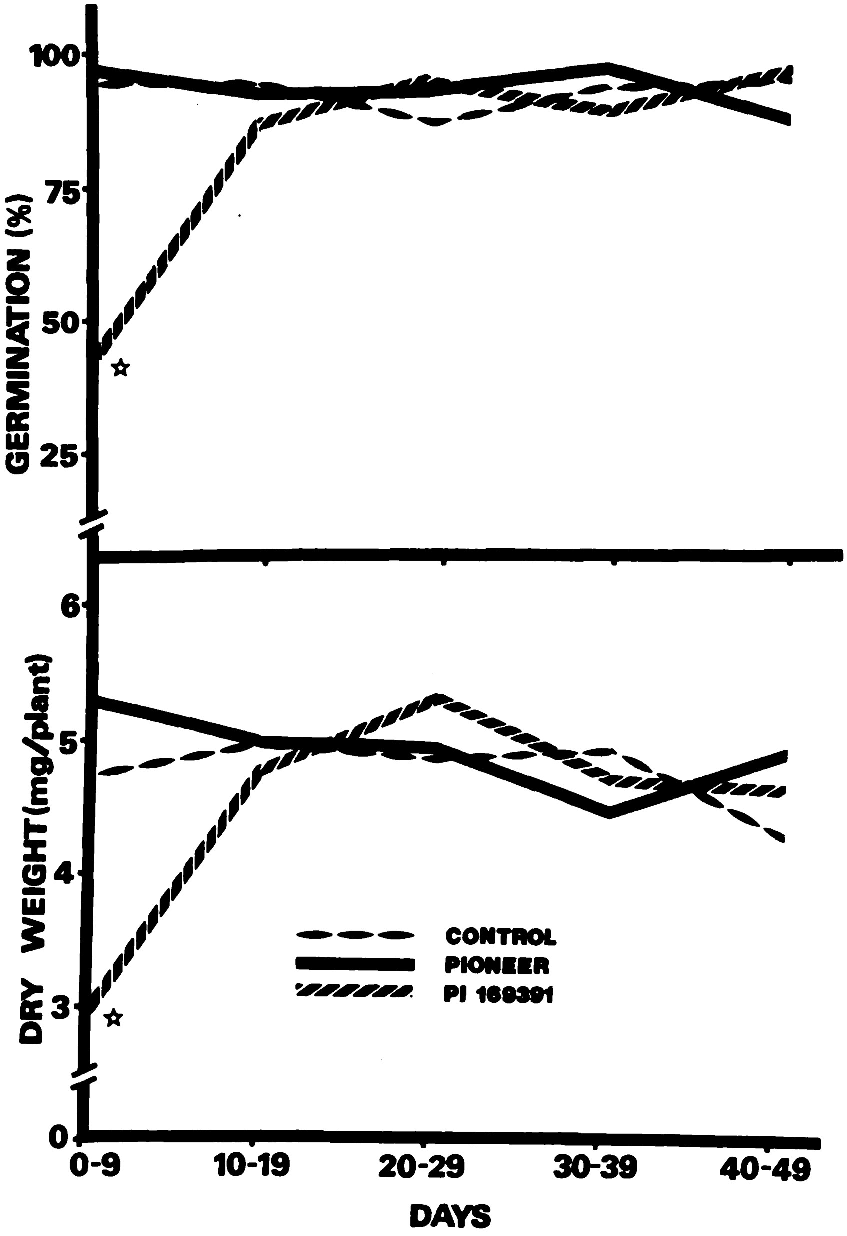
$$\mathrm{LAR} = \frac{L_1 - L_2}{W_1 - W_2}$$

The dry weight data were subjected to an analysis of variance, and means were compared with Duncan's Multiple Range test. Each bioassay was reproduced two or more times.

RESULTS AND DISCUSSION

Cucumber exudate bioassay. There was no significant difference in either the fresh weight or precent germination of proso millet receiving leachate from 'Pioneer' compared to the control (Figure 1). However, leachate collected from PI 169391 between 0 to 9 days after the onset of imbibition suppressed proso millet dry weight and percent germination 40 and 46%, respectively. PI 169391 had previously been shown to exude materials toxic to proso millet and white mustard (*Brassica hirta* (L.) Moench.) 4 to 6 days after seedling emergence under controlled environmental conditions (10). However, there was no evidence of an active toxic mechanism during the later stages of cucumber growth and development. These data indicate that toxin production or leakage terminates or the toxin is reduced qualitatively and/or quantitatively to a non-toxic level 10 days after the onset of imbibition. Further observations indicated that proso millet did not regain normal growth after exposure to a toxic cucumber leachate for 10 days. Either the initial toxic effect was persistent or competitive parameters were sufficient to suppress growth after termination of the initial allelopathic process. Competition may also become a major factor in growth interference by an allelopathic plant during the later stages

Figure 1. The effect of cucumber leachate at 10 day growth intervals on percent germination and dry weight of proso millet. Each observation is the mean of 20 plants. * indicates that means are significantly different at the 1% probability level with Duncan's Multiple Range test.


100
75
50
25
GERMINATION (%)
☆
6
5
4
3
0
DRY WEIGHT (mg/plant)
☆
CONTROL
PIONEER
PI 169391
0-9
10-19
20-29
30-39
40-49
DAYS

of its growth and development.

Growth analyses. Net assimilation and relative growth rate under distilled water and nutrient regimes indicated that the allelopathic accession was not quantitatively superior to 'Pioneer' during the time period associated with the allelopathic phenomenon (Figures 2 and 3). However, PI 169391 demonstrated a qualitative advantage under nutrient deficient conditions by initiating these processes 48 hr before 'Pioneer'. No qualitative difference was evident for NAR or RGR when nutrient solution was applied. Qualitative advantage without quantitative superiority may be beneficial in establishing early growth and development when nutrients are limited, however, the lack of quantitative advantage indicates inferior net assimilation and relative growth capabilities. Therefore, these data indicate that the allelopathic accession is not competitively superior to 'Pioneer' in total net assimilation and/or relative growth rate. Net assimilation and relative growth rate of 'Pioneer' grown in nutrient solution increased with time as compared to the initial increase and later steady-state response of PI 169391. Comparatively, 'Pioneer' shows a greater potential for acquisition and utilization of nutrients.

Leaf area ratios indicate that the allelopathic accession is qualitatively superior and quantitatively similar to 'Pioneer' in a nutrient deficient environment (Figure 4). Additionally, 'Pioneer' shows no competitive advantage when

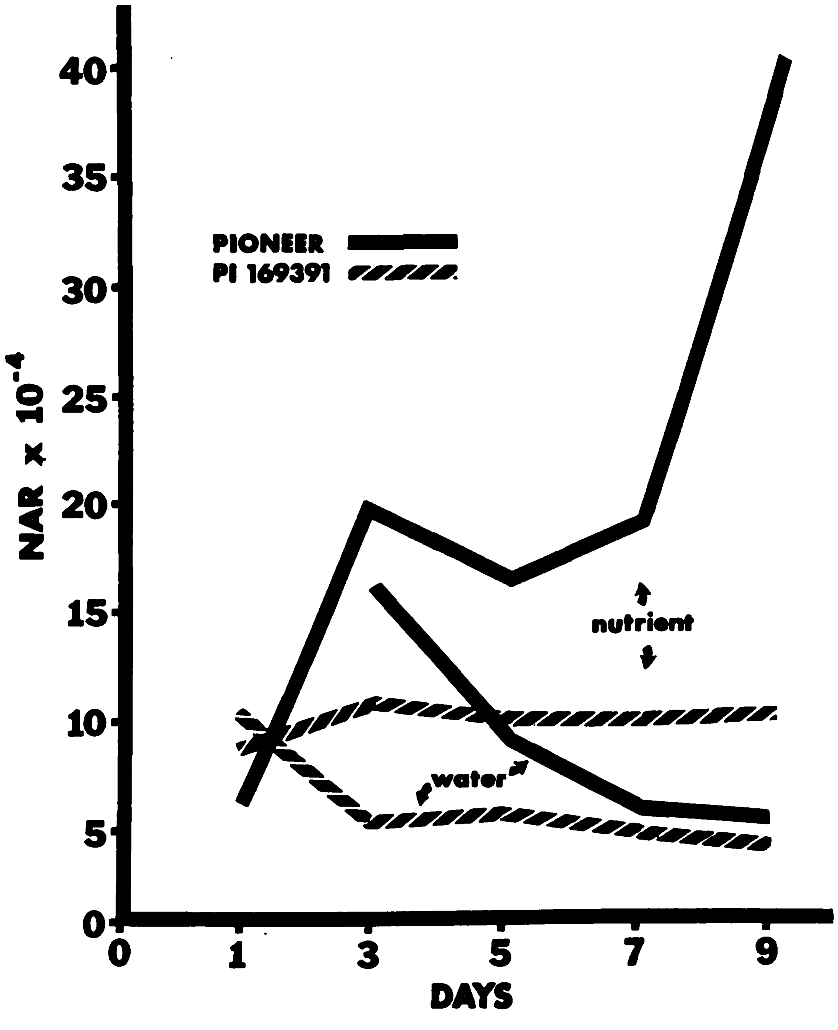


Figure 2. Net assimilation rate of cucumbers. Each observation is the mean of six plants.

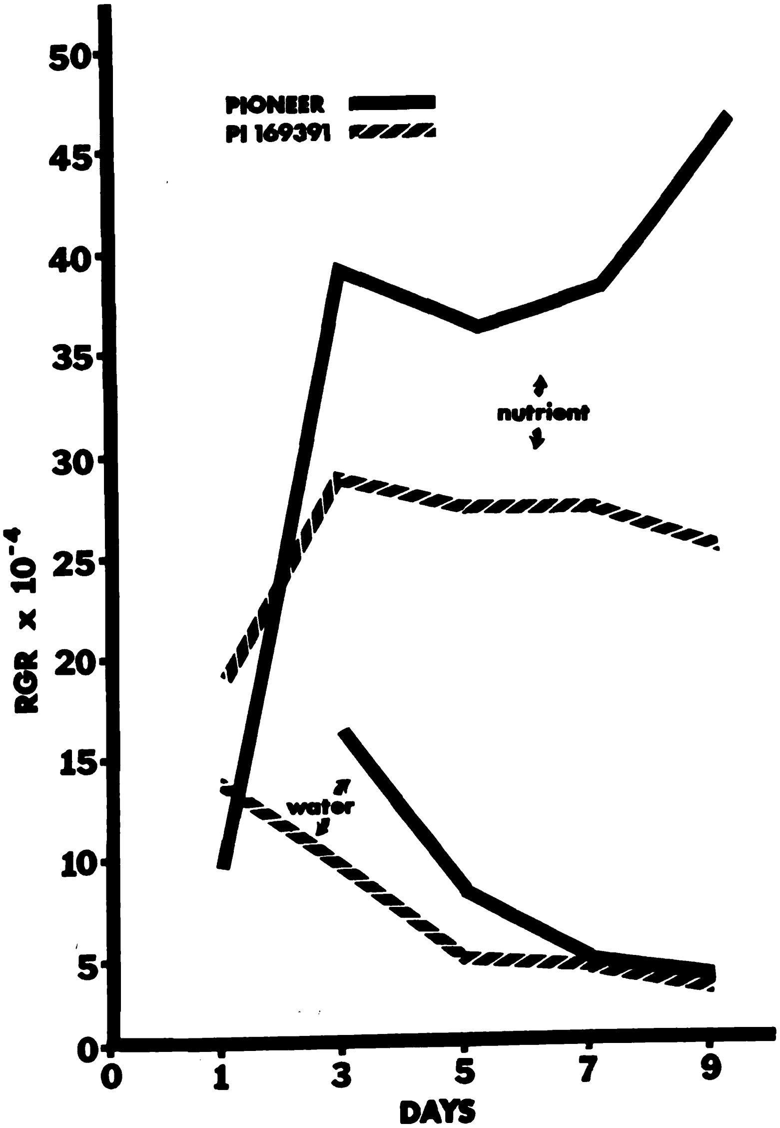


Figure 3. Relative growth rate of cucumbers. Each observation is the mean of six plants.

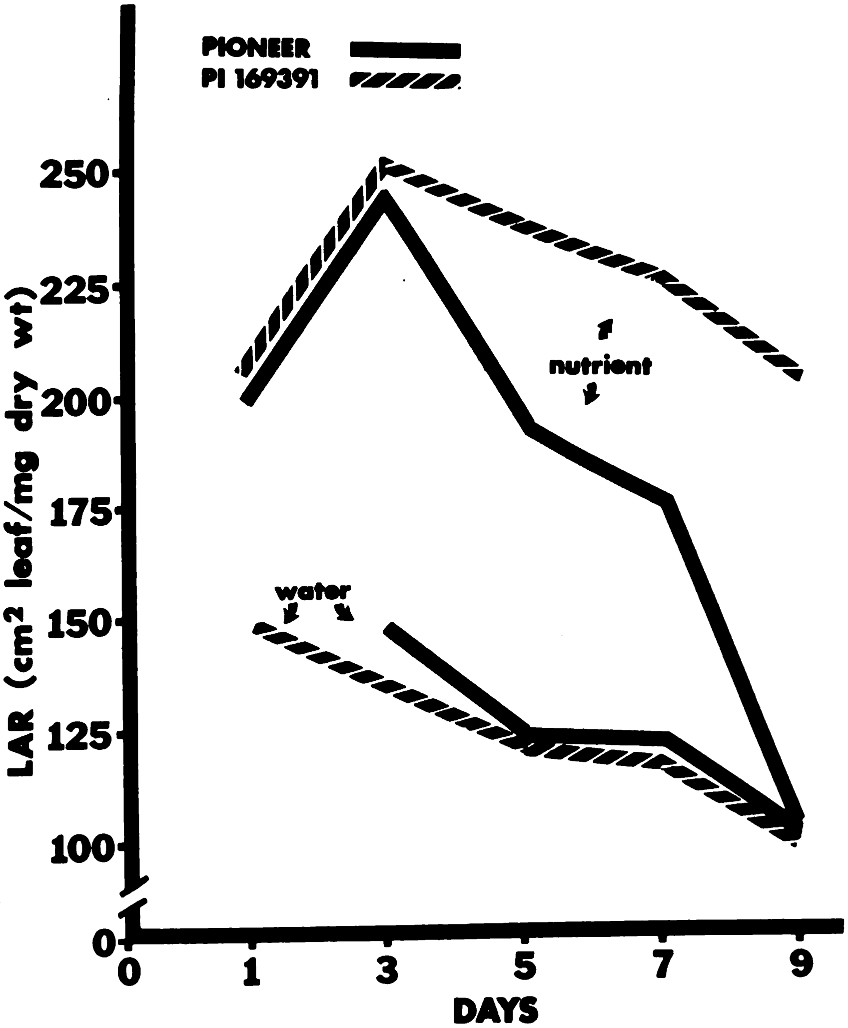


Figure 4. Leaf area ratio of cucumbers. Each observation is the mean of six plants.

nutrients are supplied. Therefore, these data do not eliminate the possibility that PI 169391 could be competitively superior to 'Pioneer' by shading.

The steady-state net assimilation and relative growth rate for PI 169391 between 3 and 9 days after the onset of imbibition does not follow the exponential increase associated with normal seedling growth and development. It is postulated that the toxin which suppresses the growth of proso millet may also inhibit some growth metabolic processes of PI 169391. Autoallelopathic effects have been reported to occur in natural environments (1,9). The early increase in NAR and RGR for PI 169391 at the onset of imbibition associated with a steady-state response may be the result of a concentration response.

The similarity of many competitive and allelopathic growth responses warrants the use of a total differential plant interference growth analysis. Growth analysis is one of the best available procedures for comparing relative plant growth and may be a useful technique to identify and measure allelopathic and competitive effects. Furthermore, steady-state or suppressive growth effects would be indicative of autoallelopathy.

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CHAPTER 4

ASSAYS FOR INHIBITORS ASSOCIATED WITH CUCUMBER SEED GERMINATION

ABSTRACT

Cucumber (Cucumis sativus L. 'PI 169391') seed contain a germination inhibitor that suppresses germination of proso millet (Panicum miliaceum L.). Seed fermentation and/or leaching with distilled water eliminated the inhibition. Non-fermented or leached cucumber seed in the presence of proso millet resulted in germination inhibitions of 57 and 43%, respectively. Inhibition of cucumber and proso millet germination decreased as the maturity of the extracted cucumber seed increased. Germination of PI 169391 in the presence of activated charcoal eliminated toxic growth effects on both cucumber and proso millet. Fermentation, leaching and activated charcoal bioassays indicate that PI 169391 seed is both autoallelopathic and allelopathic during the early stages of seedling development.

INTRODUCTION

Prototypes of many cultivars may possess biochemical mechanisms not associated with commercial crops. Seed germination is variable between wild types and cultivars. According to Horton and Kraebel (6), seeds of many annual herbs lie dormant in the soil for 40 to 50 years before germinating. It is unlikely that seeds remaining in the soil for extended periods of time could resist decomposition without biochemical as well as physical protective mechanisms. Breeding of commercial cultivars for rapid germination may have eliminated many biochemical and physical protective mechanisms.

Plant growth inhibitors are common in organs, seeds, spores and buds. For example, the fungal gemmae of *Marchantia* do not germinate within their sporogonia (13). The resistance of onion (*Allium cepa* L.) varieties to *Collectotrichum circinans* is correlated with bulb scale flavone pigments (3). Randolph et al. (16) reported that endosperm extracts of iris (*Iris Pseudacorus* L.) inhibit the germination of iris. Seed and seedling growth of many plants is inhibited by hydrogen cyanide released from almond (*Prunus amygdalus* Batsch.) and peach (*Prunus Persica* L.) seeds (2). Wheat (*Triticum aestivum* L.) seed germination is inhibited by exposure to tomato (Lycopersicon esculentum Mill.) juice for 2 hours (7). Snapdragon (Antirrhinum majus L.) and alfalfa (Medicago sativa L.) seeds are irreversibly damaged after 7 days exposure to beet (Beta vulgaris L.) seed leachates (2).

Froschel et al. (4) and Ullman (22) have shown that seeds containing inhibitors suppress the germination of other seeds when planted together. Wheat does not germinate when planted with Viola seeds (2). Rademacher (15) reported that wheat and rye (Secale cereale L.) suppress the germination of dogfennel (Anthemis arvensis L.) and Matricaria inodora L. when planted in the same pot.

Seed germination in Petri dishes with filter paper moistened by plant extracts has been used to evaluate germination inhibitors (20,21). A crucial factor is the selection of assay seeds that do not contain variable amounts of autotoxic germination inhibitors. Oat (Avena sativa L.) assays are often unreliable due to the presence of growth inhibitors in the glumes (2). Kuhn et al. (8) have shown that pollen grain germination is more sensitive to germination inhibitors than seeds. However, pollen grain bioassays are impractical due to the non-uniformity of pollen among different anthers and limited availability of test material. Conversely, activated charcoal seed bioassays are an effective means of identifying the presence of germination inhibitors. Activated charcoal readily adsorbs many organic compounds and is a reliable technique for their deactivation.

Plant introduction 169391 of cucumber (Cucumis sativus L.) releases a compound toxic to proso millet (Panicum miliaceum L.) during the early stages of germination (10,14). It was hypothesized that growth suppression of plants in the presence of PI 169391 may have resulted from the release of endogenous germination inhibitors associated with after-ripening and dormancy. Germination inhibitors appear to influence many biological processes and warrant consideration as important factors in plant ecology.

MATERIALS AND METHODS

<u>Fermentation</u>. Cucumber plant introduction 169391 was sib increased in the field in 1975 from 1973 seed obtained from the U. S. Department of Agriculture, North Central Introduction Station, Ames, Iowa. Fruit were harvested in the orange-skin maturity stage (76 days) and the seeds were fermented for 0, 2, 4 and 6 days at 23 C. Twenty proso millet and cucumber seed from each fermentation period were placed together on Whatman No. 1 filter paper moistened with 8 ml of distilled water in 92 mm Petri dishes. Controls were maintained in the absence of cucumber. Seeds were incubated in the dark at 25 C for 5 days in a randomized complete block design with three blocks. Growth effects were recorded as percent germination 5 days after the onset of imbibition.

<u>Leaching</u>. Seed from the 1975 sib increase of PI 169391 were utilized in leaching experiments. Non-fermented seed were placed in 90 ml Buchner funnels and leached continuously for 0, 4, 8, 12, and 16 hours with distilled water. Twenty proso millet and cucumber seeds for each leaching period were placed together on Whatman No. 1 filter paper moistened with 8 ml of distilled water in 92 mm Petri dishes.

Controls were maintained in the absence of cucumber. Seeds were incubated in the dark at 23 C in a randomized complete block design with four blocks. Percent germination was recorded for proso millet and PI 169391 5 days after the onset of imbibition.

Fruit maturation. Plant introduction 169391 was sib increased in the greenhouse in 1977 from 1976 seed obtained from the North Central Introduction Station. Twenty-four plants were grown in 30.5 cm Styrofoam pots containing a 1:1:1 mixture of vermiculite, peat and quartz sand. Plants were watered as needed and fertilized with solutions of 20-20-20 fertilizer. Fruit were harvested 84 days after planting and sorted into maturity groups by color (green, yellow, orange, and bronze). Twenty proso millet and nonfermented cucumber seed from each maturity group were placed together on Whatman No. 1 filter paper moistened with 8 ml of distilled water in 92 mm Petri dishes. Controls were maintained in the absence of cucumber. A randomized complete block design was utilized with three blocks. Seeds were imbibed at 23 C and growth data were recorded as percent germination 5 days after the onset of imbibition.

Seed source. Sib increases of seed from the North Central Introduction Station in 1973 and 1976 were compared with three increases made at the Horticultural Research Farm in East Lansing, Michigan in 1974, 1975, and 1977. Twenty proso millet and cucumber seed from each source were placed together on Whatman No. 1 filter paper moistened with 8 ml of distilled water in 92 mm Petri dishes. Controls were maintained in the absence of cucumber. Seeds were incubated in the dark at 25 C for 5 days in a randomized complete block design with three blocks. Growth effects were recorded as percent germination.

Activated charcoal bioassays. The cucumber cv 'Pioneer' and PI 169391 were germinated in the absence and presence of 10 mg of activated charcoal at 25 C in the growth chamber. Twenty PI 169391 seed from a 1974 sib increase and 'Pioneer' were placed separately in 250 ml aspirator bottles containing 100 ml of distilled water. Filtered air was introduced into each chamber to maintain the oxygen supply and to continuously disperse the activated charcoal. Plants were grown in a randomized complete block design with four blocks. Cucumber seedlings were removed 6 days after the onset of imbibition and 20 proso millet seed were placed in each solution containing cucumber exudates or control solutions. Percent germination, hypocotyl and epicotyl length for cucumber and proso millet were recorded 6 and 12 days after the onset of imbibition, respectively.

Cucumber interactions. Twenty 'Pioneer' and PI 169391 seed were germinated separately and in combination on Whatman No. 1 filter paper moistened with 6 ml of distilled water in 56 mm

Petri dishes. Seeds were incubated in the dark at 25 C for 5 days. A randomized complete block design was utilized with six blocks. Growth effects were recorded as percent germination.

All germination data were submitted to an analysis of variance and means were compared with Duncan's Multiple Range test. Each bioassay was reproduced two or more times.

RESULTS AND DISCUSSION

Fermentation. Suppressive germination effects for cucumber and proso millet were eliminated by fermentation of cucumber seed for 4 and 8 days, respectively (Table 1). These data indicate that proso millet has a greater range of sensitivity than cucumbers to the germination inhibitors in non-fermented cucumber seed. Non-specific germination inhibitors have been identified in the seeds of several plants (1,2,11,18,19,20). Rice (17) suggests that seed inhibition may be one of the most consistent and important ecological roles for allelopathy in annual and many perennial plants growing in natural areas.

Leaching. Additional evidence for seed germination inhibitors in PI 169391 was indicated by leaching studies with distilled water (Table 2). Four hours of continuous leaching eliminated suppressive germination effects on cucumber. The rapid removal of the suppressive effect indicates a potentially high degree of water-solubility. Additionally, the duration of germination inhibition of proso millet in the presence of cucumber seed indicated that it is more sensitive than cucumber.

Lane (9) investigated the dormancy mechanism in

Table 1. The effect of fermented cucumber seed on germination of cucumber and proso millet.

	Germination[a]	
Fermentation time (days)	Cucumber (%)	Proso millet (% of control)
0	32 a	43 a
2	63 b	57 b
4	87 c	82 c
6	90 c	82 c
8	92 c	99 d

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

Table 2. The effect of leaching cucumber seed on germination of cucumber and proso millet.

Leaching time (hr)	Germination[a] Cucumber (%)	Germination[a] Proso millet (% of control)
0	57 a	57 a
4	82 b	76 b
8	90 c	97 c
12	92 c	104 c
16	90 c	100 c

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

sunflower (Helianthus annus L.) seeds and suggested that a water-soluble inhibitor might be responsible for the dormancy. Evenari (2) states that many seeds germinate only after inhibitors contained in the seed coat are washed out by rain or degraded by microorganisms. Water solubility would be an advantage in releasing the germination inhibitors during periods of high moisture which would facilitate seedling survival. Additionally, the leaching capability of a water-soluble inhibitor would facilitate mobilization for contact with other seeds. Germination inhibitors appear to fulfill several biological functions and must be taken into consideration as important factors in plant ecology.

Fruit maturation. An increase in cucumber fruit maturity resulted in an increase in cucumber and proso millet seed germination (Table 3). This indicates that an aging or after-ripening process functions similar to fermentation and leaching in eliminating both inter- and intraspecific effects of germination inhibition. Inconsistent allelopathic effects among seeds and seedlings may be directly related to leaching, fermentation and/or aging differences among test material. Fluctuations in germination of cucumber is indicative of variable responses that are often associated with different seed sources (Table 4). Gressel and Holm (5) also found that seed lots of the same crop variety harvested in different years differed in their sensitivity to plant extracts. It is postulated that the variable germinating

Table 3. The effect of cucumber fruit maturity on cucumber and proso millet germination.

	Germination[a]	
Fruit color	Cucumber (%)	Proso millet (% of control)
green	8 a	22 a
yellow	69 b	63 b
orange	75 b	75 c
bronze	86 c	94 d

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

Table 4. The effect of seed source on cucumber and proso millet germination.

Seed source (location)	(year)	Germination[a] Cucumber (%)	Proso millet (% of control)
Iowa	1973	82 b	74 b
Michigan	1974	75 a	50 a
Michigan	1975	87 b	91 c
Iowa	1976	83 b	79 b
Michigan	1977	70 a	58 a

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

response of proso millet reflects cucumber variability since all proso millet seed were from one source.

The direct effect of fermentation on germination is indicated by differences between seed increases in 1975 and 1977. The Michigan sib increase in 1975 was fermented for 4 days to increase germination potential. Unknowingly, the utilization of fermentation to increase cucumber seed germination and subsequent field stand density resulted in the elimination of the allelopathic effect on proso millet. Non-fermented cucumber seed bioassays in 1977 confirmed the relationship of cucumber seed fermentation on germination of cucumber and proso millet.

Suppression of proso millet germination by Iowa seed in 1973 (10) was markedly greater than a bioassay of the same seed lot in 1977. This indicates the possibility of an aging effect on the activity of the toxic mechanism. The germination inhibitor may be degraded during a long storage period.

<u>Activated charcoal bioassays</u>. Germination of PI 169391 in the presence of activated charcoal increased cucumber germination and hypocotyl growth (Table 5). Activated charcoal had no effect on epicotyl growth of PI 169391 or any growth parameter of 'Pioneer'. Evenari (2) indicated that roots are generally much more sensitive to germination inhibitors than the coleoptile or plumule.

The superior growth of PI 169391 in the presence of

Table 5. The effect of cucumber leachate on growth of cucumber.

Treatment	Growth parameter[a]		
	Germination (%)	Hypocotyl (cm)	Epicotyl (cm)
'Pioneer'	92 b	4.7 b	1.0 a
'Pioneer' + charcoal	90 b	4.8 b	1.1 a
PI 169391	65 a	3.2 a	1.6 b
PI 169391 + charcoal	93 b	6.8 c	1.7 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

activated charcoal is indicative of autoallelopathy. Additionally, allelopathic effects of PI 169391 are verified by the proso millet bioassay (Table 6). The activated charcoal bioassay shows potential as a rapid and inexpensive technique for assaying a biochemical component of plant interference. Furthermore, reclamation and elution of charcoal particles may be an effective means of collecting and concentrating plant exudates in an aqueous media.

Cucumber Interactions. Exudate transfers and activated charcoal bioassays demonstrated that the commercial cv 'Pioneer' is non-allelopathic. Germination of PI 169391 separately and in combination with 'Pioneer' also indicated that the commercial cultivar was not suppressed by the allelopathic mechanism associated with PI 169391. 'Pioneer' seed germination was approximately 90% irregardless of the presence of PI 169391.

The non-toxic cucumber association between 'Pioneer' and PI 169391 may be advantageous if the allelopathic trait of PI 169391 is genetically incorporated into a commercial line as a means of supplemental weed control. The potential for manipulation and utilization of biochemical survival traits in plants to control other organisms is encouraging. It is suggested that total growth analyses in conjunction with activated charcoal bioassays will facilitate the investigation of both physical and biochemical components of differential plant interference.

Table 6. The effect of cucumber leachate on growth of proso millet.

Treatment	Growth parameter[a]		
	Germination (%)	Hypocotyl (cm)	Epicotyl (cm)
Control	90 b	2.4 b	.40 b
'Pioneer'	87 b	2.1 b	.36 b
'Pioneer' + charcoal	88 b	2.5 b	.33 b
PI 169391	55 a	1.4 a	.13 a
PI 169391 + charcoal	90 b	2.5 b	.30 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

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CHAPTER 5

GROWTH INHIBITORS IN ALLELOPATHIC CUCUMBER PLANTS

ABSTRACT

Testa from cucumber (Cucumis sativus L. 'PI 169391') seeds biochemically suppressed both cucumber and proso millet growth. Extracts of other seed tissues were non-toxic as were extracts from seed and fruit of the cv 'Pioneer'. Juice extracts from green and yellow cucumber fruit also suppressed growth of PI 169391 and proso millet. Inhibitory effects decreased with fruit maturity. Solvent partitioning demonstrated that the toxic compound is polar. Cucumber leaf and root extracts were non-suppressive to cucumber but suppressed proso millet growth. Application of leaf, root and juice extracts to soils of increasing adsorptive capacity decreased their toxicity.

INTRODUCTION

Extracts of many different plants have been reported to contain plant toxins (3,4,6,8,10,11,16). Alfalfa (*Medicago sativa* L.) hay extracts reduced root and shoot length of soybeans (*Glycine max* (L.) Merr.), peas (*Pisum sativum* L.), oats (*Avena sativa* L.), timothy (*Phleum pratense* L.) and alfalfa (17). Gressel and Holm (9) reported differential susceptibility among varieties of tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum frutescens* L.), and carrots (*Daucus carota* L.) to aqueous weed seed extracts. Fractionation of velvetleaf (*Abutilon theophrasti* Medic.) seeds demonstrated that an inhibitor is derived primarily from the embryo and endosperm. Prutenskaya (19) reported that germinating barley (*Hordeum vulgare* L.) seeds inhibit germination of white mustard (*Brassica hirta* Moench). Water-soluble germination inhibitors that suppress crop growth were also found in the glumes of several sandbur (*Cenchrus*) species (12). A germination inhibitor in the seed coat of sericea (*Lespedeza cuneata* (Dumont) G. Don.) has been implicated as being responsible for the slow germination and poor seedling establishment often associated with this plant (13).

A wide variety of plant tissues have been implicated as sources of allelopathic compounds. An ancient Japanese document indicated that rain water or dew from leaves of pine (Pinus densiflora L.) was harmful to crops growing underneath (20). Extracts of apple (Pyrus malus L.) root (5), dried rape (Brassica Napobrassica Mill.) leaves (14), oat foliage (21), wheat (Triticum aestivum L.) (15), bark of horse chestnut (Aesculus Hippocastanum L.) (22), stems of black mustard (Brassica nigra (L.) Koch.) (2), and seeds of 12 weed species (9) are all reported to contain toxins.

Many organisms convert non-toxic natural plant products into toxins (18). Conversely, water-soluble inhibitors should easily leach out of plant residues after death or maceration due to loss of differential membrane permeability. Rice (20) indicated that the majority of allelopathic occurrences involve decomposing plant residues. Stubble mulch culture has reduced the stand and growth of many plants under certain conditions (15). It has also been suggested that toxic plant residues are derived from a combination of plant and microbial derived compounds.

Inhibition of germination and growth of proso millet (Panicum miliaceum L.) has been associated with the seeds and seedlings of cucumber PI 169391. Although cucumber seeds were implicated in biochemical interactions the toxin has not been anatomically localized in any specific plant tissue. The objective of this study was to evaluate cucumber seed and various plant tissues for toxic biochemical activity and to localize the site of greatest activity.

MATERIALS AND METHODS

Seed tissue bioassay. Seeds from a 1977 sib increase of PI 169391 and the non-allelopathic cv 'Pioneer' were germinated with and without testa in a dark incubator at 25 C. Intact seeds, naked seeds and naked seeds in the presence of testa were placed separately on Whatman No. 1 filter paper moistened with 6 ml of distilled water in 56 mm Petri dishes. All seeds were imbibed for 8 hr prior to treatment to facilitate seed coat removal. A randomized complete block design was utilized with three blocks. Germination and hypocotyl length for cucumber and proso millet were determined 5 days after the onset of incubation.

Seed extract bioassay. Twenty seeds each of 'Pioneer' and a 1977 sib increase of PI 169391 were separated into testa, cotyledon and embryo. Seed tissues and 25 whole seeds of each cucumber were ground separately in a mortar and partitioned between 250 ml of water and chloroform. The aqueous extract was decanted, centrifuged, and the supernatent lyophilized. Chloroform extracts were concentrated with a flash evaporator and air dried in a 9 cm watch-glass.

Samples were hydrated in 25 ml of distilled water, divided into 5 ml aliquots and applied to 20 proso millet seed on Whatman No. 1 filter paper in 56 mm Petri dishes. Proso millet controls were maintained in distilled water. Seeds were incubated in the dark at 25 C and germination data were recorded 5 days after the onset of imbibition.

Cucumber plant bioassay. Ten plants each of PI 169391 and 'Pioneer' were grown to maturity in separate greenhouses to eliminate cross-pollination. Seeds were placed individually in 30.5 cm Styrofoam pots containing a 1:1:1 mixture of vermiculite, peat, and quartz sand. A 16 hr day-length was maintained using supplemental cool white fluorescent light (75 μ E $m^{-2}sec^{-1}$) with approximate day and night temperatures of 31 and 20 C, respectively. Water and a 20-20-20 fertilizer solution were applied as needed.

Plants were harvested 74 days after planting and sorted into leaves, stems, roots and fruit. Leaf, stem, and root samples were dried in a forced air oven at 55 C for 72 hr, and ground in a Wiley mill with a 40 mesh sieve. Juice was collected from fruit that was separated by color into green and yellow categories. Juice samples were centrifuged at 16,300 G and the supernatent was lyophilized.

Extracts were made with 100 mg dry plant tissue/100 ml of distilled water. Five ml aliquots of plant extracts were applied to 20 cucumber and proso millet seed germinated separately on Whatman No. 1 filter paper in 56 mm Petri dishes. A randomized complete block design was utilized with four blocks. Treatments were incubated in the dark at

25 c for 5 days. Growth responses recorded for cucumber and proso millet were germination and hypocotyl length.

Cucumber plant bioassay in different media. One hundred mg of each previously dried PI 169391 plant sample was hydrated with 100 ml of distilled water, divided into 25 ml aliquots and surface applied to proso millet planted in 10.2 cm Styrofoam pots in the growth chamber. Twenty proso millet seeds were planted per pot in a 5 cm diameter circle in sterilized quartz sand, Spinks loamy sand, and Miami silt loam with an organic matter content of 0.0, 1.5, and 3.0%, respectively. Proso millet controls were maintained in distilled water. Plants were grown in a randomized complete block design with four blocks. A 16 hr day-length was maintained with supplemental cool white fluorescent lamps (70 μ E m^{-2} sec^{-1}). Approximate day and night temperatures were 30 and 22 C, respectively. Growth effects were recorded as percent germination 12 days after the onset of imbibition.

All data were subjected to an analysis of variance and means were compared with Duncan's Multiple Range test. Each test was repeated two or more times. Germination was determined by radicle emergence and expressed as percent germination of the seeds that were planted.

RESULTS AND DISCUSSION

Seed tissue bioassay. Removing the testa from PI 169391 seed increased its germination and hypocotyl length 50 and 70%, respectively (Table 1). Similar seed treatment had no suppressive effect on any 'Pioneer' growth parameter. The similar self-suppressive effects of intact and naked seeds plus testa demonstrate that the inhibitor is associated with the seed coat. Baskin et al. (1) reported that leaching and removal of testa from *Psoralea subacaulus* seeds increased primary root growth 73%. Seed coat removal of cabbage seeds resulted in an increase in percent germination and radicle elongation (7).

Intraspecific growth differences among seeds with and without testa are not indicative of a biochemical mechanism. Self-suppressive effects associated with seed coats may be both physical and/or biochemical. However, growth differences for seed with and without testa compared to seeds without testa germinated in the presence of the removed seed coat is definitive evidence for biochemical activity. These data and a proso millet bioassay of cucumber seed tissue (Table 1) further demonstrate that the toxic mechanism is associated with the seed testa. Removal of PI 169391 seed testa increased proso millet germination and hypocotyl length 31 and 73%, respectively.

Table 1. The effect of cucumber seed tissue on germination and hypocotyl length of cucumber and proso millet.

		Seed source[a]			
		'Pioneer'		PI 169391	
Assay plant	Seed treatment	Germination (%)	Hypocotyl (cm)	Germination (%)	Hypocotyl (cm)
Cucumber	Intact seeds	92 a	3.5 a	60 a	2.3 a
	Naked seeds	94 a	3.8 a	90 b	3.9 b
	Naked seeds + testa	85 a	3.1 a	47 a	2.4 a
Proso millet	Intact seeds	94 a	1.7 a	59 a	1.1 a
	Naked seeds	90 a	1.9 a	92 b	1.7 b
	Naked seeds + testa	87 a	1.5 a	52 a	0.9 a

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

Seed extract bioassay. Partitioning of cucumber extracts between water and chloroform demonstrated that the toxic compound is polar and localized only in the seed testa (Table 2). Localization of a leachable toxin in seed testa leads to speculation that the inhibitor may also be a constituent of cucumber fruit juice. According to Evenari (8), many fruits contain non-specific chemicals inhibitory to seed germination such as cyanide, ammonia, ethylene, alkaloids, organic acids, aldehydes, essential oils, phenols, phenolic acids, and unsaturated lactones.

Cucumber plant bioassay. PI 169391 juice extract from green and yellow fruit suppressed cucumber germination 82 and 22%, respectively (Table 3). Hypocotyl length was also suppressed by fruit juice and decreased with maturity. All 'Pioneer' and stem, leaf and root extracts of PI 169391 had no autoinhibitory effect on germination or hypocotyl length. Conversely, PI 169391 cucumber fruit juice, leaf, and root extracts suppressed germination and hypocotyl length of proso millet (Table 4). These bioassays indicate interspecific toxicity of PI 169391 leaf and root extracts accompanied by both intra- and interspecific activity of juice. It is postulated that the plant and seed toxins and/or mechanisms are different, however, this has not been qualitatively demonstrated. Quantitative differences in susceptibility of cucumber and proso millet should not be discounted since growth responses may be a direct effect of concentration.

Table 2. The effect of partitioned cucumber seed extracts on percent germination of proso millet.

	Extraction fraction[a]			
	Water-soluble		Chloroform-soluble	
Ground tissue	'Pioneer'	PI 169391	'Pioneer'	PI 169391
Control	94 a	89 c	89 a	99 a
Seed	89 a	29 b	99 a	90 a
Cotyledon	99 a	94 c	89 a	92 a
Embryo	90 a	89 c	90 a	94 a
Testa	89 a	6 a	89 a	92 a

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

Table 3. The effect of cucumber plant tissue extracts on germination and hypocotyl length of cucumber.

	Extract source[a]			
	'Pioneer'		PI 169391	
Treatment	Germination (%)	Hypocotyl (cm)	Germination (%)	Hypocotyl (cm)
Control	89 a	2.7 a	67 b	1.9 c
Stem	89 a	3.1 a	60 b	2.2 c
Leaf	90 a	3.0 a	67 b	1.8 c
Root	90 a	2.6 a	62 b	2.0 c
Green fruit juice	90 a	3.0 a	12 a	0.7 a
Yellow fruit juice	85 a	3.3 a	52 b	1.2 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

Table 4. The effect of cucumber plant tissue extracts on germination and hypocotyl length of proso millet.

Treatment	Extract source[a]			
	'Pioneer'		PI 169391	
	Germination (%)	Hypocotyl (cm)	Germination (%)	Hypocotyl (cm)
Control	90 a	1.1 a	92 c	1.1 c
Stem	94 a	1.2 a	94 c	1.1 c
Leaf	90 a	1.3 a	65 b	0.7 b
Root	90 a	1.0 a	57 b	0.6 b
Green fruit juice	89 a	1.2 a	15 a	0.3 a
Yellow fruit juice	95 a	0.9 a	48 b	1.0 c

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

The inhibitory effect of fruit juice further indicates the testa as a possible source of the seed toxin. It is also possible that inhibitors in the juice could be absorbed by the seed testa. Qualitative analysis of juice and testa extracts must be utilized for verification.

Cucumber plant bioassay in different media. Leaf, root and juice from green and yellow fruit suppressed germination of proso millet in media with 0.0 and 1.5% organic matter (Table 5). Juice from green fruit was the most toxic and all inhibitory effects decreased as organic matter increased. The adsorptive capacity of increased amounts of organic matter and clay is suggested as the probable cause of decreased toxicity. However, persistence may have been affected by soil permeability, chemical reactions, pH, and/or microbial degradation. These data further indicate the possible use of PI 169391 leaf, root, and green fruit tissue for incorporation or soil amendment weed control mulches in low adsorptive capacity soils.

Table 5. The effect of PI 169391 plant tissue extracts on percent germination of proso millet in selected growing media.

Treatment	Media[a]		
	Quartz sand	Spinks loamy sand	Miami silt loam
Control	92 d	89 d	92 d
Leaf	62 c	65 c	90 d
Root	60 c	65 c	92 d
Green fruit juice	9 a	44 b	82 d
Yellow fruit juice	55 bc	64 c	94 d

[a] Means within a column followed by the same letter are not significantly different at the 5% level with Duncan's Multiple Range test.

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SUMMARY AND CONCLUSION

Plant introduction 169391 suppresses the growth of several plants by the combined effect of competition and allelopathy. Early inhibition of weed germination by a plant can be directly attributed to a biochemical effect, whereas, later population reductions may also result from competition. It is postulated that the combined effect of allelopathy and competition resulted in the long term interference effect of PI 169391 on both weed number and biomass. However, the fact that it was not a larger or more vigorous plant under weed-free conditions indicated that it gains advantage more by biochemical rather than competitive factors.

Although growth interference was not consistent in the field, growth suppression could be obtained under certain edaphic and environmental conditions. Growth interference on low organic and clay content soils and during periods of limited rainfall indicated potentially high adsorptive and water-soluble properties. Undoubtedly, the influence of soil type and moisture level on the action of allelopathic compounds will follow the trends already demonstrated for synthetic compounds.

The potential use of crop plants with strong interspecific growth interference as a supplemental means of weed control has increased the importance of identifying and separating allelopathic and competitive factors. The similarity of many allelopathic and competitive growth effects and their interaction demonstrates the need for a system of assessing both biochemical and physical suppressive mechanisms. One major danger of basing conclusions on the observation of one growth parameter is exemplified by the fact that two different plant responses may be inversely related at various stages of growth and development. Total growth analysis was demonstrated to be a definitive means of monitoring growth interference. Total growth analysis during the early growth stage of PI 169391 indicated that allelopathy is the main component of plant interference.

Fermentation, leaching and activated charcoal bioassays indicated that PI 169391 seed is both autoallelopathic and allelopathic during the early stages of seedling growth and development. It is hypothesized that growth suppression of plants in the presence of PI 169391 may have resulted from the release of endogenous germination inhibitors associated with after-ripening and seed dormancy. Growth differences for seed with and without testa compared to seeds without testa germinated in the presence of the removed seed coat indicated biochemical activity. Cucumber seed extract partitioning demonstrated that the toxin is polar and associated only with the testa. The inhibitory effect of fruit juice further indicated the testa as a possible source of the seed toxin. Additionally, it is possible that the inhibitors in the juice could be adsorbed and/or absorbed by the seed coat.

Plant bioassays demonstrated interspecific toxic activity for leaf and root extracts accompanied by both intra- and interspecific activity of juice. PI 169391 may possess several allelopathic systems. It is possible that compartmentalized leaf and root toxins may be released during certain natural conditions.

There are at least two important implications for allelopathic plants. Isolation and identification of the toxic products could result in synthesis of them or their analogs for use as herbicides. Additionally, incorporation of the toxic mechanism into cultivars by genetic manipulation may provide at least partial weed resistance. The utility of allelopathy may provide another strategy for integrated pest management in agricultural ecosystems.

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