AN ECOLOGICAL EVALUATION OF THE ALLELOPATHIC INFLUENCE OF *JUGLANS NIGRA* ON *LYCOPERSICON ESCULENTUM*

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ABSTRACT

AN ECOLOGICAL EVALUATION OF THE ALLELOPATHIC INFLUENCE OF *JUGLANS NIGRA* ON *LYCOPERSICON ESCULENTUM*

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The allelopathic influence of one plant on another is important not only from an ecological standpoint, but also from the standpoint of plant development and landscape plantings. The black walnut, *Juglans nigra*, is one of many plant species which exerts an allelopathic influence on other plants. This has been noted for many years, but the primary dispersal mechanism has not been determined.

The primary objective was to determine the primary method of toxin entry of *Juglans nigra* and to isolate the toxic material in wilted plant tissue. Toxins may enter the environment by crown or leaf leachates, fog drip, volatilization, plant residues, or root exudates. Root exudates seemed to be the primary mode of entry. The presumed toxic substance, juglone, was not confirmed as being present in wilted tomato plants grown beneath *Juglans nigra* trees. However, certain extraction paper chromatograms did produce the characteristic color test for juglone, but the substance could not be positively found through elution and rechromatography.

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PURPOSE

The purpose of this study was to determine the influence of the various plant parts of one genus on the growth and development of another plant genera. The influence will, of course, vary between plant genera. The inhibitory action of one plant upon another probably occurs quite frequently in natural ecosystems. This action may also occur in areas in which unrelated plant genera are brought together for their aesthetic value or crop production.

Juglans nigra was chosen for this study since it has an extensive history of inhibiting the growth of some other plants, specifically tomatoes. Much work has been done in relation to the toxic influence of *Juglans nigra*, and the question as to which plant part is responsible for the toxic action is still unanswered. Those interested in growing plants for their aesthetic value are still in doubt as to whether or not to plant beneath black walnut trees.

This work, relative to the mode of toxin entry by *Juglans nigra*, was concentrated in three areas; 1) plant residues, specifically fruit, 2) leaf leachates, and 3) root exudates.

The following plants were selected for study:

1. Lycopersicon esculentum (tomato)

2. Pyracantha coccinea (Pyracantha)

The studies were conducted at the Michigan State University horticulture farm, swine farm field, Rose Lake Wildlife Research Area, and the Graham Experiment Station in Grand Rapids, Michigan.

INTRODUCTION

Throughout time it has been observed that certain plants, including Juglans nigra, inhibited the growth of other plants when grown in close association. The nature of the factors involved in this action have been misunderstood by many interested in growing plants for food and their aesthetic value.

Much of this inhibitory action, exerted by one plant upon another, is thought to be due to the natural release of biochemical by-products. The release of such compounds, which may or may not be toxic, by higher plants is characterized as allelopathy. Ecologically, allelopathy is important relative to its influence on succession, dominance, species diversity, community structure, and productivity. It is the purpose here to evaluate the allelopathic nature of various plant parts of Juglans nigra relative to Lycopersicon esculentum.

LITERATURE REVIEW

For the past several hundred years, individuals in agriculture and related fields interested in the science of growing plants have noted that certain plant species inhibit the growth of the same or other genera and/or species of plants. This growth inhibition, usually characterized by a stunting of the plant coupled with a chlorotic appearance of the leaves, seemed to occur widely throughout the plant kingdom with one of the many presumed causative species being the black walnut, *Juglans nigra* L. *Juglans nigra* tends to exhibit both a homologous (inhibiting its own species) and a heterologous (inhibiting other plant genera and/or species) type of inhibitory action (24).

One of the first individuals to refer to the problem of growth inhibition relative to the black walnut was the Roman philosopher Pliny. He stated in his "Natural History," that "the shadow of the walnut tree is poison to all plants within its compass," and "that it may kill any plants with which it comes in contact" (28). In the 17th century, Evelyn rejected the idea of a toxic substance being produced by the walnut, stating that the roots were below the plow layer affecting neither the cultivation nor the growth of plants beneath the walnut (28). Another of the early advocates of naturally occurring plant inhibitors or phytotoxic substances was DeCandolle (17), who in 1832 observed this toxic action. DeCandolle found that spurge inhibited or reduced the growth of flax, flax reduced the growth of wheat, and oat yields were reduced by thistles (68). He believed that excretions of organic material from the roots were the cause of the growth inhibition.

The hypothesis of naturally occurring growth inhibitors was closed by Liebig in 1852, with his studies of soils relative to micronutrients (17,24). Liebig believed that nutrient balances were essential for plant growth. He found that by addition of certain elements, poor growth could be alleviated and that poor growth could be induced by removal of certain elements. His theory, the "Law of Minimum," meaning that plant growth is limited by the element present in the least amount relative to its need by the plant, was postulated in the early part of this century. During the early portion of this century, Schreiner (72) found that soils may be unproductive due to the presence of organic compounds which were toxic to growing plants. Due to this fact, he was largely responsible for reopening the question of naturally occurring growth inhibitors. Excretions of plant metabolism products from roots and other plant tissues may, if allowed to remain in the soil, exert a toxic effect on succeeding crops. Schreiner also found that meristematic portions of root tips in some soils became discolored, swollen, or slimy (72).

Currently, this toxic plant-to-plant interaction is referred to as allelopathy, which was first coined by Molisch in 1937 (8). Previously, most plant interactions were thought to be of a competitive nature for water, light, and nutrients. Allelopathy meant originally the interaction of all plant organisms and microorganisms as well as the interactions of plants relative to the production of products derived from plant metabolism processes (8). This increased the amount of study involving the relationships between higher plants.

All plants may, at some stage of development, produce substances which are toxic to other plants or which may induce a growth response, either stimulatory or inhibitory (2). The degree of toxicity may be relative to the concentration of the toxic substances produced. The concentration of the material may, in turn, be increased or decreased relative to various environmental factors, microorganisms, soil types and structures, soil pH, nutrient and moisture availability, and the plants' ability to metabolize the physiologically active substance (57). Physiologically active substances are also called growth substances and therefore can affect plant growth when applied in small amounts. The production of such substances is regulated in plants, as with cytokinins, I.A.A. (indol-acetic acid), and gibberellins.

Phytotoxic substances must also be released into the environment as well as being of a high enough concentration to cause injury. Many organic and inorganic substances which may be physiologically active are released as by-products of metabolic processes. An example of metabolic by-products are phenolic compounds (57). Entrance into the environment can be through exudates from roots and root parts, leachates from leaves and possibly other above ground parts, and decomposition of plant residues, all relative to breakdown or decomposition by various environmental conditions, soil conditions, and microorganisms. Upon entrance, the physiologically active materials may cause reactions between plants and other plants, plants and other living organisms, as well as reactions of plants or living organisms to themselves and their environment (59). This well-known definition of ecology seems to suggest that substances which are physiologically active and which are released into the environment can and do play a role in the distribution of plants within their various ecosystems through their allelopathic nature. However, for toxic substances to exert an allelopathic effect, the rate at which they enter the environment must exceed the rate that they are converted to a less toxic substance.

Since plants growing together use a mutual medium, it is presumed that there is some root competition. Because of this, the roots were one of the first areas to be considered.

Bedford and Pickering observed that grass had an inhibitory effect on the growth of fruit tree seedlings, and in some cases it caused death. The foliage of apple seedlings was chlorotic, and plant growth was poor. They disregarded such factors as soil temperature, poor or changeable soil aeration, increases in carbon dioxide, and increases in alkalinity or acidity. It was concluded from this that the grass produced a toxin which was leached into the soil from the leaves, excreted from the roots of the grass, leached into the soil from the decay of grass debris, or by an alteration of the bacteria content of the soil relative to grass decay (2).

Cubbon (68), like Pickering, believed that the grass produced a toxin since his work with grapes showed a significant inhibition of the plants relative to size. The hypothesis of Bedford and Pickering was not accepted by Russel, who found that injury characterized by slow root growth, poor nutrient absorption, chlorotic and premature abscission of leaves, and slow fruit maturity could be overcome with applications of ammonium sulfate (40). He therefore concluded that the grass had the ability to tie up excess nitrogen present in the soil.

Ahlgren and Aamodt (1) also working with grass, found that Kentucky bluegrass (Poa pratensis) is extremely sensitive to clovers and Agrostis alba (Redtop). When grown in close association, the bluegrass was almost eliminated relative to growth in a pure stand indicating that perhaps a substance is excreted by the roots of Agrostis alba.

Fletcher determined that Sesamum indicum (Sesame) failed to reach maturity when sown within two feet of Sorghum vulgare (Sorghum) (22). Sesamum died after reaching a few centimeters in height and failed to reach maturity. This was similar to Pickering's results with the apple, and Fletcher, like Pickering, believed a substance was given off by the roots. Fletcher was criticized since he believed that bacteria may have been responsible for the toxic action observed in water cultures and that an environmental stress or lack of nutrients may have been responsible for the action in the field.

Schreiner and Shorey (72), working with soils, found that dehydroxysteric acid could be isolated from poor soils. It was believed to be removed by decomposition and the precipitate was found to be insoluble in water. They were also able to isolate carboxylic, oxalic, salicylic acid, and vanillin from nonfertile soils (72).

Substances produced by quackgrass (Agropyron repens) rhizomes may be harmful to other plants based on research by Kommendahl in which the rhizomes, when mixed with soil, caused stunted and chlorotic alfalfa seedlings. When compared to oat soil leachings, the quackgrass-rhizome soil leachings reduced wheat growth. The rhizomes were frequently found to harbor root rotting fungi, which are responsible for some seedling blights and root rots. A combination of Helminthosporium sativum and quackgrass rhizomes was more inhibitory than the fungus alone, a fungus which resulted in an 80 per cent loss in stands of wheat, oat, or barley seedlings (37).

Through root leachings and related experiments, Bonner and Galston (5,6) found that guayule is inhibited by its own species but not by tomato extracts. These experiments carried out indicate that the substance is organic in nature and that it is derived from the roots. Bonner found that nutrient solutions in which guayule was grown contained a substance inhibitory to the growth of other guayule plants when applied as a soil drench. Upon isolation, the substance was found to be transcinnamic acid. In similar experiments dealing with the soil in place of nutrient solutions, no substances containing cinnamic acid were found, indicating that perhaps it was destroyed by microbial activity. Infertile soils then might be responsible for the failure of a system to destroy, change, or reduce the concentration of the toxic material present.

Proebsting and Gilmore (66), concerned with the difficulty of replanting peaches (Prunus persica) in old field sites, grew seedlings in two different soils and found that when the roots were added to virgin soil, there was a decrease in growth. They also discovered that roots of cherry and apricot trees promoted a reduction of lateral growth but not height growth. It was concluded that the bark of the root and not the wood was responsible for the toxin produced.

Since root systems of plants are in close association, it is conceivable that products of metabolic processes capable of causing inhibition or death could be excreted from living roots. It is also possible that substances once in the pedosphere, or soil, can be acted upon by various environmental factors, soils and soil types, and microorganisms in such a way that a more toxic or less toxic substance is present than that which was first excreted. This could also account for a build-up of phytotoxins capable of severe damage to other or the same species of plants and microorganisms in the ecosystem.

Aside from root contact and excretion of phytotoxins by the roots, another and more visual method of entrance into the environment is available. This is a method known as crown leaching or release of toxins from above ground parts. This includes leachates of leaves, stems, flowers, and fruits. Tukey (76) has shown that leachates are extremely important as a mechanism for the release of phytotoxins. Tukey and Mecklenburg found that inorganic and organic materials can be leached from foliage by rain, dew, or mist (75). Once they are leached, it has been determined that nutrients lost are replenished by root uptake (48). Roger del Moral (18,19), has determined that fog drip also leaches metabolites from the crown of Eucalyptus globulus (tasmanian blue gum) and hence serves as another crown release mechanism. The fog drip alone seemed to be toxic to many herbs when naturally collected beneath Eucalyptus globulus, indicating that absorption of terpenes to soil colloids or leaching of phenolic acids from litter may not need to be combined with the fog drip to develop injury. It has also been observed that nutrients can be returned to the part from which it was removed and that calcium is leached by a process of ion exchange and diffusion which involves not only the outside but also the inside of the leaf (49).

The leaves of Artemisia absinthium (wormwood), when crushed and mixed with soil, retarded the germination of Pisum sativum (peas) and Phaseolus species (bean) (23). Funke observed that thyme bordering Artemisia was reduced in size. Muller (52) found the leaves and twigs of Artemisia to be toxic in that they exerted an inhibitory effect on the germination of Avena fatua (oat) seeds. Salvia leucophylla, as well as Artemisia californica contain terpenes which, upon volatilization, inhibit the growth of seedlings in the California chaparral. McPherson (47) found that shrubs of Adenostoma fasciculatum produce toxins through normal metabolic processes which are in turn dissolved by rains and leached to the soil. Volatile materials produced by leaves of Salvia leucophylla (salvia) inhibit root growth, hypocotyl growth, and production of lateral roots in germinating herb seeds (54,56). The leaves reduced oxygen uptake by mitochondria and the number of lateral root initials in Cucumis sativus (cucumber) (55).

The leaves of Encelia farinosa (encelia) have been found to contain a substance that will inhibit the growth of tomatoes. The leaves were found to be less toxic when mixed with fertile soil than when mixed with infertile soil. Leaves which had been chopped prior to mixing with the soil exerted more of a growth inhibition than those leaves which were mixed in the unchopped state (25).

Bennett (3) determined that Thamnosma montana (thamnosma) contained toxic substances, three of which were toxic or inhibited the growth of tomato plants. Upon isolation, two of the compounds were found to be identical to byakangelicin, having an emperical formula of $C_{16}H_{15}O_6(OCH_3)$ and isopimpinellin, with the emperical formula of $C_{11}H_4O_3(OCH_3)_2$. The most lethal of the compounds was determined to be a derivative of an isobergaptene nucleus, which is an isomer of the other two compounds. The rate for lethality was determined to be 12-15 mg./l. Tomato growth was also found to be inhibited by extracts of Franseria dumosa, and the plants were characterized by wilted leaves and a destroyed root system. Muller (53) also found that Franseria dumosa, Thamnosma montana, and Encelia farinosa inhibit the growth of tomatoes grown in water solutions. Muller concluded that the toxic action is due to a lack of microbial activity, build-up due to absorption to soil colloids, or inactivation of microorganisms relative to substances released by the leaves. Went (78,79) observed that certain annuals depend on certain shrub species in desert situations and were found to grow where the largest amount of organic material had accumulated.

Mergen and others (51), have found that certain woody species also produce substances in the leaves which, when extracted, exert an inhibitory effect. Brown (11) found that of water extracts from 56 plants commonly found in a Jack Pine forest, some inhibited germination, while others stimulated germination. Cornus canadensis (dogwood), Pinus resinosa (red pine), and Pteridium aquilina (pteridium) stimulated Pinus banksiana (jack pine) germination, whereas Prunus pumila (sand cherry), Gaultheria procumbus (wintergreen), and Solidago juncea (golden rod) had an inhibiting effect on germination. Peterson (64) found that dry leaves of Kalmia angustifolia (sheep-laurel) contained a substance inhibiting the formation of primary root development in black spruce. The substance was soluble in ethanol as well as water (50). Mergen determined that the toxicity of

Ailanthus altissima (Tree of Heaven) decreased in stem and rachis extracts as compared to leaflet extracts. This might indicate production of the toxin in the leaves or translocation to the leaves from another part of the plant. The substance also moved upward when applied to a cut stem, and the substance was located in the xylem of the stem and veins of the leaf. If the substance were to move by translocation, this might account for wilting during periods of rapid growth (51).

Aside from plant interactions occurring under natural conditions as with root systems and those that can be observed, leaves and associated leachates, there is a third phase or means of environmental entrance by toxins. This third phase of toxin entry is plant residues. This phase, which we can and cannot see depending on the season and stage of decomposition of the material, is related to environmental factors and microorganisms.

Guenzi et al. (29,32,45) found that wheat, oat, sorghum, and corn residues exert an inhibitory effect on wheat seedling growth, which is important in stubble-mulch farming. In the residues, the phenolic acids (ferulic, p-coumaric, syringic, vanillic, and p-hydroxybenzoic) were present (30, 31). It was also noted that wheat, oat straw, soybean, sweetclover hay, corn and sorghum stalks, and bromegrass, and sweetclover stems all contain substances capable of inhibiting the germination and growth of sorghum, corn, and wheat. Autoclaving of the soil decreased seed germination and shoot growth, indicating that soil microorganisms may have an influence on the toxic action of allelopathic substances. It may be that acids are released in localized areas of the soil during ideal decomposition conditions.

Patrick (61) determined that inhibitory substances are released when peach root residues are combined with microorganisms. He found that the substances inhibited the respiration of excised root tips and that they influenced a necrotic condition of root meristematic cells. Toxic substances were not produced in soil which had undergone autoclaving, nor were they found in sour cherry (*Prunus cerasus*), tobacco (*Nicotiana* sp.) or pepper (Solanaceae) root residues. Patrick and Koch (62) theorized that some of the poor growth could be related to an increased susceptibility of the tissue to normally nonpathogenic organisms due to a weakening of the plant relative to the phytotoxin.

Dried apple root bark was observed by Borner to reduce apple (*Malus* sp.) seedling growth in nutrient cultures. Five phenolic compounds were isolated, of which only phlorizin could be considered a bark constituent. The other four compounds were breakdown products. Two of the four breakdown products displayed an inhibitory action, indicating that products of decomposition may also influence plant growth in an inhibitory manner (7).

Hook and Stubbs (13) examining 588 seed trees found a definite reduction in the growth of the understory vegetation beneath cherrybark oak (Quercus falcata var., 'pagidaefikua'), swamp chestnut oak (Quercus michauxii), Shumard oak (Quercus shumardii). This reduction in growth of the understory vegetation was not as pronounced under yellow poplar (Liriodendron tulipifera), sweetgum (Liquidamber styraciflua), white ash (Fraxinus americana), green ash (Fraxiums lanceolata) or loblolly pine (Pinus taeda). The retardation was more prevalent in low, wet areas than in dry areas. Root exudates, or crown leachates, were presumed to be responsible for the phytotoxic action, even though the understory growth was not repressed under all of the oaks.

Jameson (34) has determined that Blue grama, Boutelaria gracilis, radicles are inhibited by extracts of the foliage of juniper, Juniperus monosperma.

Rice et al. (60,81) have shown through experimentation that sunflower, Helianthus annuus, inhibits its own seedlings and other early weeds in old-field succession situations in central Oklahoma. Applications of extracts from decaying leaves, root exudates, leaf leachates, and soil extracts have all displayed an inhibitory action to seedling growth and seed germination. Digitaria sanguinalis (crabgrass) was found to be inhibitory to itself and to Helianthus annuus seedlings (1). The decaying crabgrass seemed to have no effect on germination, indicating that perhaps the toxic substance was exuded by living roots. This type of situation emphasizes the fact that more than one plant part could be responsible for allelopathic expression. Microorganisms, species of plant releasing the material, stage of maturity of the material, water content of the soil, pH of the soil, and length of time subject to various orders of decomposers may all influence the degree of toxicity exerted by plant litter or residues (62). Phytotoxic substances need not only be available, they must also be released into the environment or in the case of the soil, the pedosphere, where they can be reacted with actively growing plants. It is important to know from which plant part or parts phytotoxins are released and the influence it exerts on other plant species aside from the normal aspect of light, water, and nutrient competition. Allelopathic substances are important not only ecologically relative to plant community diversity but also important in commercial and noncommercial plant production. Since there are many plants either producing toxins or presumed to be producing toxins, the diversity of plants relative to phytotoxins is important. Tables I and II, taken from Garb et al. (6,22, 24,32,51,60,61,66,81) list some of the plant species reported to exhibit an allelopathic nature.

As early as the time of Pliny (28), the Roman philosopher, it was observed that the black walnut was toxic in some way to plants growing beneath its crown. Since that

TABLE I

PLANTS REPORTED TO PRODUCE GROWTH-INHIBITORS OF KNOWN COMPOSITION

Plant Source	Chemical Composition of Inhibitor	Plants and/or Parts Inhibited	Comments
Anemone pulsatilla (Spreading pasque-flower)	Protoanemonin	cress, corn	
Angelica glabra (angelica)	byakangelicin	tomato	
Avena species (oats)	scopoletin	oats	homologous
Bergenia crassifolia (Bergenia)	arbutin	wheat seedlings (roots)	inhibitor in the leaves
Dipteryx odorata (tonka bean)	coumarin	carrot, onion, lily, alfalfa (roots)	cress, cabbage resistant
Dipteryx oppositifolia (tonka bean)	coumarin	carrot, onion, lily, alfalfa (roots)	cress, cabbage resistant
Encelia farinosa (encelia)	3-acetyl-6-methoxy-benzaldehyde	tomato, pepper, corn	barley, oats, and sunflower resistant inhibitor in the leaves
Melilotus sp. (sweet clover)	coumarin	carrot, onion, lily, alfalfa (roots)	

Citrus sp. (Orange rind)	parasorbic acid	onion, tomato (roots)	
Parthenium argentatum (Guayule)	transcinnamic acid	guayule, pea	tomato resistant homologous
Pyrus communis (Pear)	arbutin	wheat seedlings (roots)	inhibitor in the leaves
Ranunculus sp. (buttercup)	protoanemonin	cress, corn	
Sorbus aucuparius (Mountain ash berries)	parasorbic acid	onion, tomato (roots)	
Thamnosma montana (thamnosma)	byakangelicin isopimpinellin $C_{16}H_{15}O_5(OCH_3)$ with bergaptene nucleus	tomato	
Vanilla bean	vanillin	wheat seedlings (roots)	
Wheat middlings	parasorbic acid	onion, tomato (roots)	

TABLE II

PLANTS REPORTED TO PRODUCE GROWTH-INHIBITORS OF UNKNOWN COMPOSITION

Plant Source	Plants and/or Parts Inhibited	Comments
Acer spp. (Maple)	Triticum spp. (Wheat)	
Agrostis alba (Redtop)	Phleum pratense (Timothy) Poa pratensis (Kentucky bluegrass) Chewing's fescue	
Ailanthus altissima (Tree-of-Heaven)	Pinus spp. (Pine) Betula spp. (Birch)	
Antennaria fallax (Cat's-ear)	Antennaria fallax	homologous
Artemisia absinthium (Wormwood)	Senecio (Groundsel)	Datura and Stellaria resistant
Aster macrophyllus (Aster)	Aster macrophyllus	homologous
Avena spp. (Oats)	tomato crown gall	
Backhousia angustifolia (Backhousia)	Araucaria cunninghamii (Hoop Pine)	
Bromus inermis (Brome Grass)	brome grass seedlings	
Castanea dentata (Chestnut)	Triticum spp. (Wheat)	
Castanopsis sempervirens (Chinquopin)	Ribes spp.	
Citrus spp. (Citrus Fruits)	Citrus spp.	homologous
Cornus spp. (Dogwood)	Triticum spp. (Wheat)	
Encelia farinosa (Encelia)	Lycopersicon esculentum (Tomato) Zea mays (Corn)	

continued

TABLE II--continued

Plant Source	Plants and/or Parts Inhibited	Comments
Encelia frutescens (Encelia)	*Lycopersicon esculentum* (Tomato)	
Erigeron pulchellus (Poor Robin's Plantain)	*Erigeron pulchellus*	homologous
Eucalyptus spp.	herbaceous spp.	
Euphorbia	flax	
Franseria dumosa (White Bur-sage)	*Lycopersicon esculentum* (Tomato)	
Fraxinus americana (White Ash)	*Pinus strobus* (White Pine)	
Grevillea robusta (Silk oak)	*Grevillea robusta*	homologous
Helianthus scaberrimus (Sunflower)	*Helianthus scaberrimus*	
Juglans cinerea (Butternut)	*Potentilla fruticosa* (Shrubby Cinquefoil) *Pinus mugo 'mughus'* (Swiss Mountain Pine)	
Juniperus osteosperma (Juniper)	*Bouteloua* spp. (Blue Gramma)	
Kalmia angustifolia (Sheep-laurel)	*Picea mariana* (Black Spruce)	
Larrea tridentata (Creosote Bush)	*Lycopersicon esculentum* (Tomato)	
Leersia hexandra (Zacate)	rice	
Liriodendron tulipifera (Yellow Poplar)	*Triticum* spp. (Wheat)	

continued

TABLE II--continued

Plant Source	Plants and/or Parts Inhibited	Comments
Lolium multiflorum (Domestic Ryegrass)	Kentucky bluegrass Chewing's fescue	
Malus spp. (Apple)	Malus spp. (Apple) Pyrus spp. (Pear)	homologous
Parthenium argentatum (Guayule rubber)	Parthenium argentatum	homologous
Pinus spp. (Pine)	Triticum spp. (Wheat)	
Poa pratensis (Kentucky Bluegrass)	Poa compressa (Canada Bluegrass) foliage and roots	
Prosopis juliflora (Mesquite)	Lycopersicon esculentum (Tomato)	
Prunus persica (Peach)	Prunus persica (Peach)	homologous
Prunus pumila (Cherry)	Pinus banksiana (Jack Pine)	
Prunus serotina (Cherry)	Pinus banksiana (Jack Pine)	
Prunus spp. (Cherry)	Prunus spp. (Cherry) Triticum spp. (Wheat)	
Quercus spp. (Oak)	Quercus spp. (Oak) Triticum spp. (Wheat) Brassica spp. (Mustard)	homologous
Robinia pseudoacacia (Black Locust)	Pinus strobus (White Pine) Hordeum vulgare (Barley)	
Salvia spp. (Salvia)	Many species	
Salix pellita (Willow)	Pinus banksiana (Jack Pine)	

continued

TABLE II--continued

Plant Source	Plants and/or Parts Inhibited	Comments
Sarcobatus vermiculatus (Greasewood)	*Lycopersicon esculentum* (Tomato)	
Sorghum vulgare (Sorghum)	other grasses	
Tridax procumbens (Tridax)	other weeds	
Trifolium spp. (Clover)	*Poa pratensis* (Kentucky Bluegrass)	
Viguiera reticulata (Viguiera)	*Lycopersicon esculentum* (Tomato)	

time, many observations relative to the toxic action of the walnut have been made and probably will continue to be made as long as plants are grown beneath Juglans nigra, L. The American Indians and the early pioneers were probably the first groups to experiment or work with the black walnut (82). They found that the black walnut husks would make a permanent brown dye for clothing when they were macerated in water (20). Salves and other various ointments were also made from the husks by these early settlers. In 1749, Kolm, perhaps one of the first people to observe the growth of the walnut, noticed that as soon as the fruits ripened in the fall, the black walnut leaves fell (36). It was also noted that cherry, apple, flax, and some vegetables planted in close proximity of the walnut, wilted and eventually died. One belief, which may not have been completely incorrect, was that dew reacted with vapors which were blown by the wind to nearby plants, which in turn were poisoned. After many considerations, it was finally theorized that the roots of the black walnut absorbed all the water and nutrients in the immediate area, thus starving other plants beneath its crown. The many and varied reports of the toxic action relative to Juglans nigra indicate that there may indeed be a phytotoxic plant interaction. Based on these observations, Table III has been prepared depicting some plants that have been reported not to grow beneath the crown of Juglans nigra. The table

TABLE III

PLANTS REPORTED NOT TO BE TOLERANT OF *JUGLANS NIGRA*

Scientific Name	Common Name
Trees:	
Betula alba	White Birch
Elaeagnus angustifolius	Russian Olive
Ilex verticillata	Black Alder,Winterberry
Magnolia soulangiana	Saucer Magnolia
Malus 'Hopa'	'Hopa' crab
Malus spp.	Apples
Pinus resinosa	Red Pine
Sambucus canadensis	Common Elder
Viburnum sieboldii	Siebold Viburnum
Shrubs, Vines and Flowers:	
Azalea spp.	Azalea
Chrysanthemum morifolium	Chrysanthemum
Hydrangea spp.	Hydrangea
Potentilla fruticosa	Shrubby Cinquefoil
Prunus tomentosa	Nanking Cherry
Pyracantha spp.	Pyracantha (Firethorn)
Rhododendron spp.	Rhododendron
Rosa chinensis 'Minima'	Fairy Rose
Syringa x *persica*	Persian Lilac
Syringa vulgaris	Common Lilac
Vaccinium corymbosum	Highbush Blueberry
Viburnum opulus	European Cranberry Bush
Vegetables and Others:	
Alfalfa spp.	Alfalfa
Beta vulgaris	Sugar Beets
Lycopersicon esculentum	Tomatoes
Solanum tuberosum	Potatoes
Vigna sesquipedalis	Asparagus

was prepared from the following references: 9,10,12,13, 21,24,26,27,35,36,41,42,44,46,58,67,69,70,71,73. Table III does not necessarily represent all plant genera or species that may be involved.

Davis determined through experimentation that the toxic substance present in Juglans nigra is juglone (16). It was found to be toxic to tomatoes, alfalfa, and apples when injected into the stems. The substance was found in all parts of Juglans nigra. A variance in the location of juglone within the plant was determined by Lee and Campbell (39). They noted that the amount of juglone varied in the leaves and in the fruit husks of different black walnut varieties. It is hypothesized that the substance was translocated from the leaves to the hulls at different times during the year.

Juglone is a napthoquinone, and only one has been isolated, having a formula of 5-hydroxy-1,4-napthoquinone, $C_{10}H_6O_3$ (14,43). Juglone has a molecular weight of 174.00 and is slightly soluble in water, being more soluble in chloroform, benzene, alcohol, and ether (74). Diethyl oxide and ether have been found to be the best extractants from walnut hulls (16).

It is conceivable that a toxic substance present in the soil could kill living cells by modifying the soil solution and perhaps effecting absorption of water and nutrients. MacDaniels and Muescher concluded that the soil pH may in turn effect the absorption of each soil site. Massey, as cited by MacDaniels, worked with root bark of Juglans nigra relative to tomatoes grown in nutrient cultures and found after 48 hours that the roots became discolored and then slimy. A similar experiment was carried out using bushel baskets filled with soil, and it was observed that the soil containing root bark inhibited the growth of tomatoes, but the soil from the field where former symptoms occurred did not inhibit growth (41).

A representative of the California Walnut Growers Association stated that toxic symptoms had been observed relative to cover crops. It was also noted that applications of excess nitrogen seemed to remove all symptoms of the toxic interaction (41). Davidson (15) of Ohio noticed that raspberry plants died beneath apple trees but grew beneath mulberry and persimmon trees. Having heard of the toxic action of the walnut, he pointed out that he has apple and walnut trees growing together with no toxic symptoms present.

Potentilla fruticosa, or shrubby cinquefoil, has been observed in many locales of Vermont, but has not been observed beneath either mature or seedling trees of Juglans nigra (35). The area beneath the walnut trees was clean of most weeds and very grassy relative to maple or apple trees in the same area. The area beneath the walnut seemed to get larger each year. Observations by A. H. Gilbert indicated that this action of inhibition is not present beneath birch, beech, maple, cherry, apple, and pine trees.

Apple trees have, for many years, been observed to suffer when planted or grown near walnut trees. The effect is often more pronounced on the side of the apple tree next to the walnut (9,21,44,70,71). Schneiderhan, observing four apple trees surrounding a black walnut, noticed that one was dead, one was dead on the side nearest the walnut, one was extremely stunted, and one exhibited very weak growth. Staymen varieties, however, displayed much more tolerance toward Juglans nigra, perhaps indicative of some genetic or varietal difference. It was also noted that corn seemed stunted when grown next to Juglans nigra.

Recently, many species of pines have been found to suffer when grown in close proximity of Juglans nigra. Red pine, Pinus resinosa, began to suffer and eventually died when planted next to Juglans nigra (7). It grew well when planted between red oak and did not show any discoloration or needle loss as was displayed next to Juglans nigra. Bruner (12) observed a loblolly pine forest on acreage formerly consisting of old homesites and noticed scattered spot-dying. Upon investigation, it was revealed that each occurrence was in close proximity to Juglans nigra. The suffering pines were first characterized by a white flow of pitch which then turned yellowish. The bark soon slipped, exposing the cambium. This would occur on the side of the pine adjacent to the walnut. Subsequently, the majority of deaths were attributed to bark beetle attack of the weakened trees. Deaths of those not attacked by bark beetles was attributed to walnut interaction. The characteristic symptoms were found in the shortleaf pine, *Pinus echinata*, as well, but did not seem as severe.

Ericaceous type plants, such as azaleas and rhododendrons, which are important in any landscape or nursery operation, have been observed to die when planted beneath black walnut trees (9,65,67). In the few isolated cases of good growth, a rock ledge has been found separating the two root systems. Pirone (9) observed that many varieties of *Rhododendron catawbiense* died but that those without root system contact did not die. In some instances, soil pH was used for an explanation. Nitrogenous applications did not create a growth response, although the symptoms seemed to be lessened.

Kentucky bluegrass, which is widely planted, has been observed to be one of the plant species enhanced by *Juglans nigra* (9,35,38,42,46). More often than not, weed or poverty grasses are found less beneath walnut crowns relative to maples (*Acer* sp.), beech (*Fagus* sp.), oaks (*Quercus* sp.), and hickories (*Carya* sp.) (9,35). This can often be observed on rich as well as poor soil sites, and good varieties of grasses, such as Kentucky bluegrass, are often found beneath Juglans nigra.

Peaches, like apples, have been noticed to be susceptible to the toxic action of walnuts but in some situations have grown well (35,46). In situations characterized by poor growth, competition for water and nutrients was theorized as the cause. McKay noticed that the growth of plums, was restricted to a lesser degree than peaches.

Two plants, more than any others, have long been associated with the toxic or inhibitory action exerted by Juglans nigra. These plants are the tomato, Lycopersicon sp. and alfalfa, Medicago sp. (4,9,10,13,16,26,27,42,58,67, 73). Tomatoes, often grown by home gardeners, were probably noticed to be inhibited when planted next to black walnuts, which may account for the many early observations. Alfalfa was probably first observed by farmers planting it along fence rows which are often habitats of black walnuts.

Like many other plant species producing growth inhibitors, the roots of Juglans nigra were the first toxin producing area to be considered as the cause. Brown (10) found roots inhibited the growth of alfalfa seedlings. The alfalfa roots were shriveled and brown, and the plant was chlorotic and stunted. Reinking (67) reported that apple, quince, Mazzard cherry seedlings, Mahaleb cherry seedlings, and Western sand cherry seedlings were inhibited in growth.

The phloem was found to be discolored, and he concluded that the substance exuded by the walnut could remain in the soil almost indefinitely, perhaps until all roots were dead, decomposed, and leached from the soil.

Walnut leaves, too, have been considered as the toxic agent. Perry (63) determined an inhibition of oxygen uptake in the bean and tomato plant species. He found respiration to be inhibited about 50 percent in each. Bode (4) hypothesized that the toxin is contained in the leaves of Juglans nigra and is leached by the rain to the environment of other plants. The leaves of Lycopersicon sp., tomato, were characterized by curling, and the yellowing which usually occurred, was not present. No inhibition of tomatoes was displayed when the plants were covered with plastic. He also determined that nutrient solutions which had contained walnut seedlings promoted tomato growth, and that catkins first inhibited, then promoted growth after twelve days.

EXPERIMENTAL PROCEDURE

Fruit Residues

The major emphasis concerning plant residues relative to *Juglans nigra* was directed toward the fruit it produces, considering it a means of toxin entry. It was thought that the characteristic wilt symptoms would occur, providing a visual interpretation, as well as being an initiation point for further work with this aspect of black walnut toxin entry. This was desired since Lee (39) had shown that certain varieties of walnut fruit do contain various quantities of juglone.

Study No. 1:

Unhusked walnut fruits were gathered, separated, and stored in the dark at $-20^{\circ}F$ on October 15, 1969.

The study was divided into two parts. Part No. I consisted of the following treatments applied to 6" plants of *Lycopersicon esculentum*, variety Spartan Red, and *Pyracantha coccinea* grown in 4" styrofoam pots containing a 1:1:1 media mix of soil, sand, and peat:

Part I.

1. Control A--tomato plants in 4" pots.
2. Control B--pyracantha plants in 4" pots.

3. Mulch A--application of 20 grams of walnut husks as a mulch to tomato plants.

4. Mulch A_1--same as 4 above using 30 grams.

5. Mulch B--application of 20 grams of walnut husks as a mulch to pyracantha plants.

6. Mulch B_1--same as 5 above using 30 grams.

Part No. II consisted of plants planted in a pot with the bottom removed and subsequently placed upon a second 4" pot of soil containing one of the following treatments:

Part II.

1. Control A--tomato plants potted in the uppermost pot with two number 8 rubber stoppers wrapped in tinfoil in the lower pot for simulation of walnut fruits.

2. Control B--same as 1 using pyracantha.

3. A_1--same as 1 with one 2-inch diameter walnut fruit in the lower pot.

4. A_2--same as above plus 20 grams of broken husks applied as a mulch to the uppermost pot.

5. A_3--same as 3, however using two walnut fruits.

6. A_4--same as above plus 20 grams of broken husks applied as a mulch as in treatment 4.

7. B_1--same as 3 using pyracantha.

8. B_2--same as above plus 20 grams of broken husk mulch.

9. B_3--same as 7 using two 2" walnut fruits.

10. B_4--same as 9 plus 20 grams of mulch.

The plants were potted on November 25, 1969, and grown to maturity. The experiment consisted of two replicates per treatment with four plants per replicate. Observations were made twice weekly throughout the experiment, and plants were watered daily as needed. Plants were fertilized once weekly with a 20:20:20 water soluble fertilizer.

Leaf Leachates

Study No. 1:

The leachate study at the Rose Lake Research area was begun on May 26, 1970, with the clearing of grassy areas and the placement of black polyethylene plastic over areas approximately 3 feet by 8 feet, which served as growing plots for tomato plants grown in containers. Four plots were established beneath mature *Juglans nigra* trees, 2 beneath *Quercus velutina* trees, 1 beneath *Carya cordiformis*, 1 beneath 3 seedling trees of *Juglans nigra* 6 feet in height, 1 in the open between a mature tree of *Juglans nigra* and *Carya cordiformis*, and 2 plots in the open field, making a total of 11 plots. On the same day, soil was removed from beneath mature *Juglans nigra* trees, of which half was sterilized and half was unsterilized. Each soil fraction was mixed with sterilized peat and sand, forming a mixture with a 1:1:1 ratio. Tomato seedlings, varieties Roma, Spartan red, and Campbell, which had been sown on May 20, 1970, were transplanted into steam sterilized No. 10 cans on June 10, 1970. Drainage holes were provided for by the use of a can-opener. At planting, each container fraction of unsterile and sterile soil mix was mixed with 2 tablespoons of osmocote fertilizer, analysis 18-9-9.

On June 15, 1970, the containers were set in the field. Each plot, except those beneath _Quercus velutina_ and _Carya cordiformis_, contained two plants of each variety; one in sterile soil and one in nonsterile soil. The _Quercus velutina_ and _Carya cordiformis_ plots contained 4 plants of each variety, 2 in sterile soil and 2 in nonsterile soil. The combined total was 42 plants in nonsterile soil and 42 plants in sterile soil.

The containers were watered every day, excluding periods of precipitation, after field placement with 1 gallon of water per container. Once every two weeks, each container was fertilized with 1 tablespoon of 5-10-10 fertilizer, and the plants were sprayed weekly with malathion and captan at the rate of 1 tablespoon per gallon of water for the duration of the experiment. Observations were made at each watering.

Root Exudates

Study No. 1:

This first study, initiated to determine the influence of root exudates, was done in conjunction with seedlings of _Juglans nigra_. This study took into account both root exudates and leaf leachates.

Forty seedlings of _Juglans nigra_ were planted, two to a container in May, 1970. The containers, made of plastic, were approximately 8 inches in diameter by 10 inches in depth.

The seedlings were planted approximately 1 inch from the sides of the container with approximately 5 inches between seedlings. The black walnut seedlings were pruned to a height of 14 inches.

Part A consisted of tomato seedlings, variety Roma, planted on June 10, 1970, between the black walnut seedlings, which were allowed to grow upright in the container. In this situation, the tomato plants would receive root exudate as well as leaf leachates. The plants were watered from above every other day as necessary, depending on the precipitation.

Part B consisted of tomato seedlings, variety Roma, planted on June 10, 1970, between black walnut seedlings, which were tied to one side of the container. This allowed for root interaction but no leachate interaction between the two plant genera. Part B as well as Part A consisted of two replicates of five plants per replicate. Two control plants were grown in similar containers with 3/4 the amount of soil.

Study No. 2:

Tomato plants of the variety Roma were grown in an open cold frame beneath seedlings of *Juglans nigra*, which were suspended above the tomatoes on a lath frame. The experimental setup was designed to determine whether or not the leachates from seedling root systems had any detrimental influence on growth of the underlying tomatoes. Each tomato plant was grown in a container and placed directly beneath a walnut seedling in order to catch any leachable root exudates.

The tomato plants, as well as the seedlings of *Juglans nigra*, were watered daily except during periods of rainfall. Daily watering of the black walnut seedlings was necessary due to rapidity of container drying caused possibly by wind exposure.

The experiment consisted of 2 replications of 4 plants per replication and 2 tomato control plants placed beneath lath without *Juglans nigra* seedlings. The plants were fertilized every other week with 2 tablespoons of 5-10-10 fertilizer per container and were sprayed every week with a mixture of captan and malathion at the rate of 1 tablespoon per gallon of water.

Study No. 3:

This experiment, established at the corner of Forest and College Roads on the campus at Michigan State University, was undertaken to determine the influence of mature tree root exudates. This was considered the primary interaction at this location due to the fact that tomato plants were planted beneath the mature trees of *Juglans nigra*. The walnut trees were planted in a straight east-west row. The tomato plants, varieties Roma and Campbell, were planted randomly in rows perpendicular to the walnut trees. Prior to planting, the plot was tilled twice with a roto-tiller.

At planting and throughout the remainder of the experiment, all plants were fertilized with a 5-10-10 fertilizer at the rate of 1 tablespoon per plant per week. All plants were sprayed once weekly with a captan and malathion mixture at the rate of 1 tablespoon per gallon and were tilled once weekly. The plants were watered daily excluding days with .5 inches of rainfall or more. The plants were not watered on days following 1 inch or more of rainfall. The plants were observed specifically for wilting twice weekly, at which time observational data was recorded. Yield data were taken at termination of the experiment.

Study No. 4:

The design of this experiment was identical to that of Study No. 3. However, the location was at the Graham Experiment Station in Grand Rapids, Michigan. The plants were grown exactly as those in Study No. 3, and data were collected the day following collection at the College Road location. The control plants in Studies No. 3 and 4 were grown in pots and arranged with plastic, having only the plant exposed, the plant and pot exposed, and neither the plant nor the pot exposed to leachates of *Juglans nigra*. None of the control plants exhibited wilt symptoms.

Chromatographic Studies

Since juglone is considered to be the toxic substance responsible for the inhibitory action exerted by *Juglans* *nigra*, the following studies were undertaken to determine whether or not juglone could be found in plant tissue of the wilted tomato plants. The tissue was obtained from tomato plants that exhibited wilt conditions in studies three and four of root exudates. The tissue was gathered in the field, frozen in dry ice, and moved to a freezing room for storage at $-20^{\circ}F$. The material was then lyophilized and ground with a 40 mesh screen in a Wiley mill.

The extraction consisted of extracting ground, lyophilized tissue twice with petroleum ether. The first extraction was for 10 hours and the second extraction was for 24 hours. The extracts were combined, evaporated to near dryness and redissolved in chloroform in preparation for chromatographic application. The previous extraction procedure applies to all materials tested. However, the quantity of material extracted and solvent system may vary with the individual study. All studies utilized systems of butanol and water 6:1 and isopropanol and water 4:1 with Part D of Study No. 3, utilizing the above mentioned plus butanol, acetic acid and water 5:1:2.2, and methanol and water 7:3.

Study No. 1:

This study was undertaken to develop the procedure for comparisons of synthetically pure juglone with extracts of wilted plant parts, and to determine whether or not juglone could be identified in tissue of wilted tomato plants.

Part A: For a standard, .01 grams of synthetically pure juglone from K & K Laboratories, Inc., Plainview, New York, was dissolved in 100 ml. of petroleum ether, evaporated to dryness, and redissolved in 100 ml. of chloroform for ascending paper chromatographic application. Whatman No. 1 grade paper was used throughout the experiment. The solvent used in Study No. 1, consisted of n-butanol and water in a ratio of 6:1 as described by Lee (39), and isopropanol and water in a ratio of 4:1. A 20 lambda portion of the solvent was applied, and the solvent was allowed to ascend a distance of 22.5 cm., at which time the chromatograms were removed, dried, and subjected to identification techniques consisting of ultraviolet light and NaOH spray.

Part B: This consisted of chromatographing an extract of leaves selected from the upper one-half portion of a nonwilted and a wilted tomato plant in conjunction with the synthetically pure juglone. One gram of material was extracted twice with 25 ml. portions of petroleum ether. A 20 lambda portion of each extract was spotted on a separate paper strip, and each was run in a separate developing cylinder.

Part C: This consisted of chromatographing an extract of leaves selected from the lower one-half portion of a nonwilted and a wilted plant in conjunction with the pure juglone. The chromatograms were run identical to the standard in Part A, and the material was extracted as in Part B.

Part D: This part was carried out as the previous part. However, stems from the upper portion of tomato plants were used. The extraction method was identical to Part B.

Part E: The test was carried out as the previous test, Part D, but stems from the lower one-half portion of the tomato plants were extracted with petroleum ether.

Part F: This test consisted of chromatographing redissolved extracts from the ripe and nonripe fruit of wilted and nonwilted tomato plants, which were grown beneath trees of *Juglans nigra*. One gram of the material was extracted twice with 25 ml. portions of petroleum ether, evaporated, and redissolved in 3 ml. of chloroform.

All of the extracts chromatographed in Study 1 of the chromatogram studies were compared to the synthetically purified juglone. The tests included all of the plant parts except for the root systems of wilted and nonwilted tomato plants.

Study No. 2:

The same extraction procedure was used for tomato tissue as in Study No. 1, and a 20 lambda portion of each redissolved extract was chromatographed in both previously mentioned systems of isopropanol and water and butanol and water. This study utilized *Juglans nigra* leaves as the standard for juglone comparison in wilted tomato plant tissue. Twenty grams of fresh walnut leaf tissue was extracted twice with 20 ml. of petroleum ether, evaporated to near dryness, redissolved in 3 ml. of chloroform, and chromatographed.

Part B: This test utilized the walnut leaves as a standard in conjunction with leaves of wilted and nonwilted tomato plants. The leaves were taken from the upper portion of the plants and extracted as Part B of Study No. 1.

Part C: This test was carried out identical to Part B, Study No. 2. However, the leaves were taken from the lower half of the tomato plants.

Part D: This part of Study No. 2 consisted of testing the extracted stem material of wilted tomato plants and comparing the results with the leaf juglone standard. The procedure was the same as that used in Parts B and C of Study No. 2.

Study No. 3:

Part A: In this study, the walnut husks were utilized for the juglone identification standard. The material was extracted and chromatographed as Part A of Study No. 2. A 20 lambda portion of each extract was chromatographed in both n-butanol and isopropanol solvents in the ratios stated previously.

Part B: The leaf tissue extracts of wilted and non-wilted tomato plants were chromatographed in both solvents with walnut-husk extracts used as the identification standard. The extracts were prepared as in Part B of Study No. 1.

Part C: This part consisted of ripe fruit tissue extracted as in Part F of Study No. 1. The extracts were chromatographed using both solvent systems.

Part D: This part consisted of chromatographing extracts from root tissue of wilted and nonwilted plants and the walnut husk standard. The root extracts consisted of extracting 5 grams of lyophilized material twice with 45 ml. of petroleum ether. The extracts were evaporated and redissolved in 3 ml. of chloroform in preparation for paper chromatographic application. Four solvent systems, n-butanol and water, butanol acetic acid and water, isopropanol and water, and methanol and water, were used in this part.

RESULTS

Fruit Residues

The study utilizing the whole walnut fruit as a source of toxin, for entry into tomato plants, did not present any visible wilt symptoms aside from occasional water stress. This slightly wilted condition could be alleviated with water applications, and therefore was not characteristic of reported walnut wilt. There were no significant differences between tomato plants relative to the various treatments, and all tomato plants grew normally to the extent of producing fruit, which matured. The pyracantha plants grew well and showed no significant wilting other than occasional water stress.

Upon examination of the root systems, it was found that the soil area surrounding the walnut fruit was darkened in some cases, due probably to the decomposition of the husk. Tomato and pyracantha roots were found growing in these areas, and there was no apparent injury to the roots of either plant genus.

Leaf Leachates

Throughout the months of July and August, there was evidence of some wilting. The wilting was more prevalent on the plots in the open field rather than on those beneath *Juglans nigra*. Walnut wilt has been characteristically expressed as a condition which cannot be alleviated even after watering. In the case of these plots, the plants did not remain wilted after watering.

On August 30, 1970, all plants were removed from the research area, and comparisons were made between plants under the same tree and plants under other tree species relative to *Juglans nigra*. All of the plants flowered and produced fruit which matured on the plants. There were no apparent differences between plant height, fruit size, or color of tomato plants of the same variety, when compared to plants beneath the same or one of the other trees used in the study. Throughout the study, the plants in the plot in the open field exhibited the most wilting, but this was due to rapidity of drying of the containers.

Root Exudates

Study No. 1:

The tomato plants grown in conjunction with and without walnut seedling leachates grew well considering the competition for water and nutrients relative to the container size. All of the plants produced fruit which matured on the plants, and there were no apparent differences in plant height, fruit amount, size, and plant color relative to the control plants. The tomato root systems did not exhibit any browning or stuntedness.

Study No. 2:

All of the tomato plants grew well, flowered, and produced fruit which matured on the plant. There were no apparent differences between plant height, fruit size or plant color of the plants grown beneath the seedlings relative to the control plants.

Study No. 3:

The plants grew well and the first sign of wilting, which occurred on July 25, 1970, was scattered throughout the planting. The plants were very slow to revive upon watering. However, they did look fairly turgid on August 1, 1970, following 3 days of very light precipitation. The plants looked progressively worse during the early part of August despite daily waterings. In fact, some were damaged to the extent of browning foliage. The condition was, as reported by others, spotty with little or no distinction between tomato variety, row location, or tree drip line. It was determined that there was an increase in the average total number of fruit and average total plant weight with increasing distance from the walnut tree trunks, as indicated in Figures 1 and 2. This increase dropped sharply at approximately the seventh plant in each row, which corresponded with the drip line of the trees.

The previous discussion of Study No. 3 is relative to distance from the tree, whereas the following results are based on a wilt-rating made at the termination of the

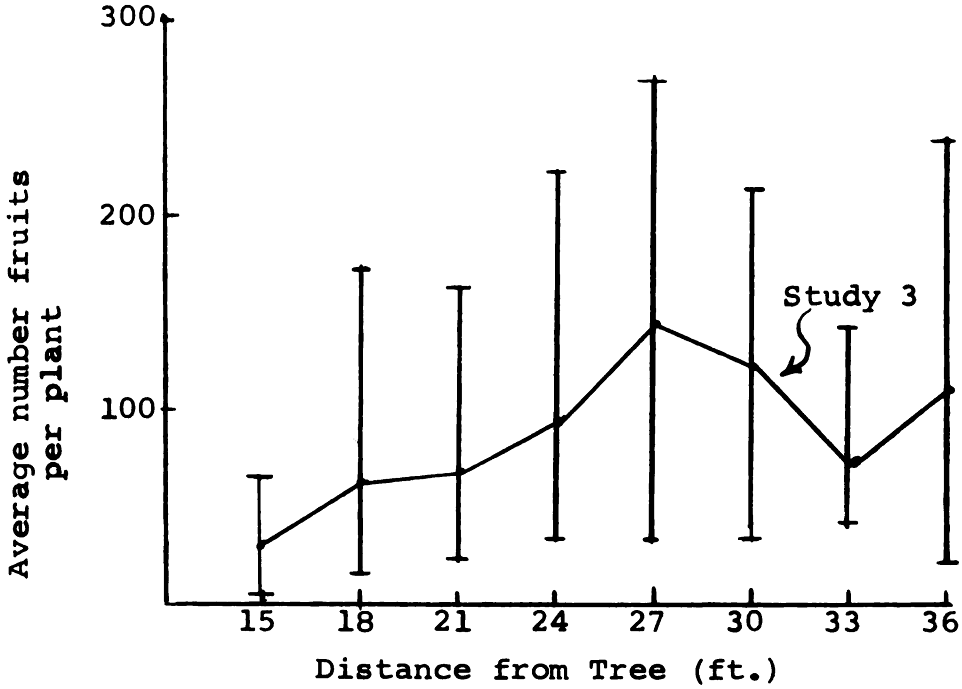

Figure 1. Average number fruits per plant with increasing distance from the walnut tree trunks.

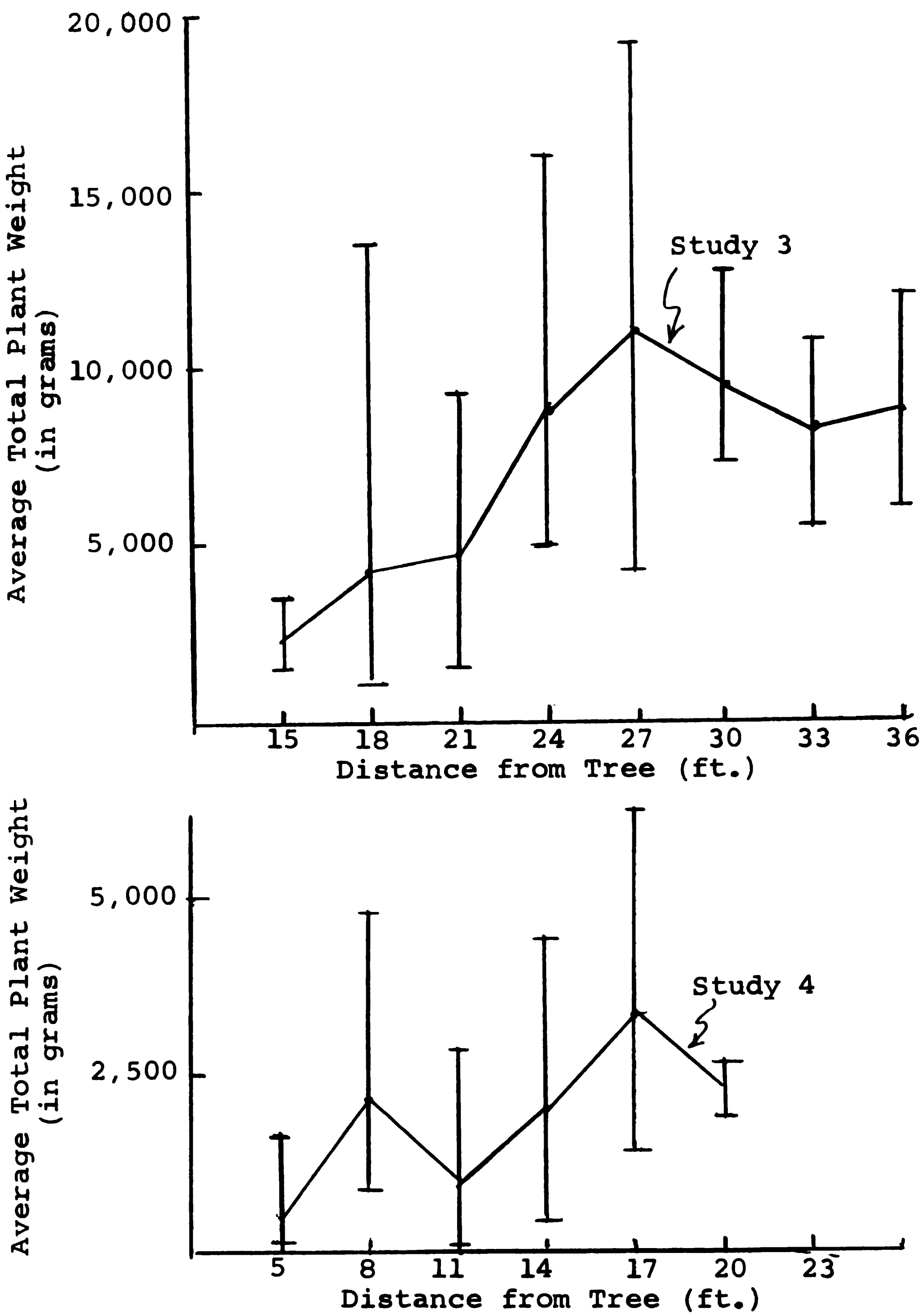

Figure 2. Average total plant weight, in grams, with increasing distance from the walnut tree trunks.

experiment and collection of data. The rating consisted of the following scale:

1. Very good growth and yield with no walnut roots less than 24" deep.

2. Good growth and yield with no walnut roots less than 18" deep.

3. Slight wilt but good growth and yield. No walnut roots less than 12" deep.

4. Wilted with some leaf browning and poor yields. No walnut roots less than 6" deep.

5. Extremely wilted with less than 40% green tissue and extremely poor yields. Walnut roots in top 6" of soil.

Figure 3 depicts the data relative to average total number of fruits and average total plant weight.

Upon examination of the wilted plants, it was found that in each case the roots of the tomato plant were in direct contact with roots of _Juglans nigra_. In this study a total of 55 tomato plants, representing wilt conditions 3, 4, and 5, were found to have roots in direct contact with black walnut roots. The remaining 45 plants did not exhibit wilt symptoms, and of these plants, 23 did not come in contact with walnut roots. The remaining 22 plants did not wilt. However, there were walnut roots beneath these plants, but in each case the roots were at least 18" in depth.

Study No. 4:

The data was collected the day following collection in Study No. 3. Results relative to fruit number and weight

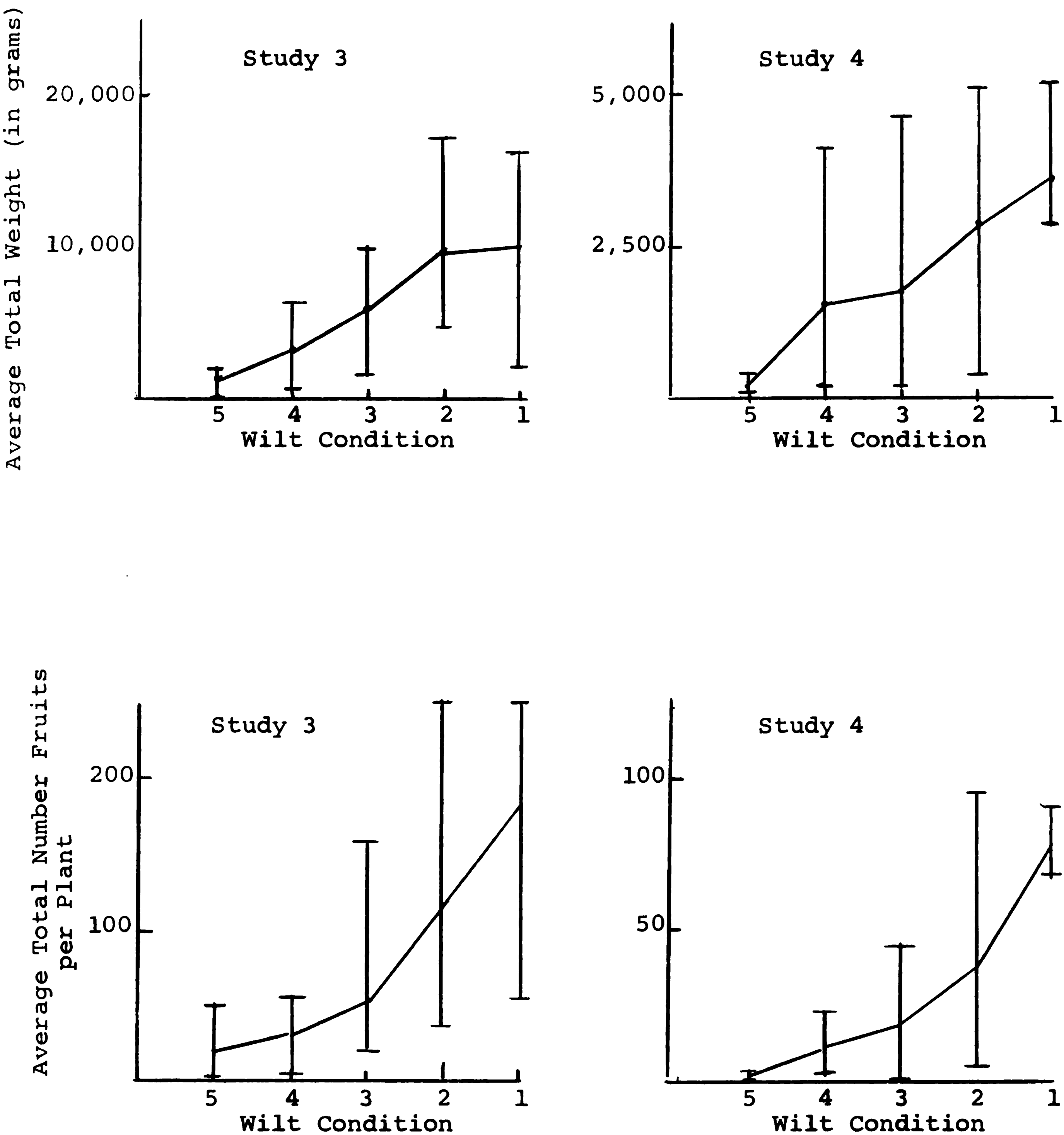

Figure 3. Data relative to average total number of fruits and average total plant weight.

were similar to the results of Study No. 3. These results were plotted in Figures 1, 2, and 3.

Results of root interactions relative to wilt rating were similar to those described in Study No. 3. A total of 59 tomato plants representing wilt conditions were found to have roots in direct contact with black walnut roots. The remaining 29 plants did not exhibit wilt symptoms and of these 29 plants, 18 did not come in contact with walnut roots. The remaining 11 plants did not wilt. However, in these 11 plants walnut roots were present at a depth of at least 18 inches.

The wilting in Studies No. 3 and 4 was spotted throughout the rows in plots. There were no apparent significant differences between the wilting of any one variety or location of the wilting in relation to the walnut trees in Studies No. 3 and 4. The most significant factor was that in each case of wilting, the walnut roots were in direct contact, or touching roots of *Lycopersicon esculentum* (tomato).

Chromatographic Studies

Study No. 1:

Fluorescence was found under shortwave ultraviolet light on all paper chromatograms and absorbance only in the zones containing synthetic juglone. Juglone was found to be a brownish spot, whereas the other materials were not characterized by any absorbance except in the case of overloading. In this instance, an orange area was found. The Rf value for juglone was determined to be .94 ± .02, while the Rf value of the material was .96 ± .02. Each area was sprayed with 5% sodium hydroxide, and juglone appeared reddish-purple, while there was no change in the unknowns. The method was considered to be valid since synthetic juglone was identified. However, there was no evidence for juglone in the tomato tissue extracts of Parts A, B, C, D, E, or F when compared chromatographically to the synthetic material.

Study No. 2:

Fluorescence was observed under shortwave ultraviolet light, but the walnut leaf extract was similar to synthetic juglone, and the tomato tissue extract was not since no brown area developed. Subsequent spraying with 5% sodium hydroxide exhibited a pinkish-purple color on the chromatogram of the walnut leaf extract. There was no indication of juglone in any chromatograms utilizing the extracts of the tomato tissue in Parts A, B, C, or D when compared to the walnut leaf chromatogram.

Study No. 3:

Neither Part A nor Part B gave any indication of juglone in tomato tissue. The fruit tissue of Part C again gave negative results. Part D deviated from all others with an increase in the amount of tomato material extracted.

Five grams instead of 1 gram were used in this part. Upon examination under ultraviolet light, a slight band was observed near that of juglone. Upon spraying with 5% sodium hydroxide, a pinkish-purple area appeared at a similar Rf to juglone. Upon further chromatography using methanol and water which lowered the Rf values, the tomato tissue extract area exhibited a drag streak. This was attributed to an overloading of the chromatogram in an attempt to get the material to show up. The characteristic color was evident upon spraying. However, it was a vertical band due possibly to the drag of the material.

An attempt to elute the material from the paper was made using petroleum ether and chloroform extracts, evaporating to near dryness, and rechromatographing the resultant material. No evidence of juglone in this paper extraction was seen under ultraviolet light, nor was there a color exhibited with the 5% sodium hydroxide spray.

DISCUSSION

The characteristic wilt, that has been exhibited by many plants growing beneath *Juglans nigra*, was found with tomato in only one experiment. This consisted of a field interaction between mature black walnut trees and tomato plants. The primary purpose of this part of the study was to determine the influence of root exudates.

The interaction of the other major modes of toxin entry, leaf leachates, and plant residues, did not inhibit tomato growth.

The chromatographic studies indicated no juglone, the presumed toxic substance, in extracts of 1 gram of tomato plant tissue. However, there was a characteristic color reaction in the 5 gram extraction chromatogram. Upon elution and rechromatography, in order to obtain a more pure substance, no color reaction occurred. This may have been due to the oxidation of the material, the low concentration level, or the inability to remove juglone from the paper.

The results of all the studies indicate that perhaps root exudates are the primary mode of toxin entry into the environment by *Juglans nigra*.

SUMMARY

Since all plants are made up primarily of chemical compounds, it would seem that plants could not exist without emission of by-products of metabolic processes. Many trees shed their leaves in the fall, exemplifying one possible method of emission. Metabolic by-products may be phytotoxic and may be released by root excretions or exudates, crown leachates, fog drip, volatilization, and plant residues.

Upon entry into the environment or immediate ecosystem, the phytotoxic substances may be acted upon by microorganisms, environmental factors, or absorbed to soil colloids. These factors may aid in a build-up or subsequent breakdown of the toxins, and if they are not broken down, it is conceivable that they might be absorbed by living plants. The resultant action displayed, either stimulatory or inhibitory, may be relative to the ability of the plant to metabolize the physiologically active substance.

The allelopathic nature of *Juglans nigra* has been observed for many years since the Roman empire. The method by which the toxin is released to the environment has been considered to be by leaf or crown leachates, plant residues, or root exudates.

It was the purpose of this study to determine the primary mode of the black walnut toxin entry into the environment and to isolate the toxin, juglone, in tissue of plants exhibiting this allelopathic action. Root interactions and subsequent exudates seemed to be the primary area responsible for the toxicity of the black walnut relative to tomato.

The toxic material was not isolated from wilted plant tissue. Further studies will need to be undertaken in order to isolate the toxin from wilted plant tissue and to determine anatomically what plant part or parts are influenced by the toxin.

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APPENDIX

STUDY No. 3

DAYS IN MONTH

PRECIPITATION/IN.

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	TOTAL or AV.
June		.35	.10									.02	2.5		.85			1.0			.08			.05		.95			.02			5.97
July								.69	2.7	.53		.08	.76		.15			.46	1.1	.04			.06	.05					.14	.15	.07	6.98
August																			1.04			.10							.44			1.58
September			.13	.02			.28						.07	.33	.38	.75	.18	.48		.02		.05	.21	.40		.14	.10	.01				4.05

AV. Soil Temperatures

DEPTH	2'	4'	8'	20'	40'	80'
June	70.5	69.4	64.6	61.1	56.6	53.3
July	75.1	72.5	67.6	66.7	62.9	58.4
August	76.3	73.5	70.9	68.8	66.3	63.4
September	68.5	66.7	65.4	65.1	64.4	62.5