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PHYTOTOXIC INHIBITION OF TREE GROWTH AND SEED GERMINATION

BY DECAYING RESIDUES OF SEVEN HERBACEOUS SPECIES

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PHYTOTOXIC INHIBITION OF TREE GROWTH AND SEED GERMINATION BY DECAYING RESIDUES OF SEVEN HERBACEOUS SPECIES

By

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ABSTRACT

PHYTOTOXIC INHIBITION OF TREE GROWTH AND SEED GERMINATION
BY DECAYING RESIDUES OF SEVEN HERBACEOUS SPECIES

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Ornamental trees are commonly grown in association with herbaceous plants containing potentially allelopathic phytotoxins with little knowledge of toxin potency, persistence, or action.

The phytotoxicity of six herbaceous associates, Kentucky bluegrass, red fescue, meadow fescue, timothy, quackgrass, and alfalfa is characterized by inhibition of cress seed germination in residue extract and honeylocust growth in residue-augmented soil. Differences among species are indicated in toxin composition, and in toxin production, persistence, or activity during decay.

The dynamics of phytotoxins during decay is studied in a model soil system. Decay of alfalfa, meadow fescue, and sudangrass is monitored by CO_2 evolution and extract opacity. Filter-sterilized soil extracts are assayed by cress germination and black locust growth in agar. Decay proceeds quickly, limited by substrate and nutrient availability, with resulting differences among species. Toxicity declines simultaneously, without evidence of toxin production or persistence. Germination and growth respond alike, with germination more sensitive.

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INTRODUCTION

Ornamental trees occupy an ecologically distinctive position among agronomic crops. While most crops are maintained in strict monoculture, trees are commonly grown in close association with a variety of herbaceous plants. In the field nursery, herbaceous associates, serving as green manure, sod, or cover crops, may be selected for their specific influence on the tree or its environment. In the landscape, herbaceous associates, chosen for their aesthetic or practical features as lawns or cover vegetation, will nevertheless directly and indirectly affect tree growth. The nurseryman or landscaper cannot eliminate the influence of other plants, but must learn to understand and use that influence to the benefit of his crop.

Associated plants might influence the growth of an ornamental tree through: (1) competition for limited resources, such as nutrients, water, or light; (2) alteration of the physical or chemical environment of the tree, such as nutrient distribution within the soil, or soil structure, organic matter, or pH; or (3) allelopathy, the release of compounds into the environment which directly or indirectly inhibit the growth of the tree.

These three mechanisms of influence, or interaction, proceed simultaneously within the tree's environment, often with conflicting effect: a sod crop may improve soil drainage, compete for nutrients, and produce toxic compounds, all at the same time and in differing proportions depending on the prevailing conditions. A nurseryman, seeking only improved soil

drainage from his sod crop, can partially compensate for lost nutrients with fertilization, or, seeking nutrient uptake by the sod to induce winter hardiness in his trees, will gladly accept the unsought changes in soil structure. Meanwhile, the impact of any toxins produced by the sod and the effect of fertilization on these are usually unknown and unconsidered.

The three mechanisms of influence, competition, alteration, and allelopathy, also proceed interactively within the tree's environment, each modifying the effect of the other: decay of the organic matter produced by a sod crop may simultaneously alter soil structure, reduce the availability of nutrients, and increase the production and persistence of toxins. The nurseryman can rarely anticipate or compensate for interactions of such complexity. Indeed, their extent and significance to tree growth are largely unknown.

The simultaneous and interactive nature of the three mechanisms has confounded research in the relative contribution of each to plant interactions. As a result, investigators have tended to specialize: nutritionists considering only their variable, with soil physicists and allelopathy researchers doing likewise.

Allelopathy is the least understood of these mechanisms. A large body of literature has been produced on the subject, and it has been the subject of numerous reviews, and yet the prevalence and relative significance of the phenomenon is widely disputed (Rice, 1974; Harper, 1975). This disputed status appears to arise from three persistent difficulties in allelopathy research. First, the soil system, through which most chemical interactions would occur, is quite complex in both structure and function, and so critical parameters are often inaccessible to analysis

with current techniques. Second, the physiological response (autecology) of a plant to any particular array of soil parameters (nutrient availability, organic molecules, water, etc.) is largely unknown. Third, due to differing perceptions and reactions to these primary difficulties, fundamental disagreements exist among researchers and reviewers as to what constitutes adequate demonstration of allelopathy. A review of the literature illustrates these problems, together with new analytical techniques which may, in some cases, eventually serve to resolve them.

In the study presented here, the above-ground residues of six common herbaceous associates of Michigan ornamental trees are screened for phytotoxicity as an indication of their allelopathic potential. Water extracts of the residues are tested against herbaceous seed germination, and soil incorporated residues are tested against tree seedling growth. Two of the more phytotoxic species, meadow fescue and alfalfa, along with the green manure plant, sudangrass, are then subjected to a controlled examination of changes in phytotoxicity during decay in soil. With residues incorporated in a model soil system at three levels of nutrition, the relative persistence of the toxic and non-toxic components of the residues are tracked, using water extraction, germination and growth assays, and gaseous and optical evidence of decay. Throughout, the experimental design and resulting data are discussed relative to the persistent problems in allelopathy research.

LITERATURE REVIEW

Molisch introduced the term "allelopathy" to refer to "biochemical interactions between all types of plants" (1937; cited in Rice, 1974). Though the word literally means "mutual harm," Molisch included both beneficial and harmful exchanges involving microorganisms as well as higher plants. This taxonomically and functionally broad usage of the term has been maintained, and even expanded, by many European researchers. Grummer and Beyer (1960), in a review of allelopathic compounds, applied subordinate terms to the interactions between various taxonomic subgroups and to the chemicals involved: for example, marasmins for substances produced by microorganisms and acting upon higher plants; kolines for compounds producing effects between higher plants. Winter (1961) proposed a functional expansion of "allelopathy" to include all exchanges of metabolic products between plants, without regard to discernable effect, arguing periodic or subtle influences might otherwise be neglected. Indeed, many authors have noted that compounds toxic at one concentration may be benign or beneficial at another (Evanari, 1949; Tukey, 1969; Larson, 1980).

Most American investigators have tended to narrow the scope of "allelopathy." Rice (1974), in his influential monograph on the subject, defined it as "the direct or indirect harmful effect by one plant on another through the production of chemical compounds that escape into the

environment," including both higher plants and microorganisms within the definition--though in a recent update (1979) Rice chose to revert to Molisch's functionally broader usage. Muller, a leading researcher in the field, has preferred to limit the term to those harmful interactions between higher plants which are directly mediated by released organic compounds (1969; 1972). In this review and thesis, a definition of allelopathy is followed which is intermediate between those of Rice and Muller: the harmful effect of one higher plant on another through the release of compounds which are phytotoxic or which become phytotoxic after some physical or chemical transformation. Although harmful effects due to compounds synthesized de novo by microorganisms are excluded, this definition recognizes the role of microorganisms in degrading complex compounds (such as proteins and lignins) into toxic constitutent units (Zemek, et al., 1979), as well as their part in removing these phytotoxins through further degradation (Dagley, 1971).

Muller (1969) has suggested that any inhibition of one plant by another should be called "interference" until the relative contribution of competition and allelopathy are determined. This terminology has been widely endorsed, though the indirect effects of environment alteration are not included. Allelopathy is by definition a more complex phenomenon than competition; it incorporates not only the dynamics of phytotoxins within the soil ("the ecology of biochemical effluvia," in the words of Muller & Chou, 1972) and phytotoxin uptake and effect upon the "receptor" plant, but also the factors affecting production and release of the allelopathic compounds by the "donor" plant. Further, unlike the immutable elements subject to competition, phytotoxins, the agents of allelopathy, are

constantly being created, degraded, stabilized, released, and altered within the soil.

Any plant substance which inhibits plant function might act as an allelopathic agent, and most plants contain a variety of such inhibitors. In a recent review of known phytotoxins, Gross (1975) listed these by group: (1) aliphatic substances such as fumaric, palmitic, and oleic acids, various neutral lipids, and 1-acetoxy-2,4-dihydroxy-n-hepta-d-deca-16-ene; (2) phenolic compounds, including acids, alcohols, glucosides, flavinoids, coumarins, tannins, and lignins; (3) stilbenes and phenanthrenes; (4) sesquiterpenes and diterpenes, including various carotinoids and lactones; (5) nitrogen-containing compounds, such as alkaloids, steroids, and cyanogenic glycosides; (6) sulfur-containing substances; and many miscellaneous compounds. Members of the first two groups are found in all higher plants, the remainder are of limited distribution. Many of these compounds have normal regulatory functions or occur as metabolic intermediates; others, without known metabolic roles, are classified as secondary compounds; all may inhibit specific or general plant processes when externally applied (Garb, 1961; Evanari, 1949). The diversity of secondary compounds in plants (over 10,000 identified) has suggested to many that they may be metabolic waste products which have been evolutionarily tailored for protective functions (Harborne, 1972; Swain, 1977; Whittaker & Fenny, 1971). Although Whittaker (1971) believes there has been no selection for allelopathic activity, the general antibiotic qualities of these compounds renders them toxic to plants (Swain, 1977). Only a few inhibitors have been specifically identified with proposed allelopathic interactions, with

simple phenolic compounds most commonly cited. This probably reflects only the limited chemical sophistication of most investigations (Muller & Chou, 1972).

Due to the ubiquity of phytotoxins and their precursers in plants, any plant or environmental process which transfers organic material into the environment is a potential mechanism of allelopathy. Five general processes have been characterized: (1) exudation of volatile compounds (Muller, 1965; Muller & Muller, 1964; Muller et al., 1968; Bell & Muller, 1973; Gant & Clebsch, 1975); (2) leaching of water-soluble substances from above-ground parts (Tukey, 1969, 1966; Tukey & Mechlenburg, 1964); (3) exudation of water-soluble substances from below-ground parts (Hale et al., 1971; Vancura et al., 1964, 1965; Rovira, 1969; Woods, 1960); (4) shedding of above-ground parts (Lodhi, 1976; Al-Naib & Rice, 1971; McPherson & Muller, 1973); (5) sloughing of below-ground parts (Curtis & Cottam, 1950; Fisher et al, 1978).

Different kinds and quantities of toxins are involved in each process. The release of compounds through volatilization is by necessity limited to certain categories of molecules, such a light terpenes and essential oils. However, movement in water can transfer both soluble compounds in solution and insoluble compounds and particulates in suspension. Living plants are surprising leaky systems; most soluble metabolites present inside a plant will move outside given proper conditions (Hale et al., 1971). Fleshy above-ground tissues can lose as much as 6% of their soluble-carbohydrates in a heavy rain (Tukey, 1969), and root systems under stress will release up to 10% of the total plant weight, and 25% of net photosynthate, into the soil (Hale et al., 1971). Toxic substances have been identified in every flow.

Many secondary compounds pose a threat of autotoxicity to their producer, and are segregated in peripheral or internal tissues (Swain, 1977). The particular pattern of segregation regulates their movement into the environment. Large or non-soluble compounds, including structural components such as tannins and lignins, enter the environment only when tissues senesce or are shed. For most plants, senescence and shedding are the primary mechanisms of organic matter transfer (Rovira, 1969).

Agricultural and nursery practices tend to amplify and concentrate each of these transfers by concentrating particular compounds with monoculture, synchronizing their release through mowing or herbiciding, and incorporating them into the soil with plowing (Altieri & Doll, 1978; DeFrank, 1979; McCalla & Haskins, 1964; Patrick et al., 1958, 1963, 1964, 1971; Putnam & Duke, 1978).

The production and release of phytotoxins near an inhibited plant is not sufficient evidence of allelopathy, though many authors cite it as such (Harper, 1975, 1977; Rice, 1974). Ideally, rate and pattern of toxin release, toxin persistence and availability within the environment, and toxin activity within the inhibited plant should be demonstrated in each case of suspected chemical influence. Unfortunately, this is an awesome task, and is considered impossible by some (Harper, 1975). Many researchers, including Rice and his students, choose instead to demonstrate phototoxin production and release, and then to eliminate competition, environmental alteration, and other factors as contributors to the observed inhibition (Al-Naib & Rice, 1971; Abdul-Wahab & Rice, 1967; Bonner, 1950; Curtis & Cottam, 1950; DeCandolle, 1834; Fisher et al., 1978; Horsley, 1977a, 1977b; Muller, 1953; Muller & Muller, 1956; Parenti & Rice, 1969; Rasmussen & Rice, 1971; Wilson & Rice, 1968). Muller and

others combine this approach with study of correlation between spatial or temporal patterns of phytotoxin release and the occurrence of inhibition (Bell & Muller, 1973; Chou & Muller, 1972; del Moral & Muller, 1970; DeBell, 1971; Gant & Clebsch, 1975; Gliessman & Muller, 1978; Hull & Muller, 1977; Lodhi, 1976; Muller & del Moral, 1966; Muller et al., 1968; Stowe, 1979; Tinnin & Muller, 1971). This latter strategy is effective especially where toxin source and inhibited plant are spatially separated and toxin release is well-defined, circumstances most frequently encountered in deserts or Mediterranean climates (Went, 1942). In such studies, as with most cases based on circumstantial evidence, there is often room for reasonable doubt whether the toxicity found is sufficient, or other explanations of the inhibition have been adequately eliminated (Harper, 1977). Subsequent work occasionally turns up alternate explanations (Kommedahl et al., 1970; Muller, 1953; Patrick, 1971; Bartholomew, 1970). Harper (1977) has suggested that detailed study of phytotoxic symptoms might allow application of Koch's postulates to prove agency.

A more analytical approach to allelopathy is required for its manipulation in the agricultural field or for proof of its occurrence in complex environments. To understand the biochemical chain between toxin production and toxic effect in a particular environment (and how to forge or break it) requires analysis of how climatic, edaphic, and physiological factors effect the release, persistence, and activity of phytotoxins (Muller & Chou, 1972). Some of these critical factors will be discussed in the introduction to Section II. This kind of analysis, by its complexity, demands a multidisciplinary and multi-front approach.

Agronomists, concerned with the impact of surface mulches or incorporated residues on succeeding crops, have emphasized toxin production

and release (Altieri & Doll, 1978; Bieber & Hoveland, 1968; Cochran et al., 1977; Hoveland, 1964; Kimber, 1973; Patrick, 1971; Patrick et al., 1964; Guenzi & McCalla, 1962, 1966), with less consideration of toxin persistance (Cochran et al., 1977; McCalla & Norstadt, 1974; Patrick et al., 1963) or toxin availability and action (McCalla & Norstadt, 1974; Patrick et al., 1964). But it is the persistence and availability of phytotoxins in the soil which have proven the critical links in most proposed models of allelopathy (Patrick, 1971). Plants phytotoxic in the laboratory and greenhouse frequently show no pattern of inhibition in the field (Muller, 1953; del Moral & Cates, 1971; Stowe, 1979). Toxins effective in water or agar culture either are degraded or immobilized when combined with soil (Bonner, 1950; Cochran et al., 1977; Martin et al., 1979; Schreiner & Sullivan, 1909b).

Questions of toxin persistance have been most elegantly addressed through radio-tracer studies by soil biochemists and microbiologists interested in dynamics of the soil organic phase, reviewed in part in Section II (Cain et al., 1968; Hackett et al., 1977; Haider & Martin, 1975; Martin & Haider, 1971, 1976; Martin et al., 1979; Verma et al., 1975). Tracer techniques have also been applied to the question of toxin availability and uptake in aqueous culture (Glass & Bohm, 1971). Melrod and others have recently reintroduced the use of agar culture for the study of root absorbtion of toxins (Bowen & Rovira, 1961; Collison & Conn, 1925; Melrod, 1977). Fusion of these varied approaches to toxin release, persistence, absorbtion, and action, may slowly shed light on the allelopathic phenomenon.

Understanding the edaphic and physiological factors which effect toxin persistence and action is crucial to the understanding of when and

where allelopathy will occur, how it can be eliminated, and how it can be used (Altieri & Doll, 1978; Bramble, 1980; Fisher, 1980; Putnam & Duke, 1978; Richards, 1973).

SECTION I

COMPARISON OF RESIDUES OF SIX HERBACEOUS SPECIES FOR PHYTOTOXICITY

INTRODUCTION

Several dozen species of herbaceous plants occur as the dominant understory vegetation in Michigan tree nurseries, some planted alone or in combination as green manures or cover crops, others springing up as weeds or remnants of earlier planting (Davidson, 1980; Voss, 1973). A smaller group of herbaceous plants are regularly planted as cover vegetation in planned landscapes (Beard, 1971). Most of these plants are aggressive growers, establishing themselves quickly and tenaciously on a site by effective interference with other species. For this study, it was hypothesized that for several of these species allelopathy serves as an effective component of that interference.

Allelopathy by a plant requires the presence of phytotoxic compounds within it, or of compounds which become phytotoxic when acted upon by the soil system. Although many other variables will effect allelopathic influence in the field, as discussed elsewhere, the presence or production of potent toxins, together with their persistence in the soil, are essential to the phenomenon. Accordingly, the nature, potency, and persistence of phytotoxicity are used as indices of allelopathic potential against trees in this first stage evaluation of selected plants.

Many of the species mentioned above as herbaceous associates have been at least cursorily investigated for their interference with the growth of other plants, some specifically for competitive abilities, others for their phytotoxic potential. Six species were chosen for this

evaluation based on: (1) evidence in the literature of either substantial phytotoxicity or significant interference against a wood plant; or (2) prevalence in the Michigan nursery or landscape environment, either as crop or weed.

Kentucky bluegrass, Poa pratensis L., and red fescue, Festuca rubra L. (together with perennial ryegrass, Lolium perenne L.) are specified as the basic components of the seed mix for establishing new cover vegetation on Michigan roadsides (Beard, 1971). Kentucky bluegrass is, of course, the dominant species in Michigan's groomed lawns and is commonly sown as a sod crop in field nurseries (Davidson, 1980). Red fescue also maintains itself in pure stands; its phytotoxicity has been suggested as its primary mechanism for dominance (Osvald, 1949, 1953, as cited in Moje, 1966). Other members of the genus Festuca are frequently cited in the literature on allelopathy (Holt & Voeller, 1975; Hoveland, 1964; Rietvald, 1975; Stowe, 1979), but meadow fescue, F. pratensis Hudson,[1] in particular, has been noted for its interference with the growth of woody species, including apple trees (Goode, 1955; Pickering, 1914, 1920; Russell, 1914), magnolia and zelkova (Harris, 1966); black walnut (Todhunter & Beineke, 1979), sweetgum (Walters & Gilmore, 1976), and black locust (Larson & Schwarz, 1980). Timothy, Phleum pratense L., like meadow

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[1]The binomial Festuca elatior L. which frequently appears in the literature, has been applied simultaneously and in succession to the species known commonly as tall fescue and meadow fescue, and so was declared "nomen ambiguum" in 1967. The species, generally considered distinct as Festuca arundinacea Schreber and Festuca pratensis Hudson, respectively, apparently interbreed and have occasionally been confused by experienced researchers, suggesting caution in interpretation of past studies (Terrell, 1967).

fescue, is a forage species introduced from Europe, which is rarely planted in nurseries, but which occasionally dominates unsown fields as a remnant species. It has been cited for interference with apple trees (Goode, 1955) and for the toxicity of its residues against germination and growth of several agricultural crops (Collison & Conn, 1925; Larson & Schwartz, 1980; Nielson et al., 1960; Patrick & Kock, 1958; Stowe; 1979). Quackgrass, Agropyron repens (L.) Beauv., with its dense, rhizomatous root system, is a persistant, agricultural weed, frequently found in nurseries. Studies have shown it an effective competitor for nutrients (Hamilton & Buchholtz, 1955; Kommedahl et al., 1970; Ohman & Kommedahl, 1964; Plhak, 1967; Welbank, 1959, 1961, 1962, 1964), but its decaying residues are also known to be quite phytotoxic (Harvey & Linscott, 1978; Kommedahl et al., 1957a, 1957b; Ohman & Kommedahl, 1960; Osvald, 1947; Stowe, 1979; Welbank, 1963). Alfalfa, Medicago sativa L., is a popular cover crop and green manure in tree production. It both adds nitrogen to the soil and scavenges deep-lying nutrients with its extensive roots, while improving soil drainage. Alfalfa residues and extracts are known to be highly toxic (Collison & Conn, 1925; Grant & Sallans, 1964; Nielson et al., 1960), and the plant is an aggressive grower in the field.

No standardized procedures exist for preparing plant residues for testing or for gauging their toxicity (Gross, 1975; Rice, 1974; Stowe, 1979). The primary procedural guideline for preparing test material should be to minimize any alteration of the toxins present in the plant when intact in the field. Certain published techniques have been shown to induce experimental artifacts, for example: (1) autoclaving, boiling, fermentation, excessive handling, and storage usually alter residue

toxicity (Collison & Conn, 1925; Patrick et al., 1964; Rice, 1974); (2) extraction by non-aqueous solvents or by buffered water solutions can sample compounds not normally mobile in the soil aqueous phase (Kaminsky & Mueller, 1978; Rice, 1974); (3) differences in residue texture and particle size can influence rates of toxin diffusion and residue decay (Allison, 1973; Martin & Focht, 1977; Rice 1974). Preparatory procedures used in this study have been chosen to minimize such artifacts.

A secondary goal in residue preparation and testing is to chose procedures which more closely reflect natural processes occurring in the field. Though the limitations of laboratory facilities and the need to minimize between-treatment variation in untested factors (such as residue texture or soil albedo) keep test conditions far from "natural," these differences should not be unnecessarily exaggerated (cf. Larson & Schwartz, 1980).

The phytotoxicity of a substance is both measured and defined by use of a specific bioassay. Researchers have developed assays to indicate interference with a variety of plant functions over a range of circumstances and time periods (Gross, 1975; Melrod, 1977; Patrick et al., 1964; Rice, 1974), usually chosen for their relevance to the process under study. Inhibition by residues in the field seems most likely to arise either from acute exposure to high initial levels of toxins present in a residue or from chronic exposure to persistent toxins or to toxins continually released during decay (Muller, 1969; Patrick, 1971; Rice, 1974). The assays used in this study, seed germination in water extract and seedling growth in residue-augmented soil, were chosen to reflect this analysis. The speed and sensitivity of a seed germination assay are well

matched to the instability of many soluble toxins (Patrick, 1964). The extended growth of a seedling in soil effectively averages the physiological effect of toxins as their concentration changes during residue decay.

Reduced growth in a seedling bioassay can be attributed to phytotoxicity only when differences between treatments in competition or environmental alteration have been accounted for. This is usually straightforward in extract assays. Unfortunately, incorporated residues effect many soil parameters other than toxicity, including water-retension, texture, microbial activity, and nutrient availability (Allison, 1973; Martin & Focht, 1977). Slight differences between residues in non-toxic characteristics such as carbohydrate composition, cellular structure, or particle size and shape, may significantly and differentially effect non-toxic soil parameters, and thereby alter seedling growth. An effective assay of phytotoxicity should either minimize or, at least, monitor between-treatment variation in the principle sources of variation.

MATERIALS AND METHODS

Six herbaceous plant species commonly found in Michigan nurseries, five grasses and one legume, were chosen for preliminary evaluation for phytotoxicity: Kentucky bluegrass, Poa pratensis L.; red fescue, Festuca rubra L.; meadow fescue, Festuca pratensis Hudson; timothy, Phleum pratense L.; quackgrass, Agropyron repens (1.) Beauv.; and alfalfa, Medicago sativa L.

Several kilograms of the above-ground portions of each species were collected from fields on the campus of Michigan State University in mid-August of 1979. The grasses were all mature, fruiting, and partially senescent when cut; the alfalfa was green and in flower. This plant material was dried for 5 days at 50°C, and ground in a Wiley mill to pass a 2 mm screen, then packaged in two layers of 4-mil polyethylene, and refrigerated at 1°C until used.

Two distinct assays of phytotoxicity were used in evaluating these plant residues: (1) seed germination in residue extract, and (2) seedling growth in residue-augmented soil.

For the germination assay, seeds of curly cress, Lepidium sativum L., a common garden green, were used. Curly cress seeds are rapid and uniform in germination, and relatively sensitive to toxic compounds (Lehle, 1981). The seeds, obtained from the Burpee Seed Co. of Warminster, Pennsylvania, were stored at 1°C, then dusted with a mixture of 43% Captan and 43% Thiram at a rate of 2.2 mg per gram of seed.

Before each assay, an extract of each species was prepared by soaking 20 g of the plant material in 400 ml of distilled water, an extraction ratio by weight of 1:20. After 4 hours of soaking at room temperature, the extract was strained through cheese cloth and vacuum-filtered through #2 Whatman's filter paper. The pH of each solution was measured, and adjusted to 6.5. Then the extracts were refrigerated at 1°C until needed. In all assays, distilled water was used as the control.

Assays were performed to gauge the effect of four variables on seed germination: (1) extract source; (2) extract concentration; (3) supplementary nutrients; and (4) time of evaluation. The germination procedures were similar for all assays. Nine centimeter plastic petri dishes, lined with one layer of #1 Whatman's filter paper, were wetted with 5 ml of test solution per dish. Fifty curly cress seeds were sprinkled over the filter paper. After inbibition had begun and the seeds had formed a gelatinous envelope, they were spaced evenly on the filter paper with a spatula. The dishes were incubated at room temperature, 19° to 21°C. Germination numbers were determined at the designated intervals by removing the newly germinated seeds from each dish; germination was defined at a 3-mm extension of the radical from the seed coat. Three replications were prepared per treatment.

For experiment 1, the 1:20 residue extracts were used to wet the cress seeds, with germination recorded at 36, 48, 72, 96, and 108 hours. For experiment 2, the 1:20 extracts were diluted to 1:25, 1:33, 1:50, and 1:100 with distilled water. These test solutions were applied to the seeds, and germination was assessed at 36, 48, and 72 hours. For germination assay 3#, the 1:20 extracts were diluted to 1:100 by the addition of

distilled water and concentrated Hoagland solution, yielding test solutions of 0 and half-Hoagland nutrition for application to the seeds. Germination was assessed at 36, 48, and 72 hours.

Growth Bioassay

Two tree species grown in Michigan nurseries were chosen as assay plants in the study of seedling growth: thornless honeylocust, Gleditsia triacanthos L. inermis Willd.; and black locust, Robinia pseudoacacia L. Both plants are easily germinated from seed, and display uniform early growth. Seeds were obtained from Herbst Brothers, Seedsmen, Inc. of Brewster, New York, in August of 1979. However, black locust was finally eliminated from this study because of poor root growth in sand culture.

Soil for potting was gathered from the A_2 horizon of a Spinks loamy sand exposed at a soil pit on the Michigan State University campus. The soil was selected for its relatively high porosity (34.6%), low cation exchange capacity, and low organic matter content. Collected in late October of 1979, it was allowed to air-dry in the greenhouse until needed.

To initiate germination, the honeylocust seeds were scarified in concentrated sulfuric acid for 60 minutes, rinsed thoroughly, then soaked in water for 48 hours in open petri dishes. The resulting 1-cm seedlings were planted in peat and vermiculite mix in flats, watered with one-tenth Hoagland solution, and grown to transplanting height (about 14 cm) in the greenhouse with supplemental fluorescent lights providing a 16-hour photoperiod.

Residue treatments in the growth assay consisted of plant material mixed with soil in a ratio of 1:150 by weight. For each test pot, 1150 g of air-dried soil was thoroughly mixed with 8.0 g of one of the six plant

materials, then transferred to a pot lined with plastic drainage screen. Dark green plastic pots, 10 cm in diameter by 15 cm deep, weighing about 35 g with screen, were used for planting. Additional sand was added to bring each pot to 1200 g dry weight, then the pot was watered with 200 ml of nutrient solution. Eight replications were prepared for each treatment and control.

Two control treatments were used as a comparison against residue effects. Sand pots were prepared as above, without the addition of organic matter. Extracted-KBG pots were prepared as above with the addition of 8.0 g of extracted-KBG in place of other organic matter. Extracted-KBG was prepared by soaking 200 g of Kentucky bluegrass in 4000 ml of distilled water for 4 hours, then straining the residue in 4 layers of cheesecloth, followed by air-drying at 50°C. Ninety-six g of fine material and water soluble compounds were removed by this process, leaving 104 g of extracted-KBG.

Honeylocust seedlings ready for transplanting were carefully removed from their flats, washed free of peat and vermiculite, and sorted by size. Seedlings were dibble-planted with roots fully extended into the moist pot soil, and settled with 50 ml of nutrient solution. An equal assortment of seedling heights having been assigned to each treatment, the plants were blocked by height and arranged by blocks on a greenhouse bench, with shorter plants closer to the central fluorescent fixtures. Individual plant heights were measured from pot rim to terminal bud, and equivalent plants were selected for dry weight determination. All pots stood in 15-cm wide by 3-cm deep plastic bowls. Fluorescent lights provided a 16-hour photoperiod throughout the experiment.

The water content of the pots was maintained between 50 g and 150 g after the initial week. Sample pots were weighed daily. When the average weight of the sample pots fell below 1250 g, all pots were weighed and watered with nutrient solution or distilled water to 1350 g. When watered from above, any drainage water was captured in the plastic bowl and soon drawn back into the pot. Beginning with the second month, water was alternately supplied to the pots via the drainage bowls, to reduce soil compaction.

During transplanting and maintenance, nutrition was supplied by a full-Hoagland solution augmented with NH_4NO_3 to raise the nitrogen content of the solution to 300 ppm or 600 ppm, for application to sand and residue-augment pots, respectively. Two hundred and fifty ml of the appropriate solution were added to each pot at planting, 100 ml at 2 weeks, and 50 ml at 4 weeks after planting, equivalent to 120 mg or 240 mg of nitrogen.

After 3 months of growth, the height of each honeylocust seedling was measured. Each plant was removed from its pot, washed of residual soil, dried at 50°C for 5 days, and divided, then root and shoot were weighed separately.

RESULTS AND DISCUSSION

Germination Bioassays

When seeds of curly cress are wetted with distilled water and incubated at 20°C, the first seeds in a dish of 50 will germinate within 30 hours after wetting, and, within 48 hours, over 90% of the seeds will have germinated. Presence of an inhibitory substance in the wetting solution may effect this pattern of germination in three ways (Lehle, 1981): (1) delay the onset of germination; (2) slow the rate of germination; or (3) reduce the total germination. The extent of each effect is an indicator of the nature, potency, and concentration of toxins within the solution.

Experiment 1

In experiment 1, cress seeds were wetted with 1:20 extracts of one of the six residues or with distilled water as a control. The results are listed in Table 1. All extracts significantly delayed cress seed germination and reduced total germination. The onset of germination was delayed in extracts of timothy, quackgrass, and meadow fescue. No germination occurred in extracts of Kentucky bluegrass, red fescue, and alfalfa. By 108 hours, bacterial or fungal colonies were actively growing in most extract dishes and further observation was discontinued.

Neither the pH of the extracts, which had been adjusted to 6.5, nor their osmotic potential, which was under 1 bar, well below the threshold of inhibition (Bell, 1974; Bieber & Hoveland, 1968; Fisher et al., 1978;

Table 1.--Germination of curly cress seeds in 1:20 extracts of six herbaceous plants relative to distilled water control. Experiment 1.

Treatment	Time (hr)				
	36	48	72	96	108
Kentucky Bluegrass	0.0	0.0[c]	0.0[d]	0.0[d]	0.0[d]
Red Fescue	0.0	0.0[c]	0.0[d]	0.0[d]	0.0[d]
Meadow Fescue	0.0	0.0[c]	6.8[c]	8.3[c]	8.9[c]
Timothy	0.0	7.7[b]	18.0[b]	22.8[b]	24.2[b]
Quackgrass	0.0	7.0[b]	13.9[b]	18.6[b]	22.8[b]
Alfalfa	0.0	0.0[c]	0.0[c]	0.0[d]	0.0[d]
Control	100.0 (54.6)	100.0[a] (95.4)	100.0[a] (96.6)	100.0[a] (96.6)	100.0[a] (96.6)

[a]Identical letters in the same column indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

Stowe, 1979), could explain these effects on germination. The delay or prevention of cress seed germination in all residue extracts was apparently due to the presence of soluble phytotoxins. The high absolute toxicity of all solutions prevented characterization or ranking of their toxicity.

Experiment 2

In experiment 2, inhibition of germination by more dilute solutions of the six extracts was examined, as 1:25, 1:33, 1:50, and 1:100 solutions, as well as 1:20 extracts, were applied to cress seeds. The results are listed in Table 2. All extracts showed significant inhibition of cress seed germination over the range of dilutions tested. Generally, the inhibitory effect of residue extract decreased with time and dilution.

The greatest inhibition for all extracts occurred during the early stage of incubation. At 36 hours, all residues exhibited significant inhibition at all dilutions. The inhibition can be interpreted as a delay in the onset of germination due to toxins present in all extracts. Dilution of the extracts produced less reduction in inhibition at this earliest stage of germination than at later stages as seen by comparison of dilution slopes in Figures 1, 2, and 3. Dilution also altered the relative toxicity of certain residues, such as timothy and quackgrass. The residues can be ranked by their ability to delay the onset of germination: alfalfa, red fescue, and Kentucky bluegrass; meadow fescue; quackgrass and timothy.

At 48 hours, the middle stage of incubation, all residues exhibited significant inhibition of cress seed germination at all concentrations. The greatest statistical difference in inhibitory effect among the residues was seen at this stage, with greatest separation at 1:100

Table 2:--Germination of curly cress seeds in extracts of six herbaceous plants relative to distilled water control.[a,b] Experiment 2.

Time (hr)	Residue Species	Extract Ratio (gm/ml)				
		1:20	1:25	1:33	1:50	1:100
36	Kentucky Bluegrass	0.0	0.0	1.1^{c}	5.8^{cd}	5.8^{cd}
	Red Fescue	0.0	0.0	1.1^{c}	0.0^{d}	2.3^{d}
	Meadow Fescue	0.0	0.0	0.0^{c}	3.4^{cd}	12.6^{cd}
	Timothy	0.0	0.0	3.4^{c}	19.4^{b}	28.3^{b}
	Quackgrass	0.0	2.3	11.2^{b}	13.6^{bc}	16.0^{c}
	Alfalfa	0.0	0.0	0.0^{c}	0.0^{d}	1.1^{d}
48	Kentucky Bluegrass	0.0^{d}	4.4^{c}	13.6^{c}	47.0^{b}	52.3^{d}
	Red Fescue	0.0^{d}	1.5^{c}	4.4^{c}	15.5^{c}	22.1^{e}
	Meadow Fescue	2.2^{cd}	2.9^{c}	21.4^{c}	53.0^{b}	81.0^{b}
	Timothy	9.5^{b}	18.3^{b}	38.2^{b}	53.0^{b}	87.9^{b}
	Quackgrass	6.0^{d}	14.8^{c}	24.9^{c}	48.6^{c}	65.6^{c}
	Alfalfa	0.0^{d}	0.7^{c}	8.8^{c}	17.0^{c}	28.7^{3}
72	Kentucky Bluegrass	0.0^{d}	14.2^{c}	37.4^{cd}	63.4^{c}	86.7^{cd}
	Red Fescue	0.0^{d}	5.7^{c}	22.6^{e}	61.9^{c}	90.0^{bc}
	Meadow Fescue	7.8^{c}	14.2^{c}	43.6^{bc}	70.4^{bc}	90.0^{bc}
	Timothy	19.0^{b}	16.8^{b}	49.3^{b}	78.2^{b}	95.1^{ab}
	Quackgrass	16.3^{b}	16.8^{b}	31.7^{de}	64.1^{c}	79.7^{d}
	Alfalfa	0.0^{d}	7.8^{c}	25.4^{e}	48.0^{d}	79.7^{d}

[a]Control germination: 36 hr, 58.6%; 48 hr, 90.6%; 72 hr, 94.6%.

[b]Identical letters in the same column indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

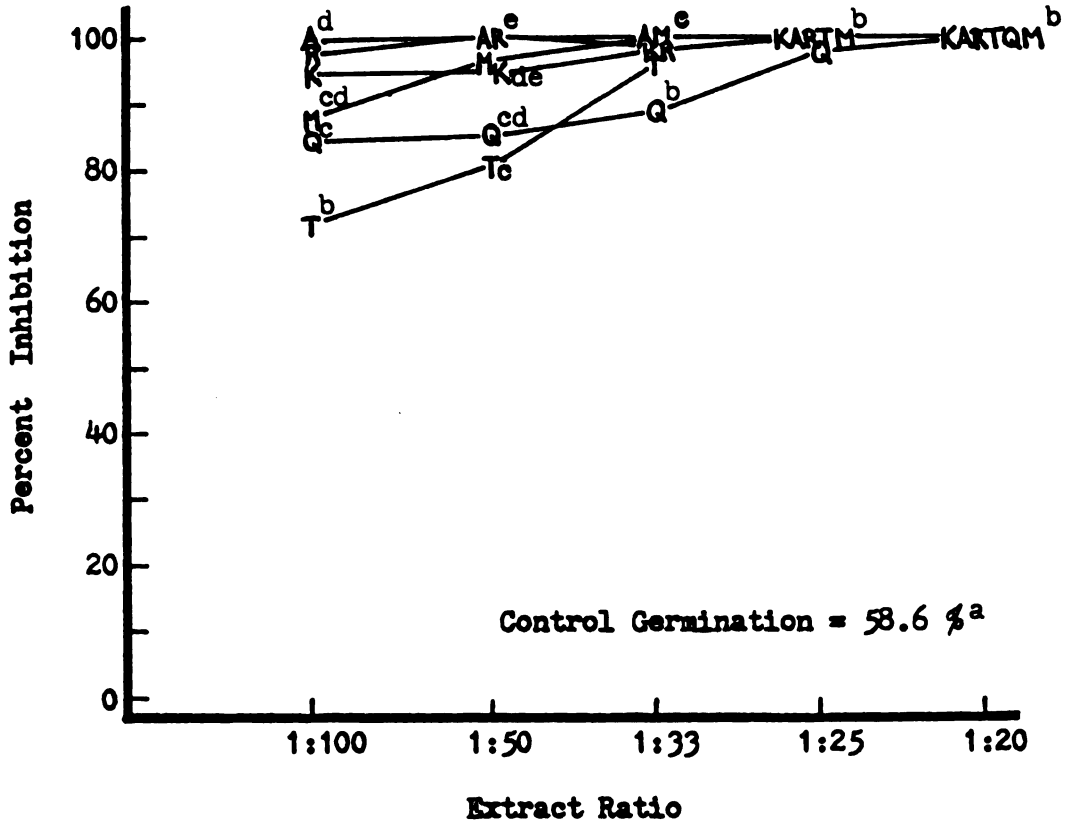
Figure 1. Inhibition of curly cress seed germination by extracts of six herbaceous species at 5 extract ratios, at 36 hours.[ab]

[a]Inhibition given as percent of distilled water control.

[b]Identical letters within the same column indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

Figure 2. Inhibition of curly cress seed germination by extracts of six herbaceous species at 5 extract ratios, at 48 hours.[a]

[a]See Figure 1 footnotes.


Percent Inhibition
100
80
60
40
20
0
1:100
1:50
1:33
1:25
1:20
Extract Ratio
Control Germination = 58.6 %a

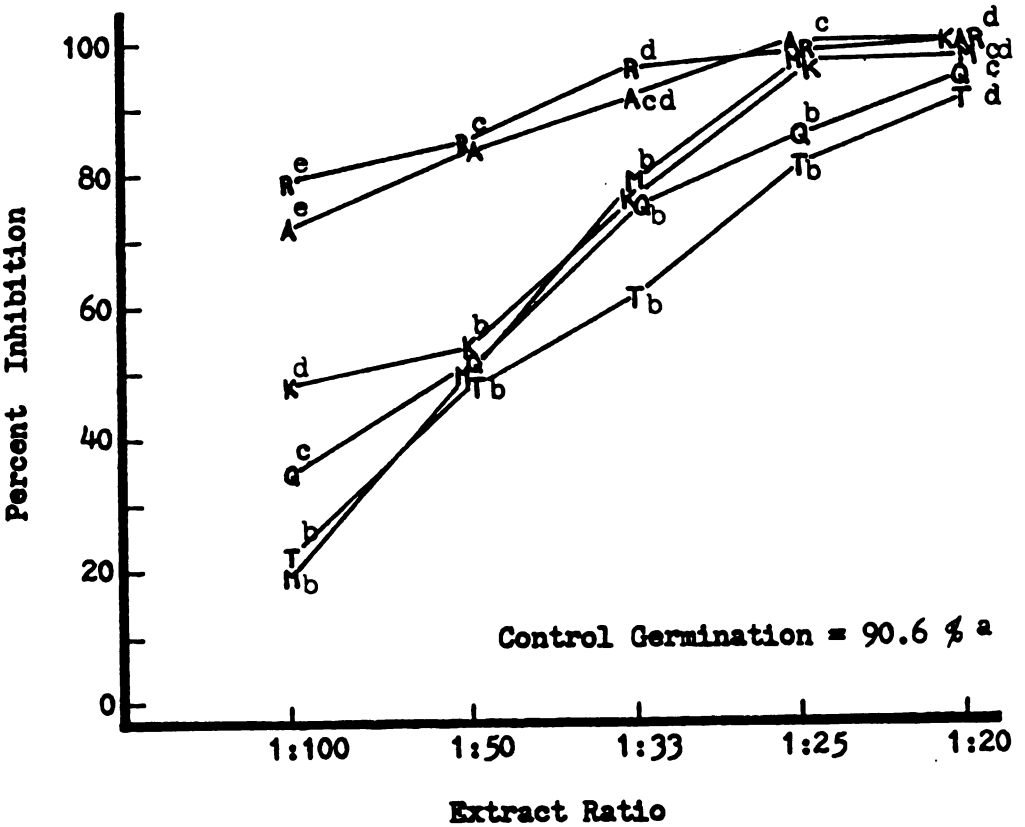
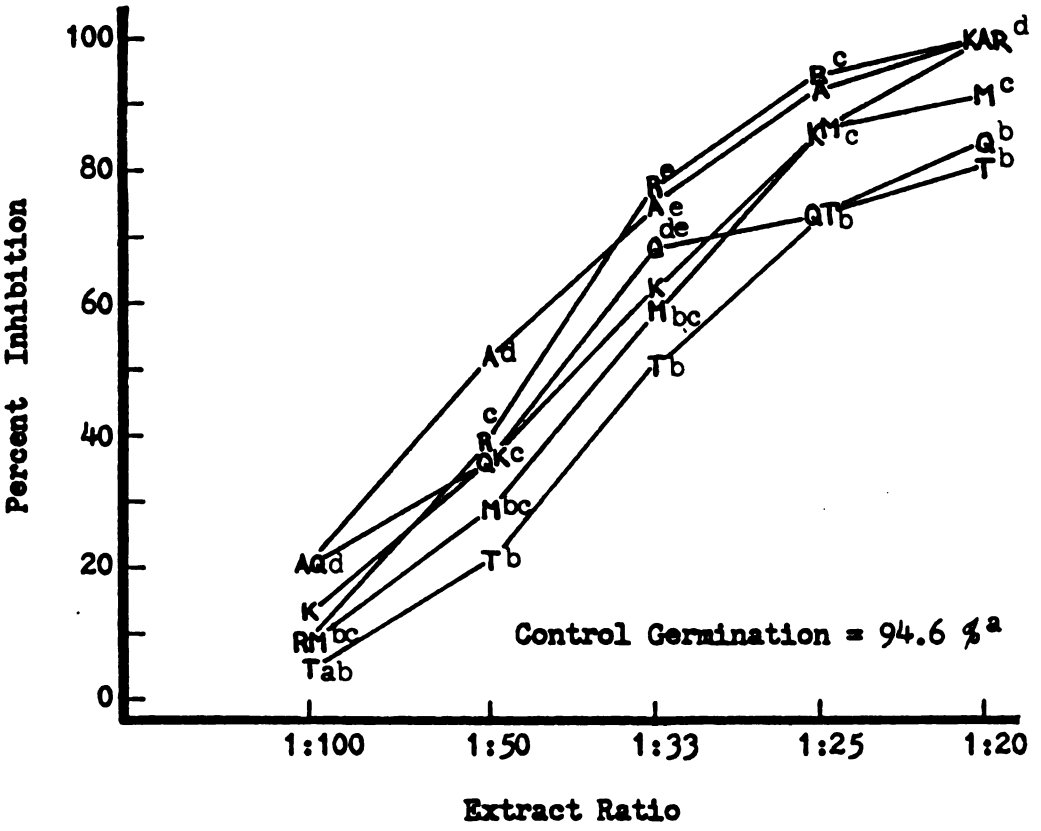

Percent Inhibition
100
80
60
40
20
0
1:100
1:50
1:33
1:25
1:20
Extract Ratio
Control Germination = 90.6 %a

Figure 3. Inhibition of curly cress seed germination by extracts of six herbaceous species at 5 extract ratios, at 72 hours.[a]

[a]Identical letters within the same column indicate no significant difference at the .05 level using Duncan's Multiple Range Test.


Percent Inhibition
100
80
60
40
20
0
1:100
1:50
1:33
1:25
1:20
Extract Ratio
Control Germination = 94.6 %a
KAR d
R c
A
M c
KM c
Q b
T b
QT b
R e
A e
Q de
K
M bc
T b
A d
c
R
QK c
M bc
T b
A d
K
RM bc
T ab

dilution. Though some residues changed in relative toxicity with dilution (meadow fescue, for example), the residue extracts can be tenatively ranked in declining toxicity at mid-germination: red fescue, alfalfa, Kentucky bluegrass, quackgrass, meadow fescue, and timothy.

Inhibition at 48 hours can be interpreted as a combined effect of delay of germination and slowing of germination rate within the population of treated seeds. Again, as shall be seen in review, the residues differ in their relative effect on each of these mechanisms. Within each residue, the diminution of inhibition with dilution is more distinct at this stage than earlier (Figures 1 and 2).

At 72 hours, all treatments, but one, significantly inhibited cress seed germination. The effect of dilution was most distinct at this final stage, with roughly equal effect among the different residues, in contrast to earlier observations (Figures 1, 2, and 3). However, some variations occurred among residues. While alfalfa, Kentucky bluegrass, meadow fescue, and timothy remained ranked in that order of decreasing toxicity with each dilution, red fescue decreased and quackgrass increased in toxicity relative to the others.

Inhibition at this stage can be considered a combined effect of delay of germination, slowing of germination rate, and reduction in total germination, though more frequent and extended observation under sterile conditions would be necessary to precisely assign the contribution of each. As indicated by experiment 1, toxins present in extracts of all residues can reduce the total germination of seeds when applied in sufficient concentration. But, using dilution data, the residues can be loosely ranked according to their ability to suppress germination:

alfalfa; red fescue; Kentucky bluegrass; quackgrass; meadow fescue; and timothy.

Certain consistent differences among the residues in their effect on the onset and rate of seed germination persist over the range of extract dilutions. As observed earlier, all the residues display an inhibition of germination onset which is not diminished in a linear manner by dilution (Figure 1). The differences in toxicity among the residues and dilutions are least in this effect on germination onset. But once germination begins, the residues differ markedly in their impact on rate of seed germination.

Red fescue and alfalfa extracts display the greatest delay of germination onset, red fescue appearing slightly, though not significantly, more inhibitory than alfalfa in dilute solution at 36 and 48 hours (Figures 1 and 2). But following initial germination, the rate of additional germination is greater in dilute red fescue extracts than in alfalfa, so that, when measured at 72 hours, red fescue is significantly less inhibitory than alfalfa (Figure 3).

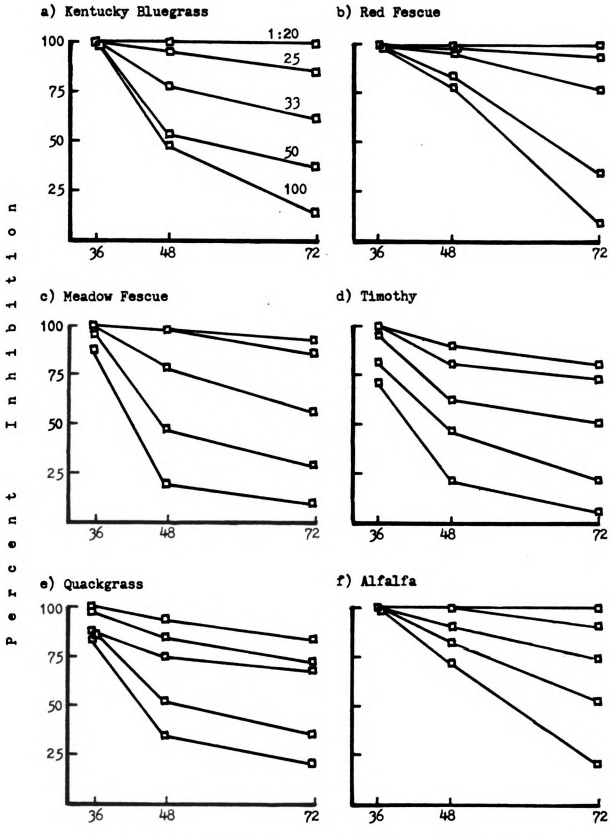
A similar pattern exists for meadow fescue. Germination onset is inhibited significantly more in dilute solutions of meadow fescue than of timothy or quackgrass. But once germination begins, it proceeds more rapidly in dilute meadow fescue extracts, so that dilute meadow fescue appears equally or less inhibitory than timothy or quackgrass at 48 and 72 hours.

The consistent and distinct patterns of germination inhibition displayed by the various residues over a range of dilutions are apparent in Figure 4a and 4f. Such distinct patterns indicate that these six

Figure 4. Inhibition of curly cress seed germination by extracts of six herbaceous plants at 5 extract ratios. Displayed by plant species.[a]

[a]Inhibition shown as percent of distilled water control.

[b]The order of extract ratios for all species are as indicated for meadow fescue.


a) Kentucky Bluegrass
b) Red Fescue
c) Meadow Fescue
d) Timothy
e) Quackgrass
f) Alfalfa
1:20
25
33
50
100
100
75
50
25
36
48
72
Percent Inhibition
Germination Period (hours)

residues distinctly differ in the nature or concentration of toxins present in soluble form within their tissues. In consequence, these plants are likely to differ as to the fate of their toxins in the soil and the nature of any allelopathic influence in the field.

Considering the taxonomic diversity of these species, the extracts of their residues are surprisingly similar in inhibitory potential. At most intervals and dilutions less than a 1-fold difference in inhibition separates the least and the most inhibitory extracts.

Experiment 3

In experiment 3, the modifying influence of supplemental nutrition on cress seed germination was examined as seeds were incubated in distilled water or 1:100 residue extracts augmented with half-Hoagland nutrition. The results are displayed in Table 3 and Figures 5a to 5f. In the absence of extract, Hoagland solution inhibited the onset of seed germination but not later germination. Conversely, nutrients significantly decreased inhibition by five of the six extracts though not at all stages. Inhibition by alfalfa extracts was significantly increased by nutrients.

Results from the three experiments illuminate the importance of the often arbitrary choices of bioassay species and assay procedures to subsequent conclusions about relative toxicity. The chemical complexity of the test solutions and the physiological complexity of the assay system make this inevitable. Toxicity can only be defined relative to plant function, and so ambiguity results.

Other investigators have examined the phytotoxicity of extracts from these plants toward seed germination or seedling growth. Nielsen et al.

Table 3.--Germination of curly cress seeds in 1:100 extracts of six herbaceous plants with and without half-Hoagland nutrition relative to distilled water control.[ab] Experiment 3.

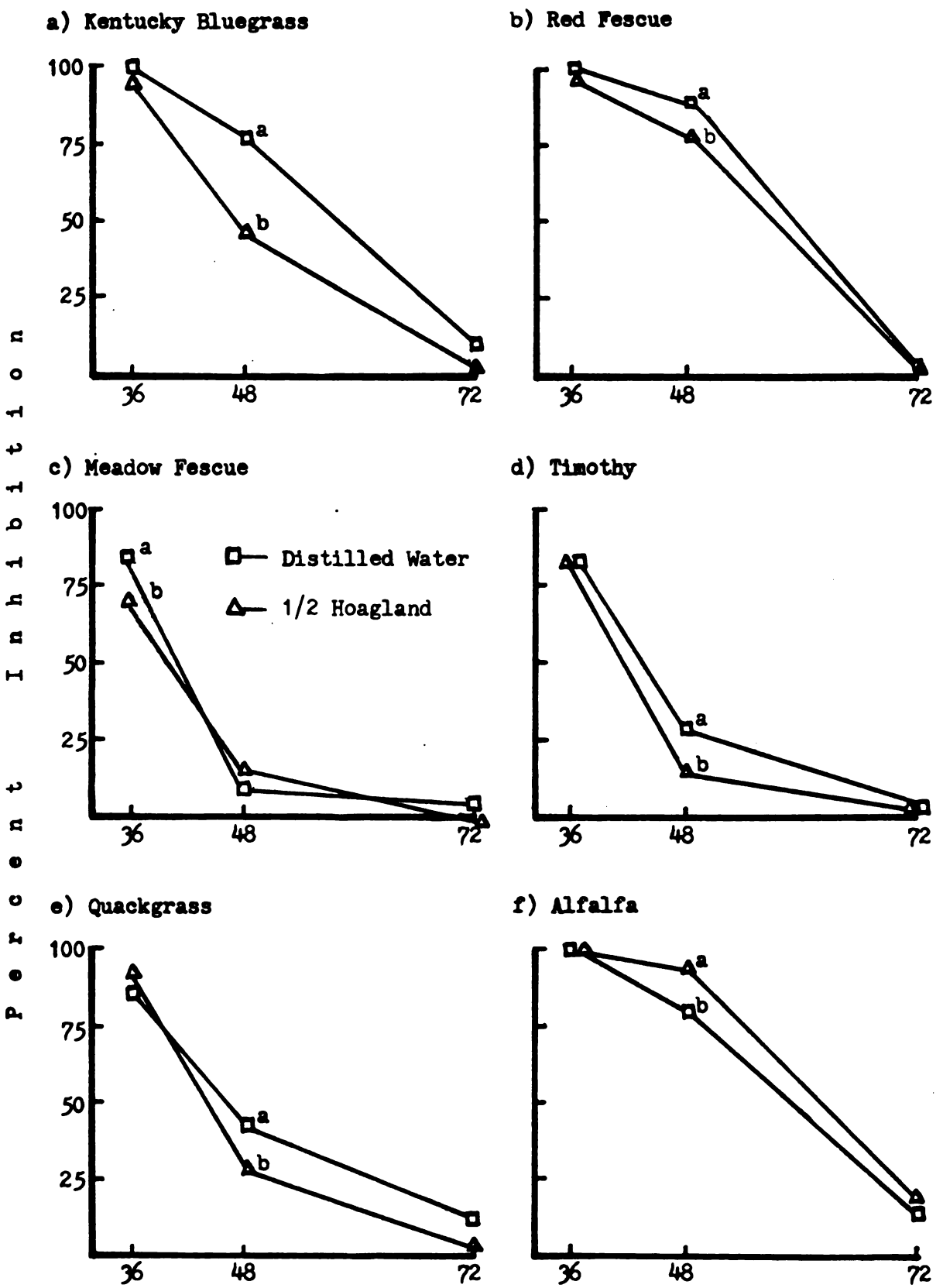
Treatment	Time (hr)		
	36	48	72
Kentucky Bluegrass	0.0	23.1[b]	90.3
with half-Hoagland	2.2	53.8[a]	99.4
Red Fescue	0.0	11.2[b]	100.6
with half-Hoagland	3.5	20.9[a]	96.3
Meadow Fescue	16.4[b]	90.4	99.4
with half-Hoagland	28.9[a]	86.8	102.2
Timothy	16.4	71.1[b]	98.5
with half-Hoagland	16.4	86.6[a]	98.5
Quackgrass	11.5	56.1[b]	88.3[b]
with half-Hoagland	7.0	70.2[a]	98.5[a]
Alfalfa	1.1	19.5[a]	84.8
with half-Hoagland	0.0	6.1[b]	82.0
Control	100.0[a] (57.4)	100.0 (89.2)	100.0 (92.0)
with half-Hoagland	44.3[b]	98.0	98.5

[a]Different letters within the same residue treatment and time indicate significant difference at the .05 level.

Figure 5. Effect of half-Hoagland nutrition on inhibition of curly cress seeds by 1:100 extracts of six herbaceous plants.[ab]

[a]Inhibition given as percent of distilled water control.

[b]Different letters indicate significant difference at the .05 level using Duncan's Multiple Range Test.


a) Kentucky Bluegrass
b) Red Fescue
c) Meadow Fescue
d) Timothy
e) Quackgrass
f) Alfalfa
Distilled Water
1/2 Hoagland
Percent Inhibition
100
75
50
25
36
48
72
a
b
Germination Period (hours)

(1960) measured the effect of 1:10 extracts of alfalfa and timothy and three other crops on the germination and seedling growth of six agricultural species incubated in quartz sand. Alfalfa extract was most inhibitory, substantially reducing the germination in 2 of 6 species and the seedling growth of all 6. Timothy was next in toxicity, reducing germination in 2 of 6 species and growth in 3 of 6. Collison and Conn (1925) found 1:20 extracts of alfalfa and timothy were markedly more toxic than other crop residues to the growth of barley seedlings, though no difference between them in toxicity was noted. Studies on quackgrass by Ohman and Kommedahl (1960) showed that 1:40 extracts of air-dried leaves reduced the germination of alfalfa seeds by an average of 50% and significantly reduced seedling growth.

However, Stowe's (1979) comparison of toxicity and distribution for six forage and weed species, including timothy, quackgrass, and meadow fescue, provides the best test of the present work. When 1:20 extracts prepared from ground, air-dried tissues were applied to seeds of all six species, germination was inhibited by meadow fescue, quackgrass, and timothy, in that declining order. With a six day incubation, inhibition averaged 50%, 40%, and 30%, respectively. In inhibition of seedling growth, the extracts ranked timothy, quackgrass, and meadow fescue.

The relative ranking of alfalfa and timothy and of timothy, quackgrass, and meadow fescue in these investigations corresponds with the results reported here. But, the degree of inhibition relative to the extract ratio appears substantially lower in all cases; the uniformity of this difference may indicate a greater sensitivity of curly cress to soluble toxins, though comparisons between studies differ in standards and procedures are problematic.

The effect of nutrition on extract toxicity seen in experiment 3 is also consistent with other studies. Early work on soil extracts and specific toxins by Schreiner (1908, 1909a, 1909b), Skinner (1916), and others of the U.S.D.A., indicated that these toxins interferred with nutrient uptake in water culture, and could be counteracted by specific nutrient supplements. Recent investigations have revealed specific mechanisms of interference by phenolic acids with phosphorus, potassium, and calcium uptake, and other related membrane functions (Demos et al., 1975; Glass, 1973, 1974a, 1974b; Newman & Miller, 1977; Rasmussen & Einhellig, 1977). Inhibition of seed germination by nutrients in distilled water, as seen here, is also common (DeFrank, 1979), and warns against generalization of germination studies to field processes.

These three experiments demonstrate that all six plant species contain potent, soluble phytotoxins differing in nature and concentration. They do not indicate whether these toxins persist, or how they might act, in a plant-soil system.

Growth Bioassay

For the growth bioassay, established seedlings of honeylocust were transplanted into soil containing residues of the six herbaceous plants under investigation to reflect, in part, the persistence and action of these toxins in soil. Change in seedling height and weight in each residue and control were measured after 90 days; these are listed in Table 4.

All residues, including extracted-KBG, significantly reduced seedling height growth, total weight change, and shoot and root weight change. Ranking of residues in effect on growth is similar for all growth

Table 4.--Growth of honeylocust seedlings transplanted into soil containing residue of six herbaceous species (8g in 1200g soil) over 90 days.[a]

Residue Species	Change In Height	Change In Total Wt.	Change In Shoot Wt.	Change In Root Wt.	root/shoot
	percent of control				
Kentucky Bluegrass	57.7^{de}	59.2^{cd}	60.4^{cd}	56.5^{cd}	.39^{bc}
Red Fescue	60.8^{cd}	61.0^{cd}	60.9^{cd}	61.2^{c}	.40^{b}
Meadow Fescue	53.0^{de}	54.1^{d}	57.0^{d}	47.1^{cd}	.35^{c}
Timothy	45.8^{e}	49.3^{d}	51.7^{d}	43.5^{d}	.36^{c}
Quackgrass	71.4^{bc}	76.4^{bc}	76.3^{bc}	76.5^{b}	.40^{b}
Alfalfa	5.3^{f}	13.7^{e}	7.2^{e}	17.6^{e}	.48^{a}
KBG Extract	81.5^{b}	80.5^{b}	80.7^{b}	80.0^{b}	.41^{b}
Control (actual)	100.0^{a} (10.5 cm)	100.0^{a} (2.92 g)	100.0^{a} (2.07 g)	100.0^{a} (0.85 g)	

[a]Identical letters in the same column indicate no significance difference at the .05 level using Duncan's Multiple Range Test.

parameters (though not all differences are significant): alfalfa, timothy, meadow fescue, Kentucky bluegrass, red fescue, quackgrass, and extracted-KBG.

The effect of incorporated alfalfa residues on seedling growth was most striking. Within a week of transplanting, all seedling in alfalfa-treated pots displayed chlorosis and wilting; by the end of 4 weeks, all had died. Acute photoxicity seemed the only explanation for this dramatic decline. To test this hypothesis, eight new pots were prepared with alfalfa residue, and watered as before, however, the transplanting of new seedlings was delayed for two weeks after incorporation. Following 4 weeks of growth, none of the new plants had died, though 3 showed moderate chlorosis, affirming the acute nature of the alfalfa toxicity. The dissimilarity between the relative effect of alfalfa and the other residues on seed germination and seedling growth indicates that different toxic mechanisms may be active in water extract and residue in soil. It is probable that the highly toxic ammonia generated by alfalfa decay was trapped in the initially water-saturated soil, and injured seedling roots. In the germination bioassay, decay may have proceeded differently and generated-ammonia could have more easily dissipated into the air. Ammonia levels in the second set of pots probably declined substantially during the 2-week planting delay. The other, less dramatic reductions in growth seen in the assay are not as easily assigned to phytotoxicity.

The moisture content of the soil was found to significantly vary among the residue treatments. Though the water content of all pots was maintained between 50 g and 150 g, the pot weights at watering showed that water content reached lower levels between weighings in certain

residues (Table 5). The treatments can be ranked by their effect on water loss (though not all differences are significant); Kentucky bluegrass, alfalfa; extracted-KBG, red fescue, meadow fescue, timothy; quackgrass; and sand. Water loss from the alfalfa pots (which were maintained after the death of their seedlings) indicates that evaporation accounted for at least 75% of the water movement, that differences in water loss between treatments were due to residue effects on physical parameters of the soil not residue effects on transpiration. In any case, comparison of ranking shows no apparent correlation between residue effects on soil water content and seedling growth. Factors other than water availability must account for the differential growth produced by the herbaceous residues.

Immobilization of nitrogen by the bacterial decay of low-nitrogen residues can reduce the growth of plants established in the same soil (Kimber, 1973). With the addition of finely ground straw to sandy loam, Allison and Klein (1962) found that nitrogen immobilization reached a maximum of 1.7% of added straw dry weight at 21 days, and averaged 1.25% over 75 days of decay. Comparable decomposition by 8 g of residue would immobilize a maximum of 136 mg of nitrogen and an average of 100 mg. This average immobilization would leave about 140 mg of available nitrogen in residue-augmented pots, compared with 120 mg in the control treatment. The degree of immobilization, however, would vary among the residues. The rate and percent of N-immobilization vary with the composition of the decaying matter, increasing with the proportion of easily degradable materials, such as soluble sugars (Allison, 1973). By this analysis, nutritional differences between the residue and control pots would seem insufficient to account for the observed growth reduction; however,

Table 5.--Water content (g) of treatment pots before watering, averaged over 11 waterings during 90 day growth assay.[a]

Sand	KBG Extract	Kentucky Bluegrass	Red Fescue	Meadow Fescue	Timothy	Quackgrass	Alfalfa
53.1[a]	67.8[c]	76.7 [d]	67.5[c]	67.4[c]	65.0[c]	59.5[b]	74.8[d]

[a]Identical letters indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

nutritional differences among the pots of the various residues might explain the smaller growth differences found between them.

Residues might alter other soil variables affecting plant growth, such as texture, microbial composition, nutrient cycling, and pH. It would be difficult to monitor or minimize all such explanations of seedling behavior. But, with the minimization of water and nutrient availability as inhibitory variables, initial, persistent, or produced toxins appear to be substantially responsible for the reduced seedling growth.

Three other investigators have considered the effect of incorporated residues of these species on seedling growth in soil. Larson and Schwartz (1980) mixed 4 g of air-dried tall fescue and timothy litter in pots containing 1 liter of vermiculite as a growth medium for young black locust seedlings. Tall fescue appeared slightly more inhibitory than timothy, though growth of all plants, including controls, was poor. Stowe (1979) added residues of timothy, quackgrass, and meadow fescue to soil in a ratio of 1:157, and grew all 3 plants plus 3 other species in the mixes for 50 days. Timothy, alfalfa, and meadow fescue inhibited growth in that order, with an average reduction of 67%, 75%, and 83%, respectively. Neither experimenter supplied sufficient nutrients to compensate for nitrogen-immobilization during decay; neglect of decay dynamics is common in allelopathy studies. When Ohman and Kommedahl (1973) incorporated 5 g, 10 g, and 15 g of dried quackgrass in 400 g of soil, growth of alfalfa and oats was progressively reduced, but addition of nitrogen substantially eliminated this reduction. None of the studies can be considered to confirm the current results.

Conclusions

All the species used in this study displayed toxicity toward seed germination and seedling growth. In the germination assay, residues at mid-germination were ranked: red fescue, alfalfa, Kentucky bluegrass, quackgrass, meadow fescue, and timothy. By growth assay, residues were ranked: alfalfa, timothy, meadow fescue, Kentucky bluegrass, red fescue, and quackgrass. There is little correlation between the relative toxicity of the six species in these distinct assay environments. Alfalfa was the only species relatively inhibitory in both assays. Several explanations for such discrepancies in residue effect have been suggested (Del Moral & Cates, 1971; McCalla & Haskins, 1964; Muller & Chou, 1977; Patrick, 1971' Rice, 1974). Toxins active against seed germination may not be active against seedling growth. The toxic constituents of certain residues may be more susceptible to decay or inactivation in soil. Some residues may release more toxic intermediates during decay. The potency of various toxic fractions may differ with dilution. Distinguishing among these alternatives is essential to understanding when and whether phytotoxic effects might occur in the field.

SECTION II

ANALYSIS OF TOXICITY DECLINE IN RESIDUES OF THREE HERBACEOUS SPECIES DECAYING IN SOIL

INTRODUCTION

The persistence and availability of phytotoxins in the soil are a function of the rates of toxin release, degradation, and stabilization within the soil complex; in this toxins are no different from the other components of decaying organic matter. But, many researchers have suggested that the toxins present in allelopathic species tend to persist in soil due to a greater resistance to decay (Bonner, 1950; Borner, 1960; McPherson & Muller, 1969; Patrick, 1971; Rice, 1974).

The residues of most plants consist primarily of polysaccharides, lignins, proteins, and fats, with smaller amounts of simple sugars, aliphatic acids, simple phenols, and other substances. The toxic subset occurring within this chemical array is potentially as diverse, but probably much different in proportionate composition (Gross, 1975). There are variations among species in the types and amounts of their constituent materials, as well as differences among the plant organs and stages of maturity. Because these organic components differ in ease of degradation, plant residues differ in their rate and pattern of decay (Allison, 1973; Martin & Focht, 1977).

Simple sugars, amino acids, aliphatic acids, some proteins, and some polysaccharides decompose quickly in soil, and may be completely utilized in a few hours or days. More resistant materials such as lignins, other phenolic substances, and waxes decay more slowly (Haider et al., 1974). Generally, 60% to 85% of the applied carbon in most residues is released

as CO_2 within a few weeks to 2 to 3 months after their incorporation into a productive agricultural soil (Jenkinson, 1971). But various residues and soil factors can alter the degree or rate of this decay, and have been considered in designing the model soil system used in this study of residue and phytotoxin decay.

The amount of added residue has little effect on decomposition rate up to 2% of the dry soil weight (Jenkinson, 1971), but the texture of those residues does. Grinding of plant material greatly increases the surface area accessable to decomposition, and reveals degradable components otherwise protected by recalcitrant tissues, thereby increasing rate of degradation (Allison, 1973).

High soil temperature, a soil pH between 5 and 9, and aerobic conditions all increase rate of decomposition. A soil water content of 50% to 100% of the water-holding capacity will usually allow good aeration and support maximum microbial activity. Addition of organic matter requires additional water to maintain this proportion (Jenkinson, 1971 and Allison & Klein, 1962 used a formula of 1:3 by weight). The nutrient content of the residue and soil can also effect degradation, if the ratios of carbon to other elements rise above those required for microbial tissues. The nitrogen content of the residue and soil is especially important, though phosphorus is occasionally limiting. If nitrogen is below about 1.7% of the added carbon, decomposition will be delayed and the process will be accelerated by supplemental nutrition (Allison & Klein, 1962).

Most organic compounds are broken down by numerous bacteria, actinomycetes, or fungi, through a series of steps characteristic of the compound, with only a few variations between organisms (Alexander, 1977).

However, certain compounds released or produced during this process may be differentially effected by certain soil conditions, such as aeration, cation exchange capacity, or pH. Phenolic monomers are an example of such compounds.

Phenolic monomers, such as vanillic, ferulic, or cinnamic acid, present as glucosides in most plant material (Harborne, 1964) and released during the degradation of lignin (Hackett et al., 1977; Ishikawa et al., 1963), have been implicated in several cases of allelopathic inhibition (Bonner, 1950; Chou & Muller, 1972; McCalla & Haskins, 1964; Patrick, 1971; Schreiner et al., 1908, 1909a, 1909b; Skinner, 1916; Zemek et al., 1979). When available in the soil aqueous phase, under aerobic conditions with sufficient nutrients, most phenolic monomers are quickly degraded to CO_2 or microbial biomass. Aerobic degradation of 100 ppm catechol in bacteria culture required less than 3 hours. In contrast, in anaerobic culture, induction of the necessary enzymes for catechol degradation alone required 5 weeks, followed by $1\frac{1}{2}$ weeks for the actual decay (Tabak et al., 1964). Since the breakdown of non-aromatic substances is much less effected by oxygen-status, anaerobic conditions, due to flooding or rapid microbial-decay, will differentially increase the persistence of phenolic compounds (Greenwood, 1968; Patrick et al., 1963). When ferulic acid and catechol were added to certain well aerated soils with a humic complex, only 67% and 25% of the added ring-carbons were released as CO_2 after 12 weeks of incubation. It was found that these compounds were differentially bound into the humic acid complex, by autooxidation in alkaline soils and by microbial phenolases in acid soils (Martin et al., 1979; Martin & Haider, 1976). While this stabilization of phenolics was relatively permanent,

Batistic and Mayaudon (1970) reported reversable stabilization of some aromatic acids by clay-colloids. Soil for the model system used in this study was chosen to minimize both differential degradation due to anaerobic conditions and differential extractability due to stabilization.

During decay, the phytotoxic component of the soil aqueous phase is in constant flux. A plant growing in such a soil responds to a constantly changing chemical environment, its cumulative growth reflects, in some sense, average soil toxicity over the growth period. But such a cumulative assay does not reveal the changes in phytotoxic concentration over that interval. The same is true, though to a lesser degree, of germination assays in soil. Only an assay procedure which samples and tests residue toxicity at discrete points has the potential of closely monitoring the changes in soil toxicity over time. In this study, water extraction sampled the readily available compounds present in the soil system at various intervals. Rapid filter-sterilization of the extract, a technique used as early as 1925 by Collison and Conn, allowed stabilization of these compounds for testing.

Two assays of phytotoxicity were used in this controlled study of residue decay: (1) inhibition of cress seed germination at 48 hours; and (2) inhibition of black locust seedling growth in agar. The germination assay is similar to that which produced the greatest differentiation among residues in Section I. Assaying of toxicity by plant growth in agar is a procedure introduced by Collison and Conn (1925), but recently developed and characterized by Bowen and Rovira (1961) and Melrod (1977).

Residues of alfalfa, meadow fescue, and sudangrass were chosen for analysis. Both alfalfa and meadow fescue were proven significantly toxic

to germination and seedling growth in Section I. Alfalfa was uniquely consistent in both assays, though only its acute effects were demonstrated in the soil assay. In contrast, the inhibition of seedling growth by meadow fescue appeared a chronic effect, since its extracts only moderately inhibited germination. Alfalfa and meadow fescue were also the only species whose inhibition of germination at 48 hours was increased by supplemental nutrition. Sundangrass was added as an experimental species because, while popular as a green manure in tree nurseries, its residues have been shown to be toxic to seedling growth in greenhouse studies, an intriguing contradiction, as in the case of alfalfa (Iyer et al., 1980).

Results in Section I suggested four hypotheses to explain the contrasting rankings of the six plant species in the germination and growth assays. The incubation and assay procedures used in this section were chosen, in part, to address those hypotheses. These hypotheses are also corollary to the broader question of the relative persistence of plant residues and their phytotoxic constituents.

MATERIALS AND METHODS

Three herbaceous plant species were chosen for the controlled study of phytotoxicity during decay: alfalfa, Medicago sativa L.; meadow fescue, Festuca pratensis Hudson; and sudangrass, Sorghum sudanense (Piper.) Stapf.

Several kilograms of the above ground portions of each of these species were collected from fields on the campus of Michigan State University: alfalfa and meadow fescue on August 15, 1980, and sudangrass on September 15, 1980. The grasses were mature and slightly senescent when cut; the alfalfa was green and in flower. The plant tissues were dried at 50°C for 5 days, ground in a Wiley mill to pass a 2-mm screen, packaged in 2 layers of 4-mil polyethylene, and stored at 1°C until used.

Soil for residue incubation was collected on September 23, 1980, from the A_2-horizon of a Spinks loamy sand (U.S.D.A., 1979) below the root zone of a stand of perennial grasses on the Michigan State University campus. After sieving with a 5-mm mesh screen, and removal of sub-samples for testing, the soil was stored in a closed container at 10°C until used. The soil had a pH of 6.8, a moisture content of 2.5% of dry weight, a total porosity of 34.6%, and a water retention porosity of 33.1%.

The field soil was modified to provide three levels of nutrition for incubation. A 5125 g batches of soil were wetted with 375 ml of distilled water, 13.3 X Hoagland solution, or 13.3 X Hoagland augmented with 8.02 g of $Ca(NO_3)_2 \cdot 4H_2O$. These three soil treatments, labelled D, H, and N,

respectively, provided 0, 210, and 400 ppm nitrogen, based on the dry weight of the soil, while H and N also supplied full-strength Hoagland nutrition, again based on soil dry weight. Soil moisture was 10% of dry weight.

Incubation and Extraction

For each incubated residue treatment, 2 g of residue were incorporated into 110 g of moistened soil and placed in a 6-ounce size, polystyrene cup. The soil was settled in the cup, moistened with 6 ml of distilled water, and covered with 1 cm of fine glass fiber to reduce evaporation. The same procedure was followed for each control treatment but without addition of organic matter or extra water. Each treatment was replicated 28 times, to provide 4 replications for sampling at 7 points during decay. The treatment cups were incubated at 16°C and high humidity in a forced-air incubator.

CO_2 evolution from sample cups was used to monitor the rate of residue decay. Two sample cups were chosen from each treatment group for analysis. At 1, 4, 7, 14, 28, 42, and 56 days of incubation, each cup was sealed in a 1-quart mason jar in the incubator. After 2 to 4 hours of gas accumulation, duplicate 2-cc samples of air were drawn from each jar into hypodermic syringes. The CO_2 content of each sample was measured using a Carle Instrument, GC 8700, Basic Gas Chromatograph, containing a thermal conductivity detector. The daily rate of CO_2 evolution from the decaying residues was estimated by subtracting control values and extrapolating to a 24-hour period. Preliminary testing had shown CO_2 release was linear over the accumulation intervals used. CO_2 analysis at 1, 4,

and 7 days was repeated with a second incubation to improve statistical separation.

Soluble compounds in the incubated soil at the various stages of residue decay were collected by water extraction. Extractions were made after 0, 4, 7, 14, 28, 42, and 56 days of incubation. Four replicate cups were selected from each treatment and watered to the specified weight (110 g for control treatment, and 118 g for residue treatment). The soil in each cup was emptied into a 250 ml, wide-mouth, Erlenmeyer flask, and diluted with 100 ml of distilled water. This slurry was briskly agitated for 30 minutes in a wrist-action shaker, at room temperature, then decanted into specially-designed, nested, centrifuge tubes. The stacked centrifuge tubes, manufactured from acrylic tubing, consisted of a 5.1-cm inside diameter, upper cup with a perforated bottom covered with a 150 um nylon screen, set in a 5.7-cm inside diameter, lower cup. The nested tubes with soil were centrifuged for 20 minutes at 2000 rpm, equivalent to a force of 672 gs at soil level (15 cm radius). With sand and silt retained in the upper cup, and coarse clay settled in the lower cup, the supernatent was decanted into a 250-ml plastic centrifuge bottle, and spun at 11,500 rpm, approximately 20,000 gs, for 20 minutes to remove fine clay and coarse organic particles. The supernatent was again decanted and briefly stored at 1°C. Between 92% and 95% of the total water in the initial slurry was recovered by this extraction procedure.

The solution of soluble compounds yielded by this extraction was concentrated to less than 20 ml using a rotating evaporator under 40 mm to 100 mm vacuum in a 50°C water bath, then rediluted to 21 ml with distilled water. The extract concentrate was then centrifuged at 30,000

rpm for 30 minutes, and filtered through a 1.2 um Gelman membrane filter to remove any turbidity, then refrigerated at 1°C.

Meadow fescue and alfalfa concentrates were measured for light absorbance as an index of extract concentration and rate of residue decay, using a Beckman DB-G Grating Spectrophotometer. Meadow fescue extracts were examined at 400 nm, alfalfa at 520 nm, and soil control at both wavelengths. An earlier incubation-extraction series had shown the greatest change in absorbtion at these respective frequencies during a 6-week period of decay. pH measurements were made of all concentrates.

Following data collection, the concentrate was cleaned and sterilized by filtering through 0.45 um and 0.2 um Gelman membranes, passing into an autoclaved, foil-sealed test tube. The sterile concentrates were refrigerated at 1°C, until used in the bioassays.

Germination Bioassay

Two separate assays were used to evaluate the toxicity of these extracts: (1) seed germination in extract; and (2) seedling growth in aseptic, extract-augmented agar.

For the germination assays, seeds of curly cress, Lepidium sativum L., were used, as in the preliminary screening. Curly cress had been shown to be rapid and uniform in germination, and relatively sensitive to toxic compounds. Seeds, obtained from the Burpee Seed Co. of Warminster, Pennsylvania, in November of 1980, were stored at 1°C, and dusted with a mixture of 43% Captan and 43% Thiram at a rate of 2.2 mg per gram of seed before use.

Procedures for the germination assays were similar to those used in the preliminary evaluation. A 9-cm glass petri dishes were lined with

Whatman's #1 filter paper, and wetted with 5 ml of test solution per dish. Fifty curly cress seeds were scattered on the wetted paper. After the seeds had imbibed, forming a gelatinous envelope (about 1 hour), they were evenly spaced with a spatula. Dishes were incubated in a growth chamber at 16°C, under low-light conditions. Germination was defined as a 3 mm extension of the radical from the seed coat. Germination numbers were counted 48 hours after the addition of seed. Three replications were prepared per treatment.

Test solutions for the germination assays were prepared by a 4-fold dilution of concentrated extract with distilled water. With the concentrated extract equivalent to a 1:10 extract ratio by weight (2 g of residue to 21 ml of water), the test solutions represented a 1:50 extract ratio. Based on amounts initially added to the soils, nutrient levels in the test solutions were equivalent to distilled water, full-strength Hoagland solution, and nitrogen-augmented Hoagland solution. Control solutions of comparable nutrition were used.

Two replicate extractions from each treatment were assayed for inhibition of seed germination. Grouped by residue type, the assays were conducted in single-replicate sets of 72 germination dishes: 3 nutrient levels x (7 incubation periods + control) x 3 replications.

Growth Bioassay

Seedlings of black locust, Robinia pseudoacacia L., were used for the growth assay of extract toxicity. While not common in the Michigan nursery industry, black locust is grown, especially for use in reclamation of disturbed sites. Black locust seeds are small, germinate easily, imbibe little water, and show relatively uniform early growth. Seeds

were obtained from Herbst Brothers, Seedsmen, Inc., of Brewster, New York, in September of 1980, and refrigerated at 1°C until used.

Seedlings for the assay were raised and maintained in aseptic culture. To surface sterilize the seeds and begin germination for each assay, seeds were scarified in concentrated sulfuric acid for 65 minutes. Under a laminar-flow hood, the acid was drained and the seeds rinsed with autoclaved, distilled water, then stored in a sterile, petri dish. After 12 to 24 hours, the dry, scarified seeds were planted with micropyle exposed in sterile 1.0% Difco-Bacto agar poured 0.75-cm deep in 9-cm petri dishes, approximately 200 seeds per dish. The dishes were inverted and maintained at room temperature (20°-21°C). In 48 hours, approximately 50 to 100 1-cm seedlings per dish were ready for transplanting into culture tubes.

Extract toxicity was assayed by measuring weight gain of black locust seedlings rooted in agar containing treatment extracts, and grown in culture tubes. Preparation of culture tubes began with mixing of 1.11% agar using Difco-Bacto agar mix and hot distilled water. Twenty ml of warm agar was dispensed into 75 ml (25 mm x 150 mm) culture tubes, sealed with metal caps. Immediately before extract addition, the agar tubes were autoclaved and then transfered to bored-out wooden racks. Under a laminar-flow hood, 5.0 ml of concentrated extract was added to each tube with a methanol-sterilized syringe with 0.2 um membrane. Agar temperature during extract addition was maintained by the wooden racks, ranging between 45° and 70°C. The warm solutions were mixed with a spatula and cooled on a slant. The tubes contained solutions equivalent to a 1:50 extract ratio with no supplemental nutrients, or with full- or

nitrogen-augmented Hoagland nutrition. Controls were prepared in a similar manner but by the addition of distilled water.

Each bioassay began with the transplanting of the small locust seedlings into the agar tubes. A 1.5-cm deep slit was made in the agar of each tube, and a seedling was inserted with root vertical and the seed half-implanted in the agar. The seedlings were planted in random order and placed randomly in the wood racks, which provided shading of the agar area for root growth. Three extracts from each soil treatment were assayed for toxicity, with 3 agar tubes prepared from each extract, requiring 72 culture tubes per single extract assay: 3 nutrient levels x 7 incubation periods and 3 replications, plus 9 control treatments.

The seedlings were grown for 120 hours in a growth chamber at 20°C with continuous lighting. At harvest, the plants were pulled from the agar substrate, grouped by treatment, dried for 4 hours at 110°C and weighed. Occasionally, the cotyledons of a seedling failed to shed their encasing seed coat, greatly inhibiting both shoot and root elongation, presumably by reducing photosynthesis. All such plants were removed before weighing.

RESULTS AND DISCUSSION

CO_2 Evolution

During the decay of organic matter under aerobic conditions, the carbon of degraded compounds may either be incorporated into microbial biomass or released as CO_2. The proportion of carbon escaping as CO_2 varies with the organic substrate and the decay environment, but for similar kinds of materials in similar environments the rate of release is usually proportional to the rate of decay (Allision, 1973; Alexander, 1977).

The daily rates of CO_2 evolution due to decay of alfalfa, meadow fescue, and sudangrass are listed in Tables 6, 7, and 8, and displayed in Figures 6 to 10. For all residues, decay and CO_2 evolution began quickly after residue incorporation, reached a peak at the fourth day sampling, then sharply declined, continuing at low levels for the remainder of incubation. But some variations occurred according to species and nutrition.

Supplemental nutrition (H and N soils) significantly increased the initial decay rates of both meadow fescue and sudangrass, the latter showing both the greatest increase and the greatest rate of decay. Alfalfa decay showed no significant response to nutrition, proceeding at rates between those of high and low nutrition for the other residues.

Uninhibited decay of 2 g of straw-like material requires approximately 34 mg of added nitrogen, though this amount will vary with the

Table 6.--CO_2 evolution from decay of 2 g of meadow fescue in 100 g of soil at 3 levels of supplemental nutrition, given as mg/day and cumulative mg.[a]

Time (days)	Supplemental Nutrition					
	Distilled Water		Hoagland		Hoag w 400 ppm N	
	mg/day	cum. mg	mg/day	cum. mg	mg/day	cum. mg
1	49.8[b]	24.9	81.0[a]	40.7	76.5[a]	38.3
4	86.7[b]	204.2	97.0[a]	308.3	105.2[a]	310.9
7	28.5[c]	351.5	43.2[b]	518.5	52.8[a]	547.9
14	15.38[b]	505.5	27.0[a]	763.9	22.7[a]	812.2
28	12.1	698.0	9.3	1017.3	14.9	1075.4
42	4.4	813.5	4.8	1116.0	5.1	1215.4
56	5.0	879.3	3.0	1170.6	3.8	1277.7

[a]Identical letters in the same line indicate no significant difference in rate of daily evolution at the .05 level using Duncan's Multiple Range Test.

Table 7.--CO_2 evolution from decay of 2 g of sudangrass in 100 g of soil at 3 levels of supplemental nutrition, given as mg/day and cumulative mg.[a]

Time (days)	Supplemental Nutrition					
	Distilled Water		Hoagland		Hoag w 400 ppm N	
	mg/day	cum. mg	mg/day	cum. mg	mg/day	cum. mg
1	67.0[b]	33.5	101.6[ab]	50.8	92.7[a]	46.4
4	87.5[b]	265.3	156.5[a]	438.1	163.9[a]	431.3
7	29.7[c]	441.1	52.3[b]	751.3	77.9[a]	794.0
14	16.0	601.1	23.1	1015.2	14.9	1118.8
28	10.0	783.1	10.0	1246.9	6.8	1270.7
42	5.0	888.1	3.0	1337.9	3.1	1292.9
56	5.3	960.2	3.5	1383.4	2.6	1332.8

[a]Identical letters in the same line indicate no significant difference in rate of daily evolution at the .05 level using Duncan's Multiple Range Test.

Table 8.--CO_2 evolution from decay of 2 g of alfalfa in 100 g of soil at 3 levels of supplemental nutrition, given as mg/day and cumulative mg.[a]

Time (days)	Supplemental Nutrition					
	Distilled Water		Hoagland		Hoag w 400 ppm N	
	mg/day	cum. mg	mg/day	cum. mg	mg/day	cum. mg
1	68.1	34.1	62.5	31.3	59.8	29.9
4	98.1	283.4	95.8	268.8	90.3	255.1
7	62.8	524.8	66.6	486.0	78.1	507.7
14	22.5[b]	823.4	29.6[a]	822.7	30.9[a]	762.0
28	9.6[b]	1048.1	15.1[a]	1135.4	17.7[a]	1102.2
42	4.9	1149.6	4.9	1275.4	3.9	1253.4
56	3.0	1204.9	4.2	1339.1	2.8	1301.7

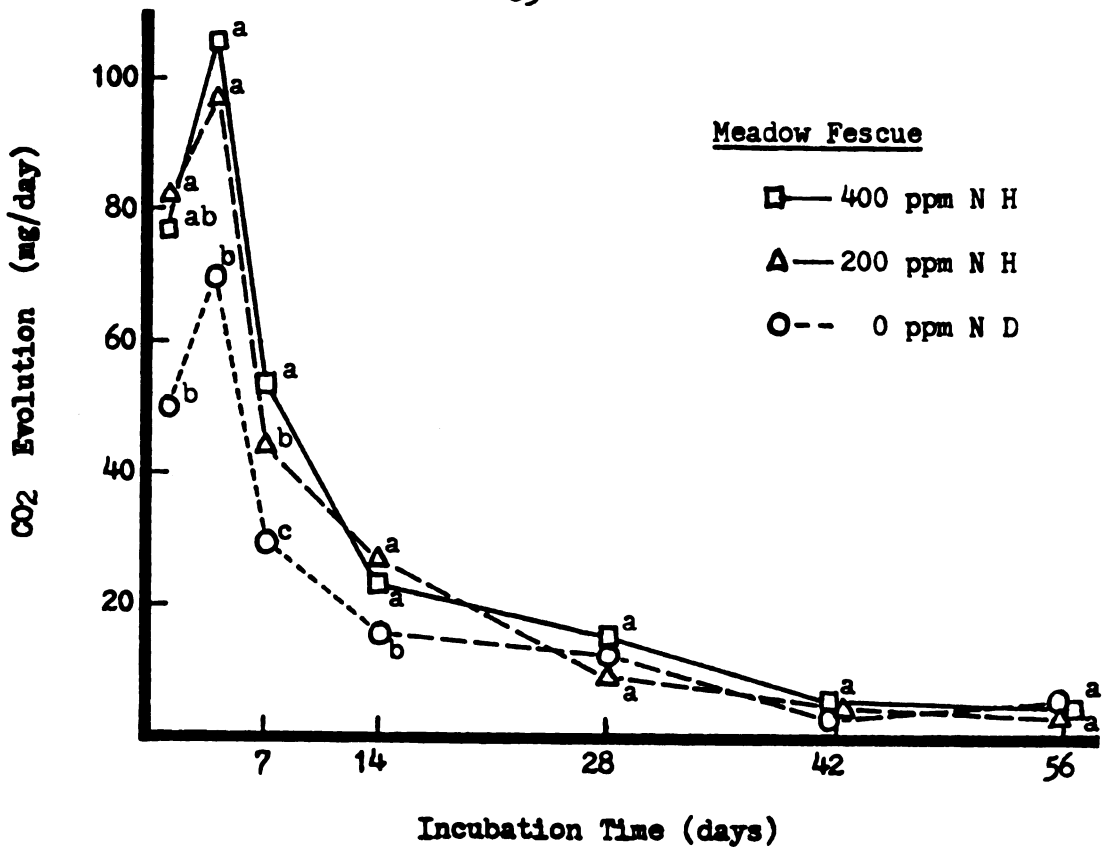
[a]Identical letters in the same line indicate no significant difference in rate of daily evolution at the .05 level using Duncan's Multiple Range Test.

Figure 6. CO_2 evolution from decaying residues of meadow fescue in soil at 3 levels of nutrition.[a]

[a]Identical letters within the same column indicates no significant difference at the .05 level using Duncan's Multiple Range Test.

Figure 7. CO_2 evolution from decaying residues of sudangrass in soil at 3 levels of nutrition.[a]

[a]See footnote, Figure 6.


Meadow Fescue
400 ppm N H
200 ppm N H
0 ppm N D
CO2 Evolution (mg/day)
Incubation Time (days)
100
80
60
40
20
7
14
28
42
56

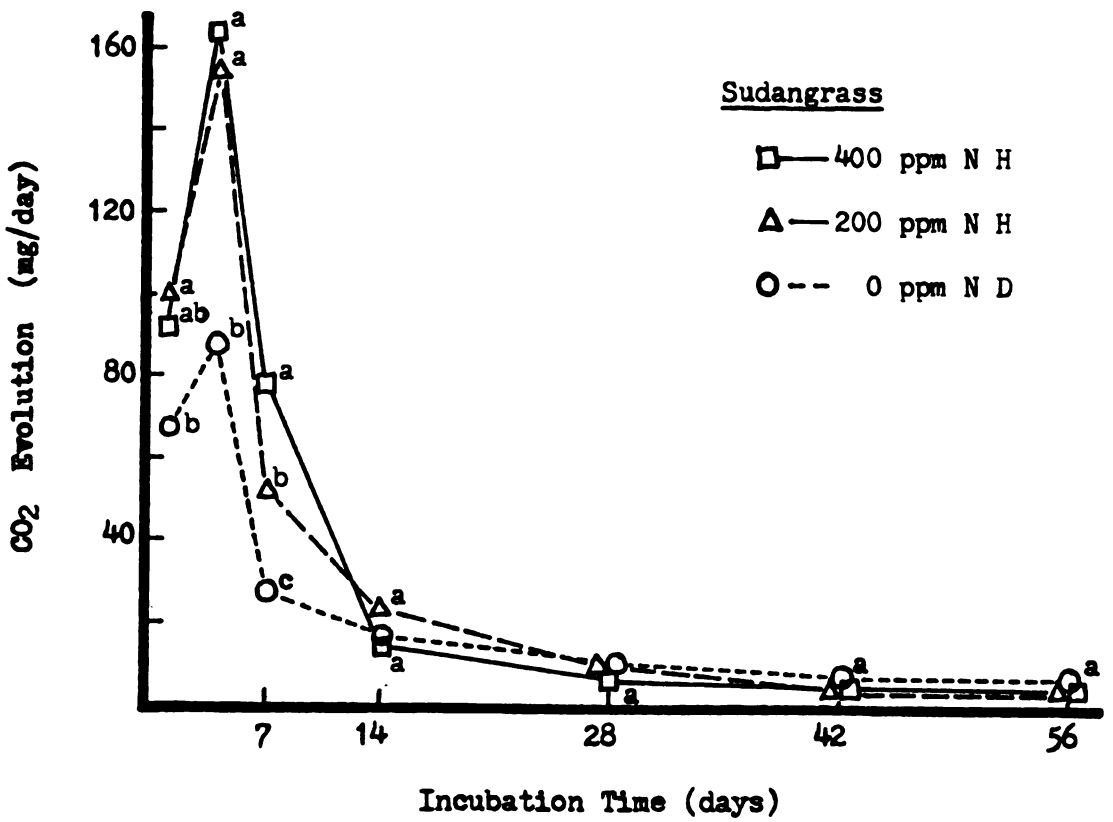

Sudangrass
400 ppm N H
200 ppm N H
0 ppm N D
CO2 Evolution (mg/day)
Incubation Time (days)
160
120
80
40
7
14
28
42
56

Figure 8. CO_2 evolution from decaying residues of alfalfa in soil at 3 levels of nutrition.[a]

[a]See footnote, Figure 6.

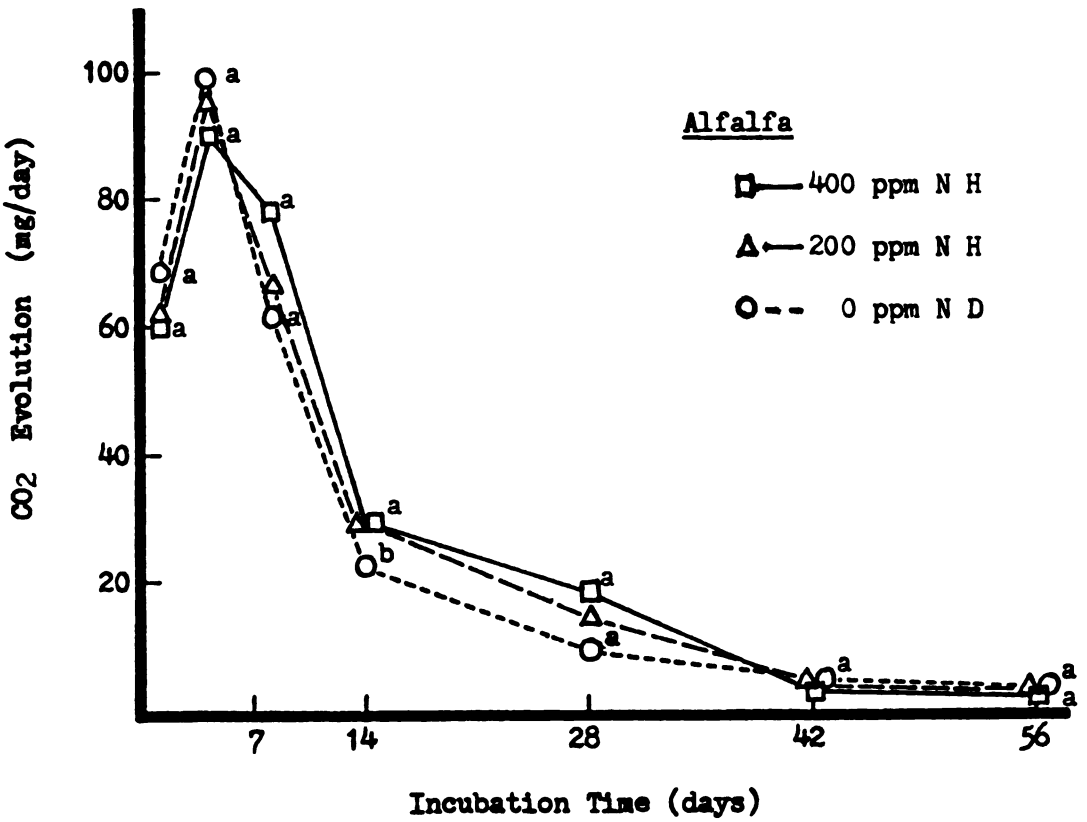
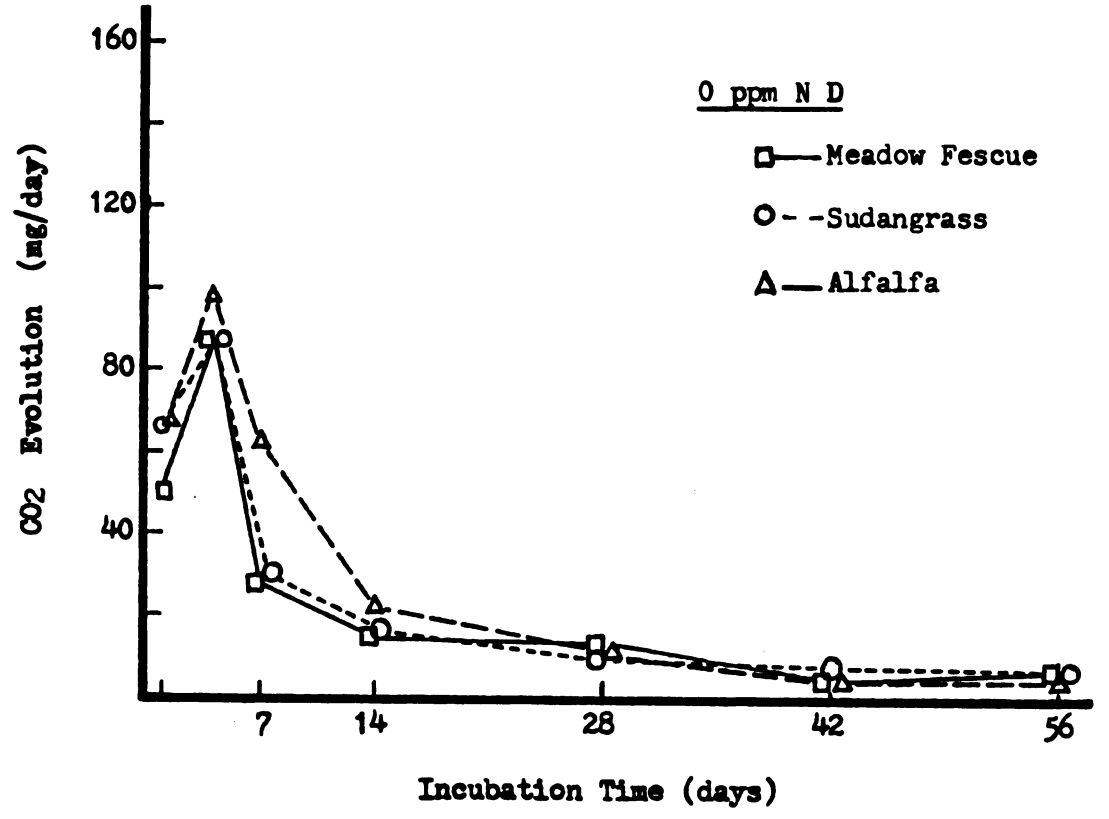

Alfalfa
400 ppm N H
200 ppm N H
0 ppm N D
CO_2 Evolution (mg/day)
100
80
60
40
20
7
14
28
42
56
Incubation Time (days)

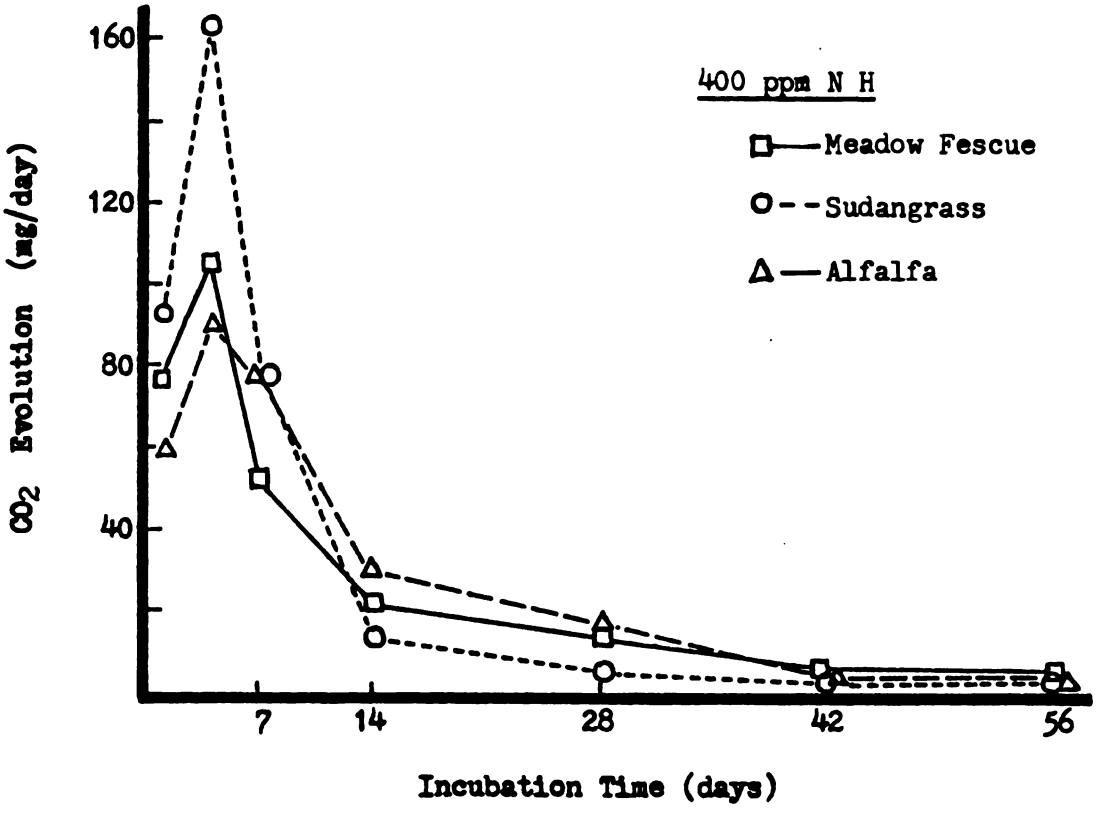
Figure 9. CO_2 evolution from decaying residues of meadow fescue, sudangrass, and alfalfa in soil without supplemental nutrition.[a]

[a]Identical letters within the same column indicates no significant difference at the .05 level using Duncan's Multiple Range Test.

Figure 10. CO_2 evolution from decaying residues of meadow fescue, sudangrass, and alfalfa in soil with Hoagland nutrition.[a]

[a]See footnote, Figure 9.


0 ppm N D
Meadow Fescue
Sudangrass
Alfalfa
CO_2 Evolution (mg/day)
160
120
80
40
7
14
28
42
56
Incubation Time (days)


400 ppm N H
Meadow Fescue
Sudangrass
Alfalfa
CO_2 Evolution (mg/day)
160
120
80
40
7
14
28
42
56
Incubation Time (days)

composition of the material and its rate of decay (Allison, 1962). If inadequate nitrogen is available, the rate of decay will decline when nitrogen becomes limiting, continuing only as the element is recycled by microbial decay. At the beginning of incubation, 0 mg, 20 mg, and 40 mg were available for decay processes in the distilled water (D), Hoagland (H), and nitrogen-augmented Hoagland (N), respectively. Nitrogen-shortage may account for the reduced rate of decay of meadow fescue and sudangrass residues from the onset of incubation in D soils and from the seventh day sampling in H soils. The uninhibited decay of the high-nitrogen alfalfa residues in D soil would tend to confirm this interpretation. However, additional factors may be involved in these release patterns: full-strength Hoagland solution would affect many nutritional and physical characteristics of the soils, residues, and microbes in addition to nitrogen balance (Hewitt, 1965).

Estimates of cumulative CO_2 evolution (Table 10) indicate that at high nutrient levels all of the residues released about 1300 mg of CO_2 over the 56-day incubation period, half during the first 10 days of decay. This corresponds to approximately 43% of the original carbon content of the 2 g of added organic matter (assuming a CH_2O composition). This proportion of the original carbon, plus the 5-25% of the carbon incorporated into new bacterial growth, represent the readily degraded component of the added residue (Allison, 1973). Assuming an immobilization maximum of 34 mg and an average microbial composition of 10% nitrogen, about 60% of the organic matter of each residue was degraded over the 56-day incubation, and about 40% persisted as resistant fractions. These estimated figures of cumulative CO_2 evolution, and therefore of the degradable fraction are

probably low. Sampling CO_2 release at the first and fourth day of decay probably underrated CO_2 evolution during the initial period of decay when most release occurred.

At low nutrition, the cumulative release of CO_2 from meadow fescue and sudangrass was about 31% and 28% less, respectively, than at high nitrogen; CO_2 release from alfalfa was off only slightly. This lower release for these residues likely reflects persistence of otherwise degradable compounds due to inavailability of nitrogen for microbial activity. Release of CO_2 from all nutrient treatments continued through the end of incubation, though at rates less than 5% of the peak rate. In N soils this release represents decay of resistant compounds, in D soils both resistant and degradable compounds may be involved. Generally, at 42 and 56 days, CO_2 release from D soils exceeded that for N soils, though not significantly.

Extract Opacity

The concentrated extracts of alfalfa and meadow fescue were tested for absorbance of light at 540 nm and 400 nm respectively. The results are given in Table 9. The absorbance of extracts of both residues increased slightly during early incubation and declined over later intervals. Absorbance declined sooner for meadow fescue extracts from H and N soils, and farther for alfalfa extracts from these same soils. The increase and decline in soluble organic compounds indicated by these figures was apparent to the eye. Meadow fescue extracts, initially medium yellow in color, and alfalfa extracts, dark blue-green, darkened in the first week of incubation and then lightened with time and level of

Table 9.--Absorbance of concentrated extracts of soil containing residues of meadow fescue (at 400 nm) or alfalfa (at 540 nm) at three levels of supplemental nutrition.[a,b]

Period of Incubation (Days)	Residue Species					
	Meadow Fescue			Alfalfa		
	D	H	N	D	H	N
0	1.48	1.43	1.43	1.58	1.45	1.40
4	2.00	1.95	1.98	1.65	1.53	1.50
7	1.93	1.73	1.73	1.45[a]	1.23[b]	1.00[c]
14	1.78[a]	1.37[b]	1.20[b]	1.30[a]	.84[b]	.71[b]
28	1.90[a]	1.22[b]	.92[c]	1.01[a]	.65[b]	.53[c]
42	1.01	1.05	.94	1.02[a]	.48[b]	.45[b]
56	.95	.82	1.00	.77[a]	.51[b]	.44[b]

[a]Key: D--distilled water, H--Hoagland nutrition, N--Hoagland with 400 ppm.

[b]Different letters within the same line and residue indicate a significant difference at the .05 level using Duncan's Multiple Range Test.

nutrition. The stable absorbance at later intervals may reflect absorbance by the recalcitrant components of the two residues.

Germination Bioassay

Germination of curly cress seeds was used to indicate changes in residue phytotoxicity during decay by assay of extracts reconstituted from concentrate. The results are listed in Table 10 and displayed in Figures 11 to 15. The inhibition of germination by all extracts decreased rapidly with time. None remained significantly inhibitory beyond 28 days of incubation.

Other sources of inhibition, such as osmotic potential, pH, and nutrient status, need to be eliminated before this reduction in germination can be assigned to phytotoxicity. The osmotic potential of the extract solutions was not monitored; however, in the preliminary screening of nursery species, solutions of at least $2\frac{1}{2}$ times greater concentration were found to be well below inhibitory levels. The pH of extracts varied, generally increasing with incubation, but all test solutions fell between pH 6.0 and 7.9, a range unlikely to differentially effect cress seed germination (Lehle, 1981). In contrast, intended nutrient differences might have altered germination results. Hoagland and nitrogen-augmented Hoagland solutions, run as controls in each assay, inhibited cress seed germination by an average of 5.2% and 6.7%, respectively. When combined with residues in 0-day extracts, both solutions significantly increased the inhibition of all three species (Table 10). Although, the nutrient status of the later extracts are uncertain, having changed during incubation, these known inhibitory effects of nutrients on germination would tend to minimize, rather than enhance, the pattern of inhibition seen at

Table 10.--Germination of curly cress seeds in extracts of soil containing residues of meadow fescue, alfalfa, or sudangrass at three levels of nutrition.[a,b,c]

Period of Incubation (Days)	Residue Species								
	Meadow Fescue			Alfalfa			Sudangrass		
	D	H	N	D	H	N	D	H	N
0	30.5[b]	19.7[b]	15.0[b]	9.3[b]	3.7[c]	3.9[c]	4.3[b]	1.5[c]	1.1[c]
4	44.8[b]	40.3[b]	43.3[b]	19.0[b]	19.4[b]	18.6[b]	19.2[c]	34.5[b]	27.5[bc]
7	60.1[c]	78.0[b]	78.8[b]	36.9[b]	49.2[b]	49.7[b]	40.6[c]	65.8[b]	63.3[b]
14	73.5[c]	86.0[b]	88.2[b]	66.9[b]	78.9[b]	75.5[b]	70.0[c]	85.2[b]	96.5[ab]
28	87.1[c]	93.5[bc]	96.5[b]	95.8[a]	96.6[a]	89.1[b]	88.2[b]	95.7[ab]	100.1[a]
42	91.9	94.3	95.4	97.9	93.8	97.1	98.6	96.4	98.8
56	91.9	97.5	97.3	98.5	99.8	93.7	100.7	100.8	100.0

[a]Key: D--distilled water; H--210 ppm N Hoagland; N--400 ppm Hoagland.

[b]Inhibition given as percent of distilled water control, which varied between 88.6% and 96.6% over the six trials represented here.

[c]Identical letters within the same line and residue indicate no significant difference at the .05 level using Duncan's Multiple Range Test. Control treatments carry statistical letter "a".

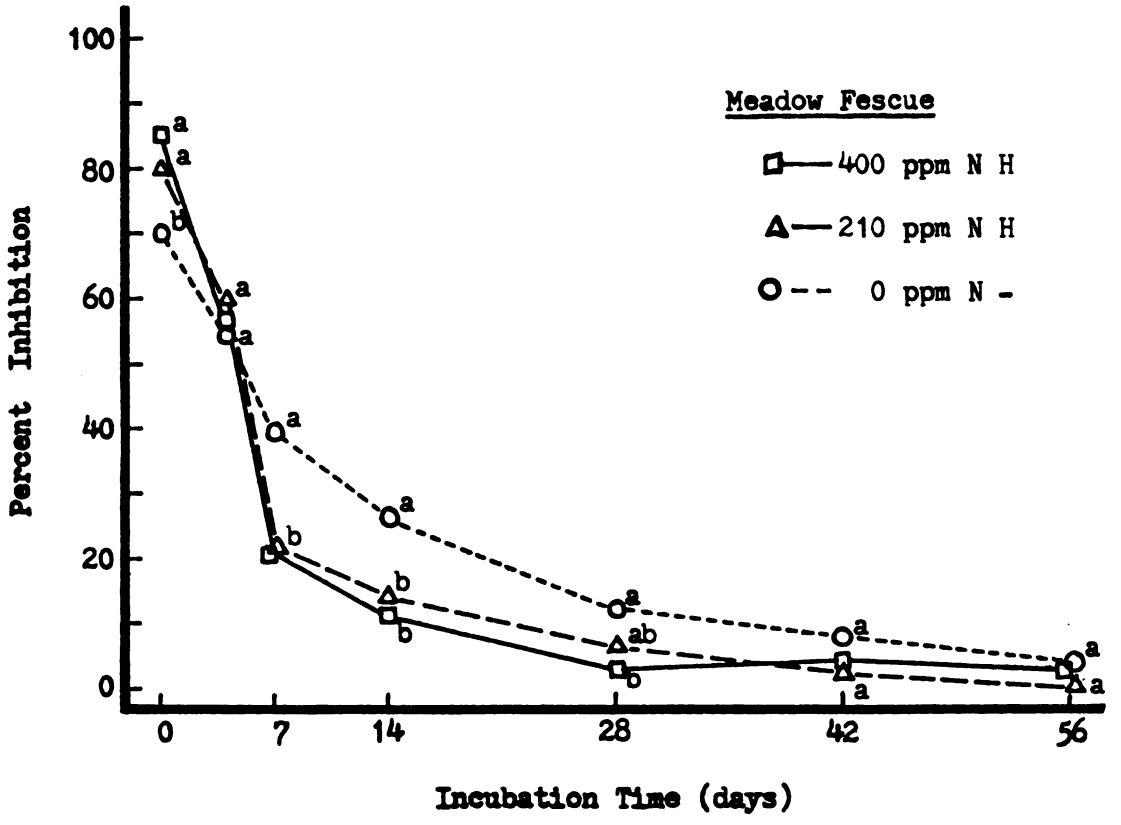
Figure 11. Inhibition of cress seed germination by extracts of decaying residues of meadow fescue in soil at three levels of nutrition.[a,b]

[a]Inhibition given as percent of distilled water control.

[b]Identical letters within the same column indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

Figure 12. Inhibition of cress seed germination by extracts of decaying residues of sudangrass in soil at three levels of nutrition.[a]

[a]See footnotes, Figure 11.


Meadow Fescue
400 ppm N H
210 ppm N H
0 ppm N -
Percent Inhibition
Incubation Time (days)
100
80
60
40
20
0
0
7
14
28
42
56

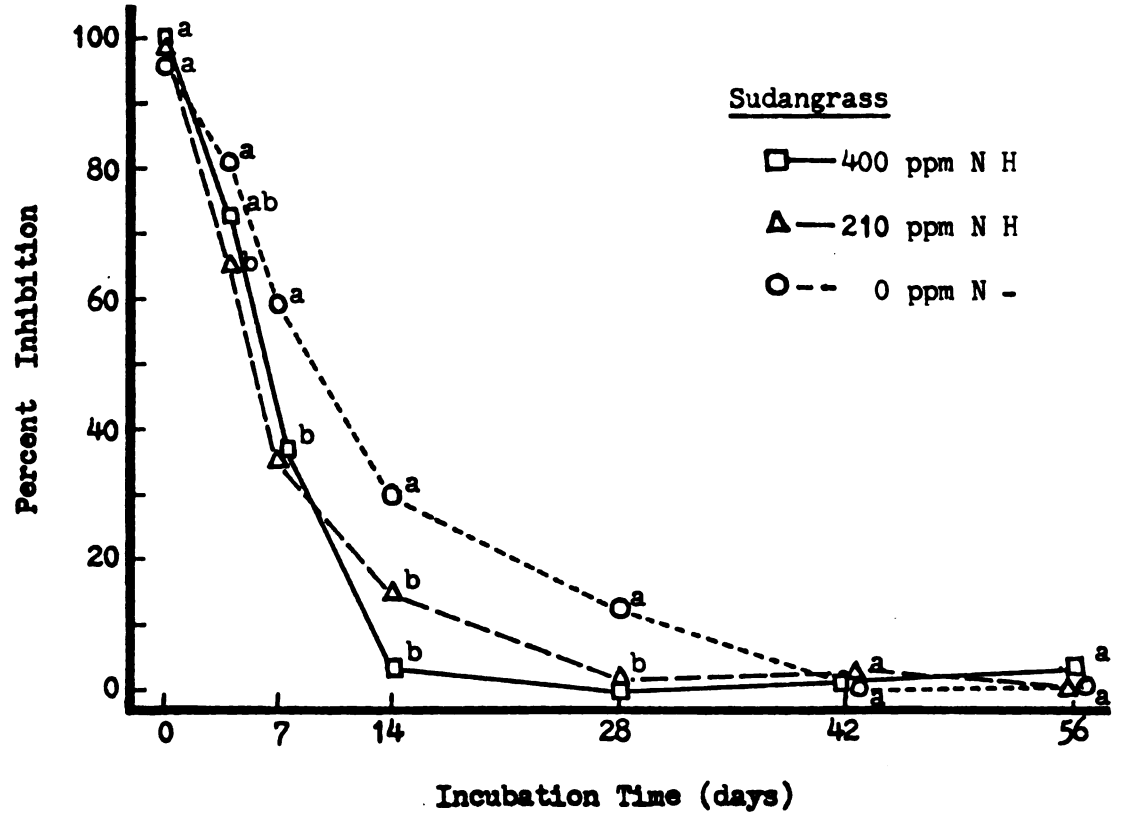

Sudangrass
400 ppm N H
210 ppm N H
0 ppm N -
Percent Inhibition
Incubation Time (days)
100
80
60
40
20
0
0
7
14
28
42
56

Figure 13. Inhibiton of curly cress seed germination by extracts of decaying residues of alfalfa in soil at three levels of nutrition.[a,b]

[a]Inhibition given as percent of distilled water control.

[b]Identical letters within the same column indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

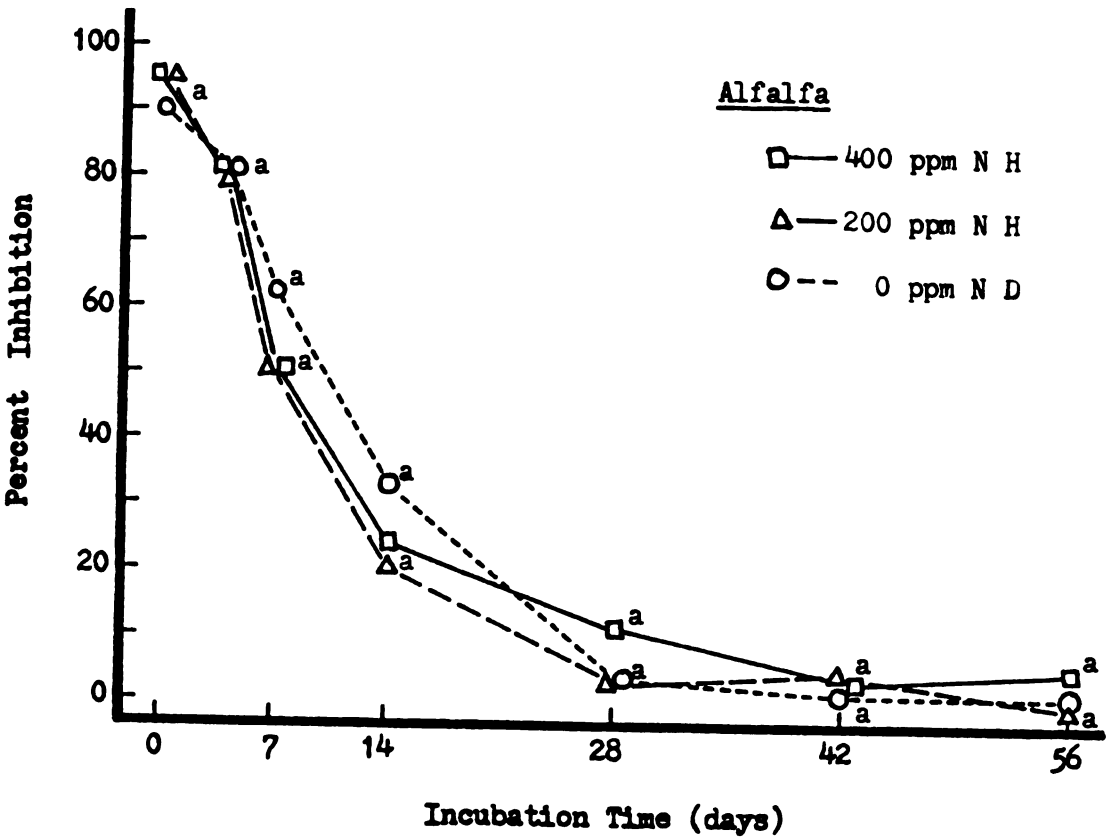

Alfalfa
400 ppm N H
200 ppm N H
0 ppm N D
Percent Inhibition
100
80
60
40
20
0
0
7
14
28
42
56
Incubation Time (days)

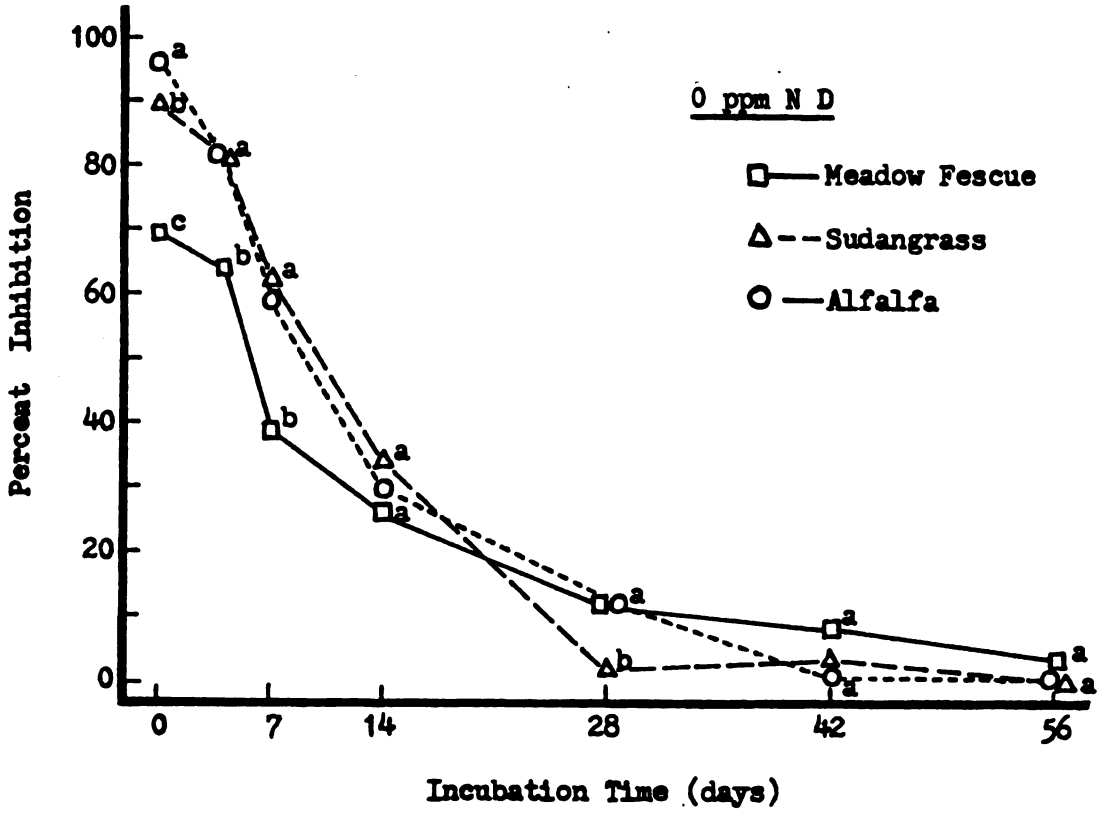
Figure 14. Inhibition of curly cress seed germination by extracts of decaying residues of meadow fescue, sudangrass, and alfalfa in soil without supplemental nutrition.[a,b]

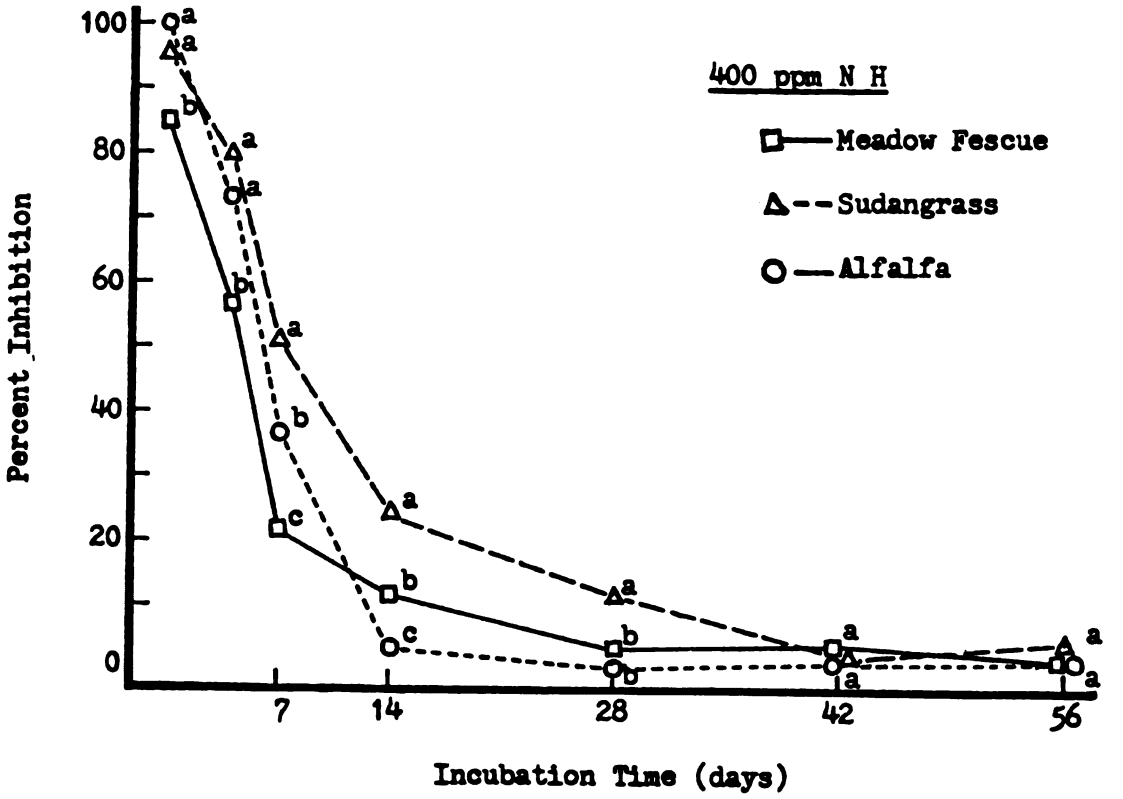
[a]Inhibition given as percent of distilled water control.

[b]Identical letters within the same column indicate no significant differences at the .05 level using Duncan's Multiple Range Test.

Figure 15. Inhibition of curly cress seed germination by extracts of decaying residues of meadow fescue, sudangrass, and alfalfa in soil with nitrogen-augmented Hoagland nutrition.[a]

[a]See footnotes, Figure 14.


0 ppm N D
Meadow Fescue
Sudangrass
Alfalfa
Percent Inhibition
Incubation Time (days)


400 ppm N H
Meadow Fescue
Sudangrass
Alfalfa
Percent Inhibition
Incubation Time (days)

longer incubations. Consequently, while nutritional contributions to germination trends cannot be eliminated, they are unlikely to have been substantial. At all intervals, extracts of D, H, and N soils without residues did not effect germination differently than the comparable nutrient solutions. Germination rate in soil extracts appears primarily an index of residue toxicity.

Although the toxicity of all residues declined quickly with incubation in soil, slight differences among residues and nutrient treatments did occur. Meadow fescue and sudangrass toxicity declined more rapidly in H and N soils than in D soils; while, relative to meadow fescue, sudangrass toxicity declined more quickly in all soils. In contrast, decline in alfalfa toxicity did not respond to nutrient level, declining less rapidly than meadow fescue and sudangrass toxicity in H and N soils, and more rapidly than in D soils This pattern of toxicity decline among residues and nutrient treatments closely follows that seen for general residue decay.

Comparison of cumulative CO_2 evolution and percent of total inhibition decline at each test interval indicates that residue decay and toxic decline were significantly correlated at the .01 level for all treatments. From this correlation and the pattern of relative decay, it can be inferred that the decline of residue toxicity in these soils was largely due to degradation of the responsible toxins. Furthermore, most of the toxins present in these residues were not more resistant to degradation than the other readily accessible plant constituents. Indeed, a greater susceptibility of toxins to degradation or detoxification might be indicated by the continued decline of meadow fescue and sudangrass toxicity

in low nutrient soils when one-third of the readily degradable compounds remained. There is no evidence of significant production of new toxins during decay. Since toxic phenolic compounds are known intermediates in lignin degradation (Hackett, 1977; Henderson, 1961; Ishikawa et al., 1963; Zemek, 1979), their effective concentration must be below detectable levels.

Due to sampling only at 48 hours, this assay of residue phytotoxicity does not distinguish patterns of differential effect on delay, slowing, or reduction of germination among the residues or nutrient treatments such as seen in the preliminary screening. Differential degradation of particular toxic components might produce such patterns. The inhibition tested by this sensitive assay was interference with early stages of germination. Casual observation indicated that only 0-day and 4-day extracts of these residues significantly reduced cress seed germination at 72 hours.

Growth Bioassay

Growth of young black locust seedlings in agar was significantly effected by addition of residue extracts. A 1-cm seedling, weighing about 15 mg dry weight without its seed coat, when rooted in plain agar under continuous light and at 20°C, will grow to about 10 cm in length (5-cm root, 5-cm shoot) and over 50 mg in weight in 120 hours. In preliminary testing, addition of extracts of all three residues to plain agar, in a 1:25 final dilution, resulted in visible interference with seedling growth, including slight yellowing of leaves, in sudangrass and alfalfa extracts, and reduced root expansion in all three. Reduction in dry weight was found to be the most sensitive parameter to changes in the agar substrate.

Weight gain of young black locust seedlings grown in extract dissolved in aseptic agar was used as an assay of decaying residue toxicity toward seedling growth. Assay results are given in Table 11. All undecayed residues were significantly inhibitory to seedling growth, but inhibition declined quickly with incubation, none remaining significantly inhibitory beyond 14 days.

As with the germination assay, other sources of inhibition must be excluded before this reduction in seedling growth can be assigned to phytotoxicity. Neither osmotic potential nor pH seem likely inhibitors of seedling growth. The agar itself was probably more osomotically negative than any extract used, and the observed extract pH was well within the growth range of this tree species. Nutrition did effect the weight gain of locust seedlings (Table 12). Contrary to the case with germination, increased nitrogen-nutrition progressively increased average seedling weight over the range used in the assay. Hoagland and nitrogen-augmented Hoagland nutrition, when combined with residues in 0-day extracts, reduced extract inhibition of seedling growth to an even greater degree (Table 11). Rather than contribute to inhibition, these nutrient effects would tend to counter the apparent toxin concentration in extracts of H and N soils. For these soils, phytotoxin and available nutrient levels in extract would both decline with decay, effecting seedling inhibition according to their relative rates of decrease. Measurement of toxicity decline for residues in D soils would be unobscured by these complications.

Based on 0-day extracts, sudangrass was significantly more toxic than alfalfa or meadow fescue, but alfalfa only slightly more toxic than meadow fescue. The toxicity of the three residues persisted in a parallel

Table 11.--Inhibition of black locust seedling growth in agar with extracts of soil containing residues of meadow fescue, alfalfa, or sudangrass at three levels of supplemental nutrition.[a,b,c]

Period of Incubation (days)	Residue Species								
	Meadow Fescue			Sudangrass			Alfalfa		
	D	H	N	D	H	N	D	H	N
0	41.7^{cd}	26.6^{bc}	19.3^{b}	63.0^{e}	49.7^{d}	46.4^{d}	44.5^{d}	31.0^{bc}	33.2^{bc}
4	23.6^{bcd}	12.1^{ab}	15.1^{bc}	34.9^{d}	25.8^{cd}	24.0^{bcd}	27.4^{bcd}	19.5^{bc}	22.4^{bcd}
7	8.0^{abc}	4.7^{ab}	5.6^{ab}	16.7^{bc}	13.6^{bc}	19.1^{c}	15.0^{bc}	17.0^{bc}	12.5^{abc}
14	-1.2^{a}	4.5^{ab}	-4.0^{ab}	13.7^{b}	6.4^{ab}	7.8^{b}	6.3^{ab}	-5.7^{a}	-5.0^{a}
28	-0.5	3.6	0.9	-1.2	2.9	-5.7	-2.7	0.8	4.5
42	-3.8	-7.0	-6.2	4.3	1.7	0.0	-3.6	1.1	-8.2
56	0.3	3.2	-3.2	-4.2	-5.9	4.7	0.5	-5.1	-7.0

[a]Key: D--distilled water, H--210 ppm N Hoagland, N--400 ppm N Hoagland.

[b]Control seedling weight for the nine trials represented here varied between 51 mg and 63 mg.

[c]Identical letters or no letters within the same line indicate no significant difference at the .05 level using Duncan's Multiple Range Test.

Table 12.--Effect of nutrition on weight gain by black locust seedlings grown in agar.[a,b]

Nutrition Level					
D	½ H	H	N	2xH	2xN
100.0	104.2	119.3	127.8	97.6	95.0

[a]Key: D--distilled water; H--210 ppm N Hoagland; N--400 ppm N Hoagland.

[b]Control (D) seedling weight averaged 52 mg.

manner. D-soil extracts remained significantly toxic for 4, 7, and 14 days for sudangrass, alfalfa, and meadow fescue, respectively, though between residue differences within nutrient levels were not themselves significant at these points. The greater inhibition by D-soil extracts cannot be ascribed to relative persistence of phytotoxins under low nutrition (as in the germination trials), because of the known effect of nutrients on toxin expression and the unknown concentration of nutrients.

There were no consistent differences in seedling appearance among the treatments, such as seen during growth in 1:25 agar. Seedlings in 0-day extract, for example, appeared healthy at 120 hours, although slightly reduced in size. Unfortunately, a doubling in residue concentration to a 1:25 extract ratio would have simultaneously complicated interpretation of nutrient effects on inhibition because of reduced seedling growth in 2x Hoagland nutrition (Table 12).

Conclusions

The integrated measurement of CO_2 evolution, extract opacity, and inhibition of seed germination and seedling growth combine to produce a relatively cohesive picture of the dynamics of residue and phytotoxin decay in this model soil system.

For all residues, decay began quickly after residue incorporation, slowing only as microbial activity became limited by the availability of nitrogen or of easily-degradable organic matter. Differences in the initial status of the residue-soil system of each treatment relative to these two variables determined the progression of decay, including peak rate and total degradation.

For all residues, the compounds responsible for extract toxicity were no less susceptable to degradation than the bulk of degradable organic matter. When decay became nitrogen-limited, residue toxicity continued to decline, perhaps due to processes other than degradation. Production of new toxins through residue decay was not detected. When the easily degradable fraction of the organic matter of each species had been broken down, only a persistent, non-toxic fraction remained.

Locust seedling growth was less sensitive to extracted toxins than cress seed germination as indicated by the respective assays. However, both species and both processes ranked the species similarly according to the toxicity of their residues: sudangrass, alfalfa, and meadow fescue.

The model soil system, designed to allow easy extraction of water-soluble toxins, was homogeneous, well-aerated, and low in cation-exchange capacity. In consequence, it maximized rates of organic decay and nutrient-immobilization for all residues and minimized the persistence and stabilization of various toxic constituents.

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