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Biochemical Frontiers of Allelopathy

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Abstract. Allelopathic interactions between plants and other organisms have been recognized by scientists worldwide because they offer alternative uses in agriculture, such as decreasing our reliance on synthetic herbicides, insecticides, and nematicides for disease and insect control. The recognition of the role that allelopathy can have in producing optimum crop yields is of fundamental importance. Despite much optimism and some progress in unravelling the complexities of biochemical interactions between species, a firm foundation for the scientific rationale of the existence and function of the allelopathic phenomenon has not been developed. Allelopathic chemicals are primarily secondary products of plant metabolism which have been an enigma to plant scientists; however, they undergo a variety of reactions with plant, insect and animal species that inhibit or stimulate their growth and development. Examples of some allelochemicals and their basis of molecular and biological action are shown: interaction between the unicorn plant (*Proboscidea louisianica* L.) and cotton (*Gossypium hirsutum* L.); diterpenoid alkaloids (from *Delphinium ajacis* L.) as allelochemicals; substances that occur in wheat (*Triticum aestivum*) and wheat soil that cause autotoxic effects; alfalfa (*Medicago sativa* L.) root saponins as allelochemicals; humic acids from wheat soil as allelochemicals; and structure–function of flavonols serving as allelochemicals in chloroplast-mediated electron transport and phosphorylation. This paper concludes with a discussion of some frontier areas of research in allelopathy.

Allelopathy involves multiple disciplines. Its recent advances have been made through the use of modern instrumentation, and it is moving from practical biology to basic molecular biology quite rapidly. The allelopathy and/or allelochemical area is an important emerging one. I would like to give my perception of the conceptual setting for production of food and fiber in the world today. In the history of the Earth there has never been a species so successful as *Homo sapiens* in establishing and maintaining itself (BENTLEY 1987). Meeting the essential dietary requirements of the increasing numbers of human beings is a global, persistent and arduous challenge for agricultural research. Most of us are familiar with how well the challenge has been met: fewer people on the land are feeding more of the world's population than ever before. It could not have been done without advances in knowledge through research and development and ultimately the practical application by the farmers of the world.

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Allelopathic interactions between plants and other organisms* have been recognized by scientists worldwide because they offer alternative remedies in agriculture, such as decreasing our reliance on synthetic herbicides, insecticides, and nematicides for disease and insect control. Recognition of the role of plant allelochemicals produced by one species that affect members of another species, either detrimentally or beneficially, is known as allelopathy. The recognition of the role that allelopathy can have in producing maximum crop yields is of fundamental importance to the welfare of mankind. Despite much optimism and some progress in unravelling the complexities of biochemical interactions between species, a firm foundation for the scientific rationale of the existence and function of the allelopathic phenomenon has not been developed. Some progress has been made toward recognizing allelopathic phenomena, such as forest regeneration, herbicide and pest resistance, the effect of crop residues on growth of seeds, weeds and other plants, detoxification mechanisms, inhibition of cell division, chloroplast and mitochondria inhibition of development by neighboring plants, the plant–soil–plant relationship.

ALLELOPATHIC CHEMICALS (ALLELOCHEMICALS) IN AGRICULTURE

It is important to recognize several aspects, such as allelopathy in (a) the capacity of species and varieties to produce allelochemicals, (b) the capabilities of various plants to synthesize allelopathic compounds, and (c) production of these by plants during the various stages of the life cycle. The producing and receiving plants may be the same or different species. The receiving plants may by growing intermingled with the producers, or they may be found adjacent to the producers in the same or a different location. Crop plants most often contact allelochemicals as soil components. The schematic Fig. 1 illustrates some of the potential field interrelationships and complexities. As allelochemicals move through the environment their quantity, residence time, and biological activity fluctuate widely (EINHELLIG 1985, 1987).

Some investigators have suggested that allelopathic substances in higher plants are immediately detoxified after release. Transformations can occur at times that can cause higher toxicity; *e.g.*, hydrojuglone is oxidized to juglone, a potent quinone that is inhibitory to some species at 10^{-6} M levels (RIETVELD 1983). Amygdalin, a cyanogenic glucoside of peach roots, yields hydrogen cyanide and benzaldehyde upon hydrolytic enzyme cleavage; other inhibitors are formed from amygdalin which are associated with the peach replant problem (RICE 1984). Microbial

* Some scientists believe that it is unfortunate to expand the definition of allelopathy so broadly, but there is a growing tendency to do so. Adherence to Molisch's definition (RICE 1984) has certain advantages, since in the past, most researchers have followed his definition, which includes interactions between bacteria, algae, fungi and plants, but not animals. Actually a check into the current literature of *Chemical Abstracts* indicates that the words allelopathy and allelochemical are being used as sub-titles, although, and appropriately so, they are not used synonymously; the terms also include effects on insects and to some extent higher grazers (HEDIN *et al.* 1985, GREEN and HEDIN 1986, WALLER 1987).

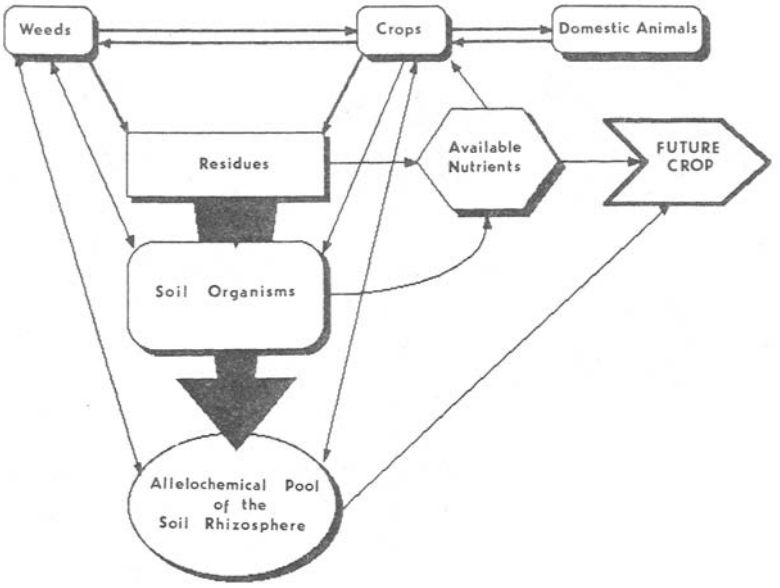


Fig 1. Field interrelationships of sources (boxes, transfers) (heavy lines), and direct and indirect impacts (thin lines) of allelopathic chemicals in agriculture (modified after EINHELLIG 1985, 1987).

transformations of chemicals, and the metabolic production of diverse allelochemicals by many microorganisms, add to the complications in determining the role of allelopathic interference with crop production. During residence in the soil they may be modified and impacts of such compounds on crop plants may be influenced by moisture, temperature, and other soil factors (PATRICK and KOCH 1958, PATRICK *et al.* 1964, MCCALLA and NORSTADT 1974, BHOWMIK and DOLL 1983, EINHELLIG and ECKRICH 1984).

Plants produce and store large numbers of secondary metabolic products (alkaloids, essential oils, phenolic compounds, steroids, terpenoids, coumarins, flavonoids, cyanohydrins, polyacetylenes, *etc.*), which vary in their biosynthetic pathways, concentration, and localization according to species. These are predominantly of protective value against predators (insects or higher grazers), bacteria, algae and fungi; they may also affect the growth of other neighboring plants, thus serving as allelochemicals (or allelopathic chemicals).

The number and diversity of compounds that are known to serve as allelochemicals is expanding. Primary metabolites such as palmitic and stearic acids (actually fatty acids up to twenty-two carbons in chain length) have been isolated from soil by WALLER *et al.* (1987 a, b), and have been shown to be allelopathic (RICE 1984, WALLER *et al.* 1987 a, b). Acetic acid is produced in large quantities from decaying plant residues; however, it is difficult to correlate soil acetic acid level with

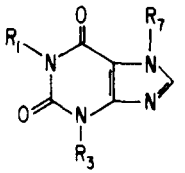
phytotoxicity (LYNCH 1987). Compounds differ widely in activity, and probably only a small fraction of naturally occurring ones may be considered allelochemicals. Some, such as essential oils (predominantly terpenes and terpenoids) and polyacetylenes, may function in a vapor state; however, most literature reports on allelochemical nature are of water-soluble compounds. Many phenolic compounds have been shown to be allelopathic; of these, naturally occurring derivatives of cinnamic acid, benzoic acid, and coumarin have been most often identified from plants (EINHELLIG and ECKRICH 1984).

EXAMPLES OF SOME ALLELOCHEMICALS AND THEIR BASIS OF MOLECULAR AND BIOLOGICAL ACTION

A) Caffeine Autotoxicity in Coffee (*Coffea arabica* L.)

Coffee plantations are managed in different ways, ranging from intensive cultivation to maintenance as natural forest-like ecosystems, but little or no attention has been given to adjusting their management for the allelopathic effects that are produced by secondary metabolites such as caffeine. Caffeine is present to the greatest extent in fruits (1.5 ± 0.3 %) in *Coffea arabica*, the plant from which most commercial coffee is made. Structures of coffee alkaloids are shown below.

	Trivial Name	R_1	R_3	R_7
Xanthine		H	H	H
1,3 Dimethylxanthine	Theophylline	CH_3	CH_3	H
3,7 Dimethylxanthine	Theobromine	H	CH_3	CH_3
1,7 Dimethylxanthine	Paraxanthine	CH_3	H	CH_3
1,3,7 Trimethylxanthine	Caffeine	CH_3	CH_3	CH_3



Plant–insect interaction involving caffeine (and other purine alkaloids and some synthetic methylxanthines) was shown by NATHANSON (1984), who used it to inhibit the feeding by larvae of *Manduca sexta* (tobacco hornworm), *Tenebrio* spp. (mealworm), *Vanessa cardui* (butterfly), nymphs of *Ancopeltus fasciatus* (milkweed bug), and adult *Tribolium confusum* and *T. castaneum* (flour beetles). Caffeine was toxic at the concentration that occurs in *C. arabica* (0.4%) in the whole plant; but all insects were affected by methylxanthines, although the ED_{50} ranged from 0.007 to 3%. There was an inhibition of phosphodiesterase (PDE) activity and an increase in intracellular cyclic adenosine monophosphate (cyclic AMP) in the insects; DUFFUS and DUFFUS (1969) had previously shown that theophylline inhibits PDE activity in barley endosperm. At lower concentrations NATHANSON (1984) demonstrated that methylxanthines are synergistic with certain other insecticides (e.g., chlorodimeform) and they are known to activate adenylate in insects.

The natural insecticidal effects of caffeine occur through an increase in concentration of cyclic AMP in tissue and an inhibition of PDE activity.

To determine the phytotoxic effect of the purine alkaloids, aqueous solutions of each compound in a series of concentrations (100, 200, 300, and 400 ppm) were bioassayed with lettuce seeds. The tested compounds exhibited significant phytotoxicity, as manifested by decreased radicle growth, at concentrations below 100 ppm (Table 1).

TABLE 1
Inhibitory effects of four alkaloids in aqueous solution of radicle growth of lettuce after 48 h at 25 °C

Compound	Phytotoxicity (% inhibition compared to distilled water) at 4 concentrations [ppm]				Statistical value [F]
	100	200	300	400	
Distilled water (control)	9	0	0	0	
Caffeine	54	78	90	84	101.3[a]
Theobromine	41	51	32	64	10.2[a]
Theophylline	58	69	81	76	76.1[a]
Paraxanthine	62	66	66	85	40.9[a]

Source: CHOU and WALLER (1989a).
[a]Statistical significance below 1 % ($F = 6.0$) level. All compounds showed significant inhibition below 1 % level at 100 ppm.

Phytotoxins with autotoxic activity are often present in the outer parts of seeds, or diaspores. If these toxins are not sufficiently leached out by rainfall or metabolized by soil microflora, they hold germination in check and ensure that this will occur only after the amounts of rainfall are sufficient for the establishment of seedlings. Other toxins, however, are stored within the seeds and are difficult to leach; these are natural protectants (*e.g.*, caffeine in *C. arabica* and strychnine in *Strychnos nux vomica*). When either of these compounds is applied exogenously to the seed that stores it, germination is inhibited, even when the concentration of the exogenous inhibitor is much lower than that found endogenously in the imbibed seed (EVONARI 1949). This suggests that such seeds have some means to avoid the effect of their own phytotoxins. FOWDEN and LEA (1979) described mechanisms by which plants avoid self-poisoning by their secondary metabolites, especially by nonprotein amino acids.

When seeds of coffee were allowed to germinate in aqueous solutions of caffeine of various concentrations, elongation of hypocotyls was reduced in all cases and growth

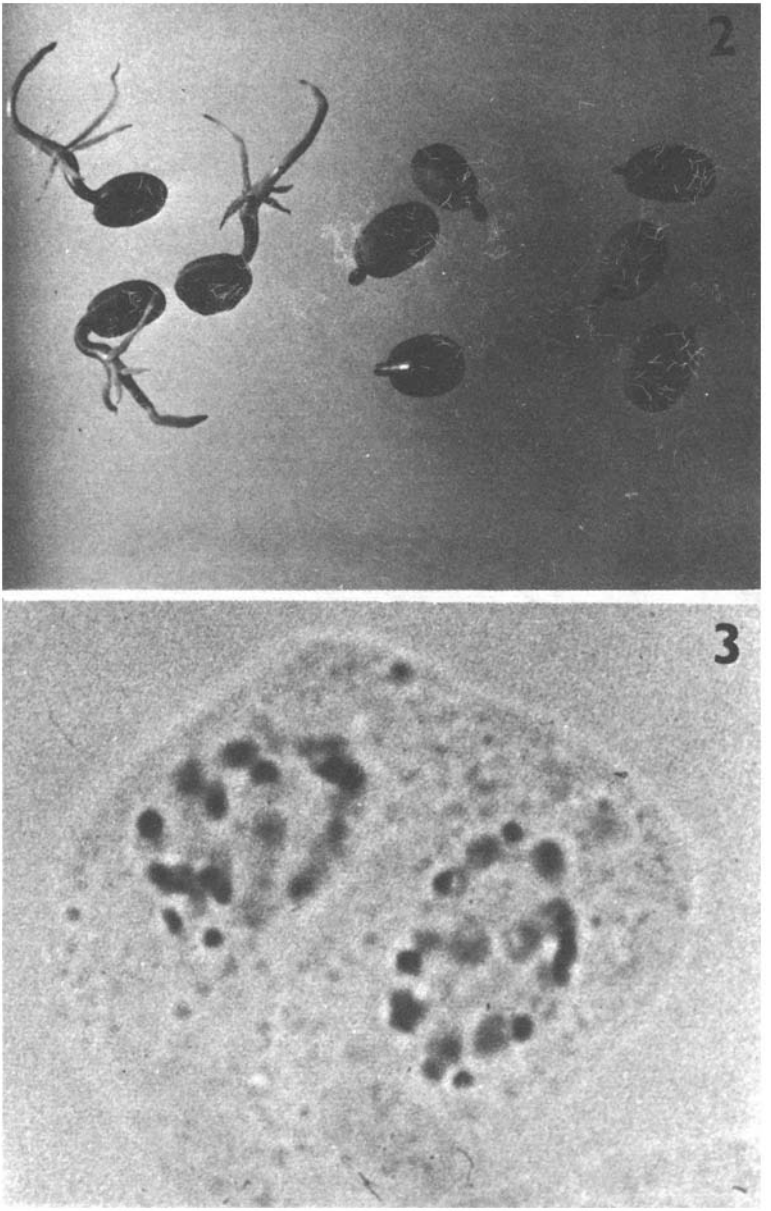


Fig. 2. Seedlings of *Coffea arabica* L. cv. Bourbon produced in distilled water (left) and in 4.25 mM (center) and 25 mM (right) caffeine, 3 weeks after wetting, in the dark. In all caffeine-treated seeds, emergence of the hypocotyl did occur but rootlet growth was arrested. (FRIEDMAN and WALLER 1983a).

Fig. 3. Inhibition of cell-plate formation in root tips of coffee (*Coffea arabica* L. cv. Bourbon) seedlings after exposure to 10 mM caffeine for 248 h (× 1200) (FRIEDMAN and WALLER 1983a).

of rootlets was almost completely inhibited by 10 mM caffeine (Fig. 2, Table 2). Root tips darkened and 4–5 days later deteriorated. A similar although milder inhibition was caused by theophylline. Suppression of growth occurred, although the concentration of the endogenous caffeine in the imbibed coffee seeds was 40–60 mM. This indicated that embryos of germinating coffee seeds must be able to avoid autotoxic hazards from their endogenous caffeine.

TABLE 2
Growth of rootlet and of hypocotyl of 4-week-old seedlings of coffee (*Coffea arabica* L. cv. Bourbon) seedlings in various concentrations (5, 10, 20 mM) of caffeine or theophylline[a]

		Concentration [mM]		
		5	10	20
Caffeine	Rootlet	72 ± 8	3 ± 2	3 ± 3
	Hypocotyl	152 ± 7	61 ± 19	39 ± 12
Theophylline	Rootlet	59 ± 7	27 ± 4	14 ± 7
	Hypocotyl	158 ± 4	48 ± 11	54 ± 6

Source: FRIEDMAN and WALLER (1983a).
[a] Data presented as percentage of growth (length in mm) of the control (distilled water); average of 50 seedlings.

Inhibitors may be sequestered in specific tissues, dead or alive. For example, in the fruit of *Ammi majus* (Bishop's weed, *Umbelliferae*), 8-methoxypsoralen is stored mainly in the outer dead layers of the fruit (FRIEDMAN *et al.* 1982). Autoinhibition occurs only after the inner fruit shell is pierced; otherwise, an impenetrable barrier keeps the embryo away from autotoxic agents. Germinating coffee seeds contain an average of 40 mM caffeine; however, mitosis in root tips is arrested by 10 mM caffeine, in a manner similar to the response of roots of onion [(KIHLMAN and LEVAN 1949) (Fig. 3)]. The caffeine of the seeds (1–2 % dry mass) is stored within the endosperm and the dormant embryo is nearly devoid of the alkaloid. During the first stages of germination no cell division occurs in the root tip; it starts only after the tip is pushed outside (1–3 mm away from the caffeine-rich endosperm) as a result of cell expansion and elongation of the hypocotyl. During this stage the cotyledons of the embryo are about 2 mm in diameter and mitosis proceeds until the third week of germination. At the end of this stage caffeine disappears from the endosperm and is accumulated within the cotyledons (Fig. 4) when mitosis is arrested. At the final stage of germination most of the caffeine of the seedling is in cotyledons (85 %) and the hypocotyl (13 %) with only a small amount in the roots. Studies by BAUMANN and GABRIEL (1984) using sterile conditions did not support the loss of 13 % in the hypocotyl, presumably because the system used by FRIEDMAN and WALLER (1983a, b) was nonsterile. Caffeine is thought to be sequestered from the sites where mitosis occurs, but how this is effected is not yet known.

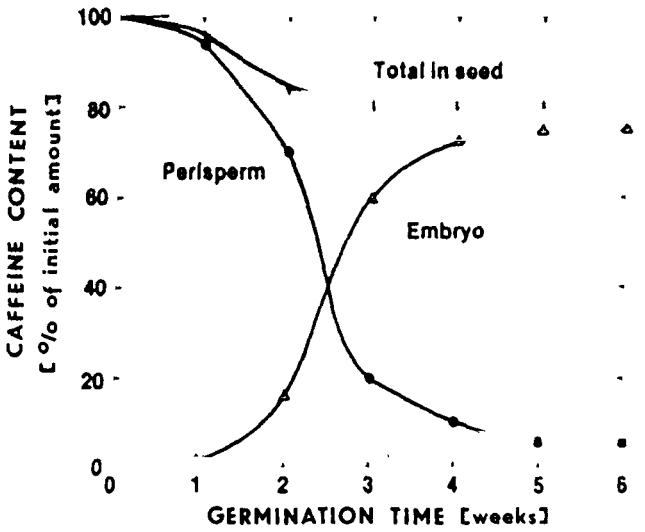


Fig. 4. Caffeine content in the endosperm (perisperm) and in the embryo during the germination and growth of seeds of coffee (*Coffea arabica* L. cv. Bourbon) (FRIEDMAN and WALLER 1983a).

Despite starvation, caffeine is preserved, which indicates that the alkaloid is not easily utilized either for energy or as a nitrogen source. Uptake of the alkaloid into the cotyledons, despite its potential hazard, suggests that caffeine is an important protective agent for the plant. The nature of the interaction between biochemical molecules and soil matrices depends upon the chemistry of the compounds and the properties of the matrix near the soil surface. Such interaction between plants and soil microorganisms is less complicated but little investigated; the allelochemical effect is governed by the soil environment and climatic conditions, which may vary by geographical regions.

Attempts to extract endogenous caffeine by the method used for fruit or seed were not successful when applied to soil from a coffee plantation of Mexican origin (Fig. 5). Soil was treated with caffeine in varying amounts and then incubated for 24–48 h at 28 °C. This gave higher recoveries of caffeine from canopy soil by refluxing with aqueous HF and HCl for 20 min, a recovery of nearly 100 % (Fig. 5) was obtained. Caffeine should be determined to ascertain what the survival of the alkaloid in the Mexican soil may be. It is remarkable that soil from under coffee trees allows complete recovery of added caffeine! An understanding of the differences between the soils from the canopy and outside the canopy is needed. There is a possibility that soil microbes in canopy soil have adapted to metabolizing caffeine, but rather slowly, since it never gets below the "saturation" level in soil.

These preliminary analytical data on the soils suggest that their binding of caffeine is very complicated. They imply that the canopy soil already contains much caffeine while the outside soil of the canopy is far from being saturated. The term

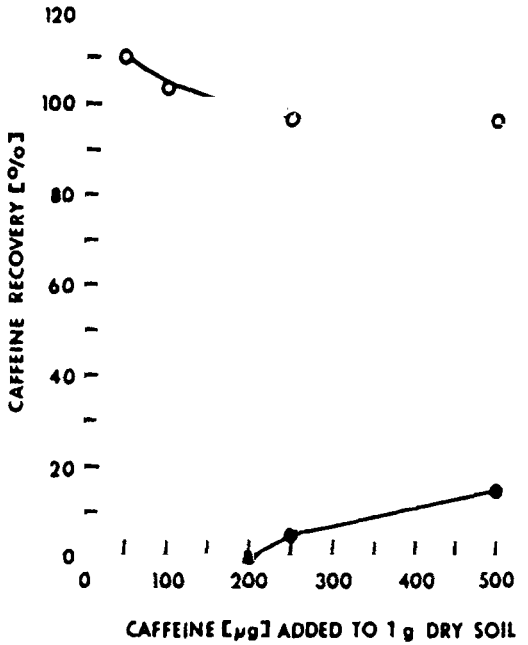


Fig. 5. Caffeine recovery [%] from soil samples previously treated with different levels of caffeine, by the HF-HCl method: soil under coffee canopy (o); soil 2 m away from coffee tree (•) (WALLER *et al.* 1986).

"saturation" refers to the amount of caffeine bound to the soil matrices by covalent or ionic bonds or sorption and to humic and fulvic acids. For the sake of simplicity, two different groups of sites that immobilize caffeine can be distinguished, those that have a very strong binding capacity (covalent or strong ionic bonding), and those that have a weaker binding capacity (weak ionic and sorption bonding). In canopy soil under old trees, the first sites are regarded as saturated with caffeine and related alkaloids, but these are not removable by our methods; any added amount of caffeine is more weakly held on the second group of sites and therefore is recoverable by our extraction. In a rather simplified manner we have calculated the amount of "saturation" of caffeine to be 2–4 mg g^{-1}. This level in soil is enough to inhibit the growth and development of roots of the coffee plant.

We suggest that caffeine can be incorporated into the nucleic acid chains of DNA and RNA and thus prevent the normal cell division. LANG (1976) observed a decrease in the T_m of DNA in the presence of 20 μM caffeine or higher concentrations. The destabilizing effect increased with increasing concentrations of caffeine. Since these changes serve as recognition sites for repair endonucleases, their reversal by caffeine prevents excision repair (Fig. 6).

In conclusion, the results show that autotoxicity caused primarily by caffeine may occur in nature. The growth of coffee seedlings exposed to 10 mM caffeine was inhibited in the rootlets, where mitosis and cell plate formation were blocked. Since

concentrations of endogenous caffeine in the imbibed seed were 40–60 mM, 4–6 times as high as in the seedlings, we conclude that coffee embryos have specific means of avoiding caffeine autotoxicity. Caffeine levels in Mexican canopy soil indicated that around old coffee trees considerable amounts of the alkaloid may be released from the tree litter and accumulate in the vicinity of roots over the years. The annual amount of litter (mostly leaves) produced, plus about 10 % of lost fruits, in old coffee trees is 150–200 g dry matter m^{-2} $year^{-1}$ (EPIFANIO 1981). We reason that this litter may release 1–2 g caffeine m^{-2} $year^{-1}$, with some additional amounts of other purine alkaloids and compounds. The antimicrobial activity of caffeine may reduce catabolism of the alkaloid in the soil and thus prolong its retention and increase its accumulation.

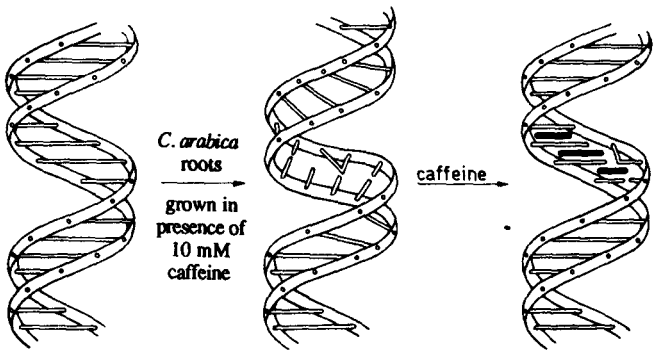


Fig. 6. Schematic representation of conformation of root DNA and its reversal by insertion of caffeine molecules. Modified from LANG (1975, 1976).

Since most roots of coffee trees develop in the upper soil layer immediately under the tree's own litter, autotoxicity can occur. Soil that is "saturated" with caffeine can also continue building up other naturally occurring compounds from the action of microorganisms. Some of these products are toxic, particularly if formed in high concentrations. This toxicity of allelochemicals may provide an explanation for the worldwide phenomenon of early degeneration of coffee plantations at 10–25 years of age (WELLMAN 1961). Caffeine, the compound most associated with the production of coffee, is the factor that allows for survival of coffee plants in a hostile environment, but it may also be responsible for shortening the lives of mature trees.

B) Biochemical Interaction Between Unicorn Plant (*Proboscidea louisianica*) and Cotton (*Gossypium hirsutum*)

The unicorn plant (*Proboscidea louisianica* (MILL.) THELL.) is an annual broadleaf weed of the family *Martyniaceae*, found in the cotton-growing areas of Texas and Oklahoma, and has been found to reduce cotton yields up to 83 %. This viscid,

Fig. 7. Picture of cotton leaf and unicorn plant leaves and flowers in the field near Perkins, Oklahoma, July 1987. (Note that the necrotic and senescent tissue is only on the cotton.)

pubescent plant is difficult to control with conventional herbicides, and often hand labor or spot treatment with more concentrated herbicides is used. It can reach heights of 1 meter and the canopy spread is approximately 2 meters. There is evidence that the effect of allelochemicals upon the cotton plant can be a contributing factor when unicorn plant grows in the presence of cotton. Leaves and stems of the unicorn plant were inhibitory in the bioassay described below on cotton leaf growth by 31 %, roots 27 %, and pods 35 %.

The unicorn plant is densely covered with glandular hairs, each tipped with a droplet of oil. A strong acrid odor of the unicorn plant when growing in the field can be detected. Fig. 7 shows that the cotton plant can be deleteriously affected by coming in contact with the unicorn plant (RIFFLE 1988). Senescence and finally death occur in a portion of the cotton leaves exposed to the unicorn plant leaf only a few centimeters away (when the wind blows, actual physical contact is made between the unicorn plant leaf and the cotton leaf). When the cotton plant is more than 3–10 cm away, the volatiles emitted are rapidly dispersed in the surrounding air. Some cotton plants show only a small response to the unicorn plant, indicating that they have ability to rapidly metabolize compounds produced by the weed.

The essential oil was isolated by steam distillation of the leaves, stems, pods, and roots of the unicorn plant for 5 h, and subsequent extraction. Mass spectrometric data were acquired and analyzed with a Kratos DS–55 data system.* Identifications were based on comparison of known with unknown spectra and visual interpretation of the fragmentation patterns.* The Kratos MS–50 mass spectrometer coupled with a Varian Model 3700 gas chromatograph containing an OV–1 fused silica column was used to obtain the profile of the essential oils which required approximately 140 min and 3 500 spectra for each sample (WALLER *et al.* 1987). The following unknown peaks were produced from the essential oil and assigned: vanillin (Fig. 8), perillyl acetate, Δ-cadinene (Fig. 9) α-bisabolol, traxolide, 2-methyl-1,4-naphthoquinone, 1-hydroxy-2(or 3)-hydroxymethylanthraquinone, and hexadecanoic acid, with small amounts of 6-methyl-5-hepten-2-one and piperitenone; the remaining compounds were mostly terpenoids, terpenes, and other hydrocarbons.

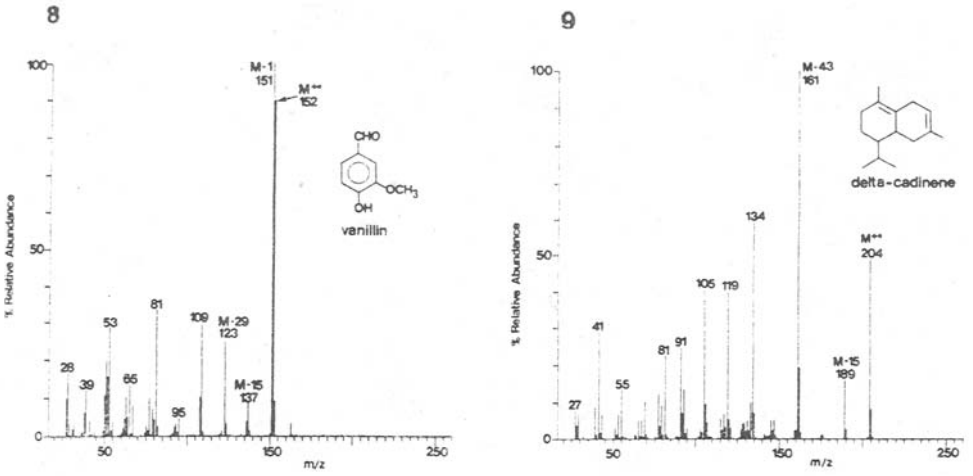


Fig. 8. Mass spectrum of vanillin from the essential oil of unicorn plant. Column background subtracted; retention time: 39.3 min; scan no. 980.

Fig. 9. Mass spectrum of Δ-cadinene from the essential oil of unicorn plant. Column background subtracted; retention time: 79.33 min; scan no. 1907.

The results of the bioassay against cotton and wheat using unicorn plant (UCP) steam distillates, were 15 and 6 %, respectively. Table 3 shows the bioassay results of some pure compounds identified thence, both as individuals and as synergistic mixtures on the radicle length of cotton (Table 4).

* The mass spectrometry analysis and interpretation were done by Dr. Richard Sgaramello, International Flavors and Fragrances, Union Beach, NJ, USA.

TABLE 3
Effects of compounds at 1 mM concentration on cotton radicle growth

Compound	Inhibition [%]
Control	0
Vanillin	20[b]
Phenylethyl alcohol	14
Δ-Cadinene	5
6-Methyl-5-hepten-2-one	17
UCP essential oil	9

[a] An average M_r of 200 was assumed for making the 1 mM concentration.
[b] Significantly different at a level of 95 % confidence.

TABLE 4
Effects of mixtures of compounds at 2 mM total concentration of cotton radicle growth

Compoud	Inhibition [%]
Control	0
Vanillin + vanillin	18[b]
Vanillin + 2-phenylethyl alcohol	12
Vanillin + Δ-cadinene	18[b]
Vanillin + 6-methyl-5-hepten-2-one	12
Vanillin + UCP essential oil[a]	15

[a] An average M_r of 200 was assumed for making the 2 mM concentration.
[b] Significantly different at a level of 95 % confidence.

We are somewhat at a loss to explain the death of cotton leaf that comes in contact with the unicorn plant leaf. A possible sequence of events associated with senescence and ultimately death of the plant cells (and animal cells) is shown in Table 5, taken from WOODHOUSE (1987). It is probable that the pathway involves the failure of a series of functions. According to Woodhouse, the synthesis of some RNA and protein occurs right up to the later stages of leaf senescence; physiological stress (as indicated by declining photosynthetic activity), retention of mitochondrial integrity, respiratory activity, plastid envelope, and leaf turgor are maintained until the very last stages of senescence, when the leaf is completely yellow. With this picture of specific pattern of events leading to the death of the cell, the probable sequence involves the preservation to a late stage of structure required for the translocation of the products of degradation to other living parts of the cotton plant.

C) Diterpenoid Alkaloids as Allelochemicals

WALLER and BURSTROM (1969) have shown that certain diterpenoid alkaloids from *Delphinium ajacis* exhibit allelochemical effects on pea cambium growth (Table 6). The diterpenoid alkaloids are related in chemical structure to the

TABLE 5
Hypothesized sequences of events in cell death (WOODHOUSE 1987)

PROGRAMMED CELL DEATH

Physiological signal provokes alteration in metabolism

PHYSIOLOGICAL CELL DEATH

Altered physiological state of cell detected

↓

Response of cell unsatisfactory, condition deteriorates

↓

Condition of cell reaches minimum defensible limits

↓

Failure mode invoked

↓

New RNA synthesized, new protein synthesized

↓

New protein suppresses further homeostatic synthesis

↓

Condition of cell deteriorates until exhaustion of most labile component

↓

Membrane defenses fail: ionic regulation fails

↓

Endonuclease activates

↓

Colapse of cell

↓

Removal of cell

gibberellins in that configuration at the A/B ring junction is similar in the two groups of compounds and is antipodal to that of most naturally occurring steroids. These results clearly indicated that delcosine and delsoline (SASTRY and WALLER 1971) were growth-inhibiting for both phloem and xylem tissue. Particularly significant

TABLE 6
Effect of *Delphinium ajacis* diterpenoid alkaloids[a] on pea cambium growth[b]

Compound[c]	Surface in transverse section [mm²]		Percent inhibition	
	Phloem	Xylem	Phloem	Xylem
Initially	0.147	0.033		
Control	0.301	0.065		
Control + delcosine	0.229	0.051	24	22
Control + delsoline[d]	0.202	0.041	33	37
Control + ajaconine	0.301	0.065	nil	nil
Standard error	±4 %	±6 %		

Source: WALLER and BURSTROM (1969).
[a]Concentration: 5×10^{-4} M.
[b]This test is quite sensitive, as indicated by the alkaloid concentration used for the inhibition studies.
[c]These are different treatments and not different results for the same treatment.
[d]This series of tests also delayed or inhibited initiation of the cambium.

was the inhibitory or delaying effect on the initiation of cambium by delsoline. In contrast, ajaconine had no significant effect on growth (Table 6).

In further studies of the relationship of diterpenoid alkaloids and gibberellic acids (GA), LAWRENCE and WALLER (1973) showed that the *Delphinium ajacis* diterpenoid alkaloids inhibited the action of gibberellic acid (GA_3) in cucumber hypocotyl bioassays. Comparison of the effects of diterpenoid alkaloids with those of abscisic acid (ABA) (a known inhibitor of GA action) and 2-isopropyl-4-trimethylammonio-5-(methylphenyl)piperidine-1-carboxylate chloride (AMO-1618) (a known inhibitor of gibberellic acid, GA_3) indicated that the presence of GA_3 inhibited α-amylase synthesis in germinating seeds and induced elongation of the hypocotyl and stem internode of lettuce seedlings in the light and dark, as well as that of cucumber hypocotyl; however, these authors did not suggest that diterpenoid alkaloids alter plant development through their influence on GA biosynthesis and/or transport within the plant.

This allelochemical action can be explained in several ways. Delcosine and delsoline may compete with the gibberellic acids for enzyme active sites, which changes the catalytic function of the latter. Such interaction may be at or near the active site or at secondary allosteric sites. Another mode of action may be feedback control of gibberellic acid(s) production by delcosine and delsoline (Fig. 10).

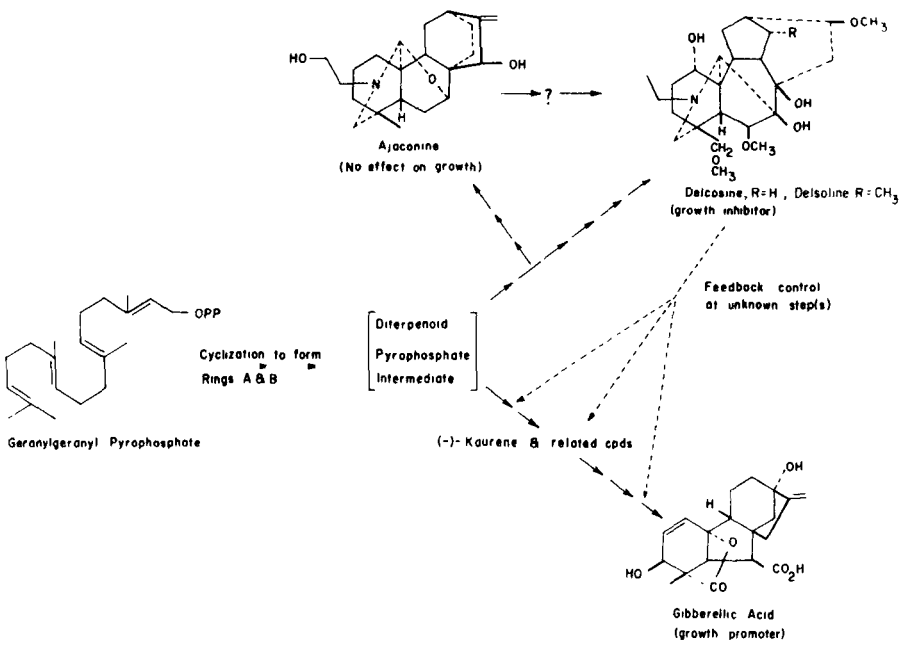


Fig. 10. Proposed relationship between *Delphinium ajacis* diterpenoid alkaloids and gibberellins.

Each group of compounds probably originates from the same diterpenoid pyrophosphate intermediate. In the usual method of feedback control, the end product controls its production at one of the initial steps in biosynthesis (such as the branch point). It is conceivable that the diterpenoid alkaloid control could be exerted at some other step on the gibberellic acid biosynthesis branch of the pathway. These nitrogenous bases can be isolated from plants of the *Aconitum* and *Delphinium* genera of *Ranunculaceae*, the *Garrya* genus of *Garryaceae*, and *Inula royleana* of the *Compositae*, thus, they might exert an effect on the plants which were endogenous to gibberellic acid on ABA.

D) Isolation and Partial Purification of Substances that Act as Allelochemical Agents in Wheat Straw and Soil

No-tillage management of wheat farming reduces the growth and yield of successive wheat crops when compared to conventional tillage. An allelochemical effect of wheat straw residues is thought to be one factor in the yield reduction. Field soil and straw samples of no-tillage and conventional tillage wheat plots were collected at monthly intervals during February 1986 and January 1987. The soil and wheat straw were extracted with water under mild conditions that most nearly

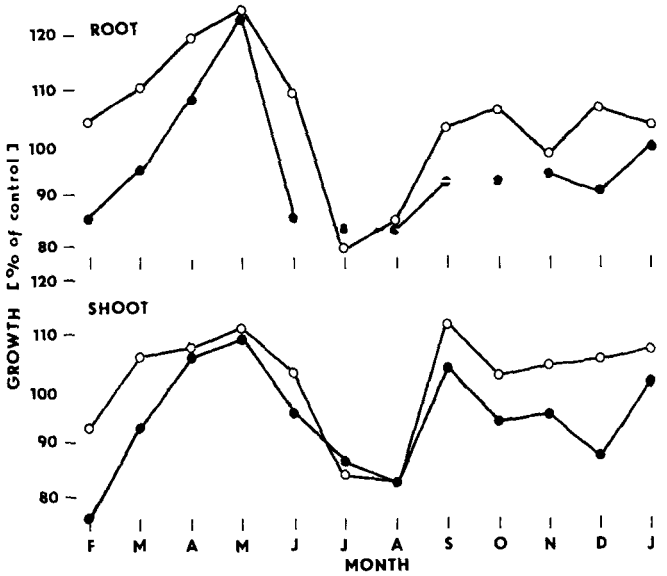


Fig. 11. Growth as a percentage of control from bioassay of direct aqueous extract of NT (•) and CT (o) soils from February 1986 to January 1987.

approximated the natural situation. The extracts were fractionated by using organic solvents. All fractions were subjected to wheat bioassay to assess the extent of inhibition or stimulation of growth. The organic compounds in the solvent extracts were separated by capillary gas liquid chromatography and analyzed with a low-resolution mass spectrometer equipped with a data system (CGC/MS/DA).

Extracting soil under slightly basic conditions (pH 8) was most effective. When the results of the wheat soil experiments were examined for monthly variation (Figs. 11 and 12), the most significant inhibition was found in bioassays of soil collected in June, July and August (CAST 1988). Fig. 12 shows that the water-soluble compounds were, for the most part, transferred to the methanol-soluble portion. Only the methanol-soluble portion of the extract was analyzed with CGC/MS/DA. The mass spectrometric analysis (Fig. 13) showed fatty acids (C_{14} through C_{19}) as the most abundant compounds, with palmitic and stearic acids dominant. Some of the compounds that are represented at 1190, 1252, 1300, 1351 and 1400 mass spectral peaks in Fig. 13, are probably active allelochemically, although their identification remains unknown.

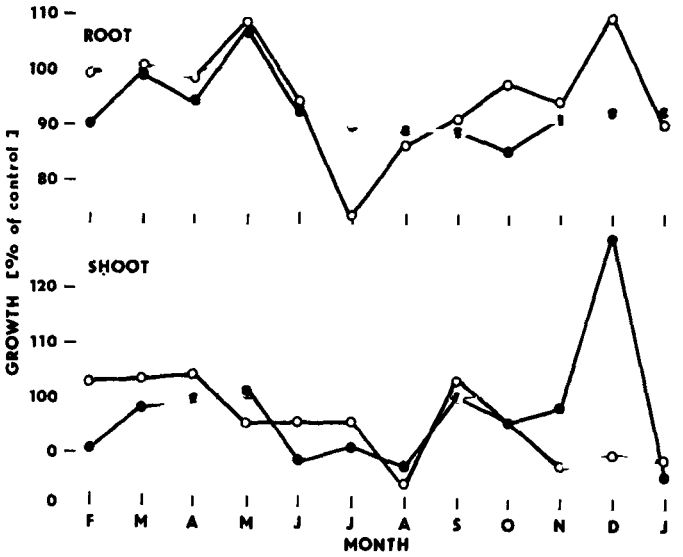


Fig. 12. Growth as a percentage of control from bioassay of methanol extract of lyophilized residue at approximately the same concentration as the aqueous bioassay of NT (•) and CT (o) soils, from February 1986 to January 1987.

Caffeine was added as an internal standard and all samples were treated with diazomethane, which methylates the carboxylic acid groups, but not the other hydroxyl groups. The free fatty acids occurred in soil, as indicated when the samples of the soil extract were analyzed on the CGC/MS/DA system prior to methylation. Myristic, palmitic, heptadecanoic, stearic, and eicosanoic acids were not inhibitory at

1.0 mM in the wheat bioassay at 1.0 mM (all bioassay results were statistically analyzed), nor were the synergistic mixtures (CAST 1987). Every possible combination of two, three, four and five acids was tested as well as all five fatty acids separately at concentrations of 1.0 mM and 5.0 mM, but their inhibitory or stimulatory activity was nil.

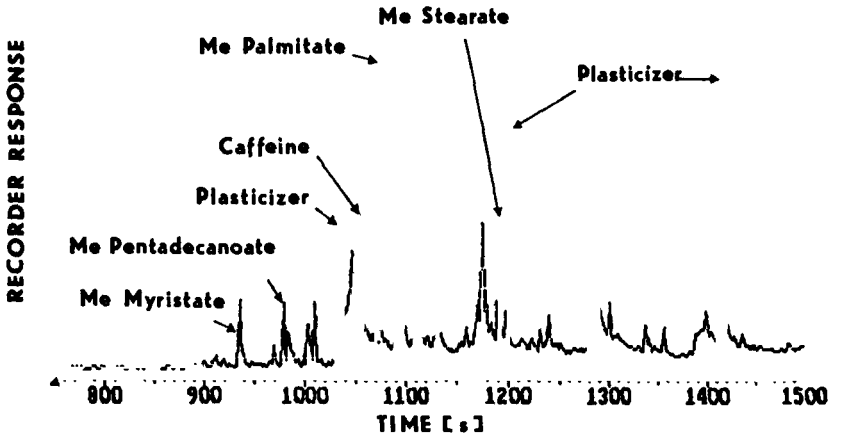


Fig. 13. Reconstructed total ion current chromatogram of the methanol-soluble CT soil extract (LKB–2091 CGC/MS/DA): Peaks represent compounds; identified peaks labeled. Soil sample: June 1986.

It was previously reported (CAST 1987), that allelochemicals were extracted from conventional-tillage and no-tillage soils for one year (1986) at monthly intervals at pH 8. The same plots were used for collecting wheat straw (freshly harvested) and the extraction was done with distilled water. The bioassay results indicated that toxic compounds occurred in both the wheat straw and wheat soil: the new wheat straw extracts had the strongest inhibition on wheat seedling growth, the wheat soil showing less inhibition (THORNE 1987). As the bioassays of organic fractions were followed, the wheat straw extract showed more inhibition when the more hydrophobic solvents (methylene chloride and chloroform) were used, rather than a hydrophilic solvent (methanol) (Fig. 14).

One unknown compound that was found in wheat straw and soil is thought to be an allelopathic substance. This compound is represented by peak 468 in Fig. 15 in the reconstructed total ion current of the capillary gas chromatogram of the methylene chloride-soluble fraction of new wheat straw. This mass spectrum of this compound obtained with the LKB mass spectrometer showed a molecular mass of 286 and corresponds to the molecular formula composed of carbon, hydrogen and oxygen. It is possible to draw some tentative conclusions: 1) we think that it is a methoxyben-

zoic ester of a C_{10} alcohol or a C_{10} ene-yne corresponding to a molecular formula of $C_{18}H_{22}O_3$; 2) the partial interpretation of the mass spectra indicates that the loss of a methyl group ($-CH_3$) probably comes from the methoxy group to give rise to the most abundant ion at m/z 271 which is further fragmented to give ion m/z 193 which corresponds to the loss of a C_6H_5 (– 73) moiety; 3) this interpretation would agree with the allelochemical activity (32 % of shoots and 11 % inhibition of root growth) of germinating wheat seeds (THORNE 1987). The reason for some excitement over this ester of benzoic acid is that it appears in each of the soil extracts during June, July, and August, but not before or after during the year. This means that this compound or its precursor was derived from the wheat plant at the time of maturity and has some capability of resisting microorganisms at least for around three months. This also corresponds to the time of the year, in Oklahoma, when the soil moisture is at its lowest level; usually in late August or early September when the rain begins, the compound is probably broken down by soil microorganisms. Attempts to reproduce this finding has been frustrating since the wheat crops of 1986, 1987, and 1988 have all shown negative for this compound; and we haven't had the time to check the soil yet for its occurrence. It must be active at microgram or picogram levels; if it does meet this criterion it might be used for the synthesis of herbicides or nematocides. The fact that it occurs relatively early in the elution pattern of the column indicates that it is moderately volatile.

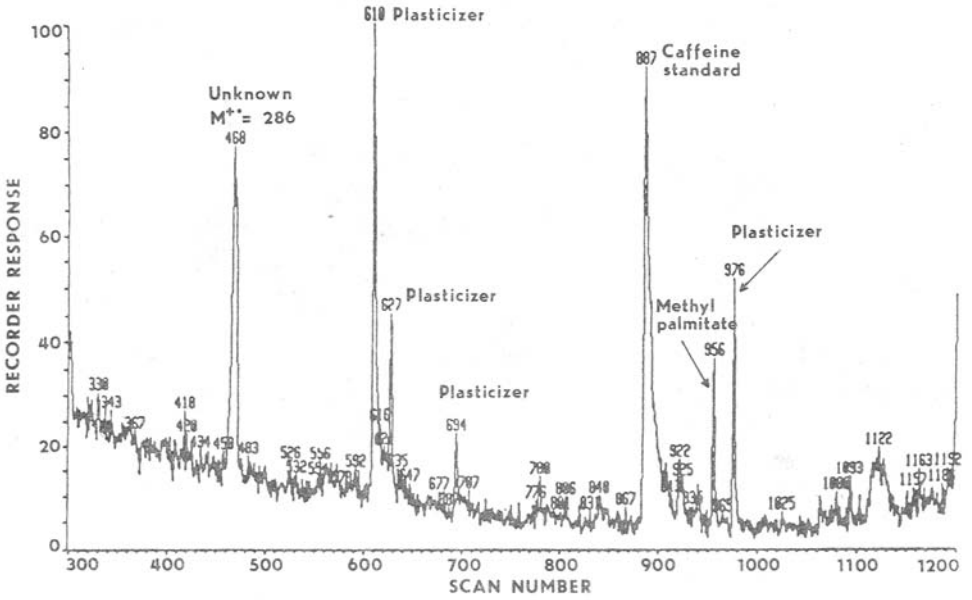


Fig. 14. Reconstructed total ion current chromatogram of the methylene chloride extract of the aqueous leachate of wheat straw collected on June 10, 1985 (peaks labeled represent the number of the mass spectrum no. and the identity of the compound).

The fact that the old wheat straw had none or only traces of the compounds identified in new wheat straw and soil meant that most of these compounds had either been leached out by rainfall or, since the wheat was lying on top of the soil, been decomposed photochemically or by microorganisms in or on the wheat straw. Unfortunately, there was no difference in the bioassays noted for the presumably active compound in the aqueous and methanol extracts of wheat straw, so at this time it is not possible for us to suggest a type of action other than a general inhibition of growth and development.

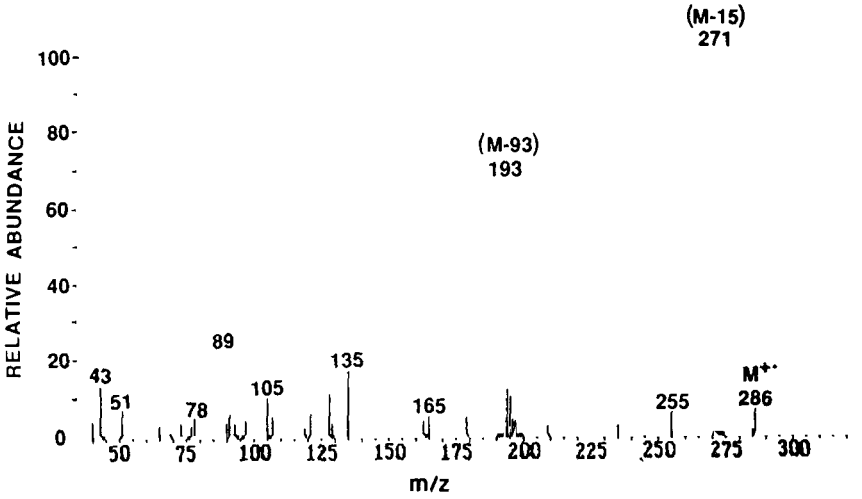


Fig. 15. Mass spectrum of an unknown compound, which was M 286, corresponding to peak at scan 468, on Fig. 14.

E) Alfalfa Root Saponins as Allelochemicals

Saponins are oligosaccharide derivatives of steroids and triterpenoids which have been characterized as possessing unique properties such as marked foam formation in aqueous solution, hemolytic and surface activities, complex formation with cholesterol, pronounced antifungal action, antinutritional effect on livestock, toxic effects on mammals and fish, and most recently as an allelopathic agent. They are widely distributed in the plant kingdom (nearly 100 families are represented) and have long been regarded as the active principle of many medicinal herbs (*i.e.*, ginseng, *Panax ginseng*). Among foodstuffs for man, saponins are found in tomatoes, oats, peanuts, spinach, soybeans, and at least 10–15 other foods; among animal feeds, saponins are found in alfalfa, clover forage, other cover crops and so on (PRICE *et al.* 1987). A great deal of attention has been paid to saponins in alfalfa, primarily because of the antinutritional and other adverse physiological properties

that have been ascribed to their presence. Alfalfa has been found to have allelopathic effects on wheat and lucerne as well as on itself (MISHUSTIN and NAUMOVA 1955, JURZYSTA 1970).

OLESZEK and JURZYSTA (1987) isolated saponins from alfalfa roots and separated them into cholesterol-precipitable and noncholesterol-precipitable fractions. They obtained crystalline medicagenic acid glycosides, 9–15 of them, depending upon the variety of alfalfa, and 4–19 hederagenin and soyasapogenol glycosides, again depending upon the variety of alfalfa. Upon acid hydrolysis they produced crystalline medicagenic acid; further analysis showed that glucose, arabinose, xylose, and rhamnose were sugar components for the cholesterol-precipitable fraction. Upon acid hydrolysis of the hederagenin and soyasapogenol glycosides they obtained the aglycons in crystalline form and analysis of the sugars showed the presence of glucose, arabinose, xylose, galactose, and glucuronic acid. The medicagenic acid glycosides made up around 6 % of the root dry matter and showed high biological activity in red blood cell lysis (hemolytic index of 3 000), completely inhibited *Trichoderma viride* growth at a concentration of 25 mg ml^{-1} of growth medium, and inhibited wheat growth at concentrations as low as 100 ppm. The noncholesterol-precipitable fraction caused no blood cell lysis or fungus growth inhibition; however, it did inhibit wheat germination, although to a much lesser extent than the other fraction.

The role of saponins as an allelopathic agent was first reported by MISHUSTIN and NAUMOVA (1955), who demonstrated a lower yield of cotton following alfalfa. This decrease in yield of cotton was more when the alfalfa was allowed to grow longer (2–4 years) as compared to 1–2 years, which suggested the accumulation of root products, they thought might be alfalfa root saponins. MISHUSTIN and NAUMOVA (1955) also observed that alfalfa saponins were inhibitory to cotton seed; % germination was 63, 61, and 51 % respectively for 2–, 3–, and 4-year-old alfalfa. In 1962, LAWRENCE and KICHLER (1962) found that alfalfa roots contained water-soluble substances toxic to crested wheatgrass, Russian wild ryegrass, sorghum, alfalfa, sweet clover, wild barley, dandelion, wheat, oats, and barley seedlings. GUENZI *et al.* (1964) used Buffaloe and Ranger cultivars of alfalfa at 3 cuttings and 6 stages of growth and found that the aqueous extracts were inhibitory to corn seedlings. There were no differences between cultivars although there were differences at various stages of growth and cuttings. PEDERSON (1965) reported some differences in allelopathic potential of alfalfa cultivars, with leaf saponins showing a greater toxicity than those isolated from the stems. SHANY *et al.* (1970) showed that the germination of cotton seeds was inhibited in soils on which alfalfa was grown on soils supplemented with lucerne meal prepared from tops and roots.

The Polish scientist JURZYSTA (1970) isolated saponin fractions from alfalfa (*Medicago sativa* and *Medicago lupina*) seeds and found that the extracts reduced the germination and seedling growth of barley, oats, wheat and rye when applied at

concentrations of 0.1 and 0.5 %. Identical concentrations of *M. media* retarded growth of barley and oat seedlings, but had no influence on rye and wheat growth.

WYMAN (1988) has also found alfalfa root water extracts to be much more inhibitory to cheat growth than to wheat growth. In preliminary bioassays involving water extracts from six cultivars of alfalfa ranging from dormant to nondormant (Advantage, WL 318, Baron, WL 515, Granada, and Cuf 101), she found that 2 ml of an extract of 4 g ground alfalfa roots in 100 ml of water* inhibited wheat growth by 30 % and *Bromus secalinus* (cheat) growth by 40 % compared with a distilled water control, whereas 2 ml of a similar extract of 8 g ground alfalfa roots inhibited both wheat and cheat seedling growth by 65–75 %**. The preliminary findings indicate significant differences in activity of the cultivars at different stages of growth but no significant difference between cultivars. These findings are similar to those of GUENZI *et al.* (1964).

The allelopathic effect of alfalfa root saponins on winter wheat seedling growth and the fate of these chemicals in soil environments were studied in detail by OLESZEK and JURZYSTA (1987). Seed germination of wheat (alfalfa seed are very small, making seed measurement difficult) and of spores of *Trichoderma viride*, a test fungus, were inhibited by water and alcohol extracts of alfalfa roots, and by crude saponin mixtures from alfalfa roots, indicating that medicagenic acid glycosides were the primary inhibitors. These glycosides strongly inhibited *Trichoderma viride* and wheat growth. Alfalfa root saponins, like alfalfa roots, lost toxicity when incubated in water in the presence of wheat and *T. viride* at 25 °C for 2 and 4 days. This loss of allelochemical activity could be followed by TLC and is related to the loss of the sugar moieties, giving medicagenic acid as the final product. It was possible to ascertain the rate and the particular sugars lost but this was not reported. This remarkable finding showed that medicagenic acid glycosides express their allelochemical activity in winter wheat as long as they stay in the soil in an unbound form, but they can be hydrolyzed slowly by enzymes of the wheat plant and microorganisms. Adsorption of saponins on soil particles, including humic substances, is intimately involved in the process of preserving them from attack by soil enzymes. In other words, loss of inhibitory effect depends on gradual hydrolysis of longer-sugar-chain glycosides that are readily soluble in water; thus more hydrophobic glycosides are formed, and finally pure medicagenic acid, which is insoluble in water and has almost no inhibitory activity as shown in Fig. 16. OLESZEK and JURZYSTA (1987) demonstrated these results in synthesized textured soils rather than alfalfa soils from the experimental field, which might contain small roots (even microscopic ones) of the alfalfa plant, and confuse the results. In practical terms this

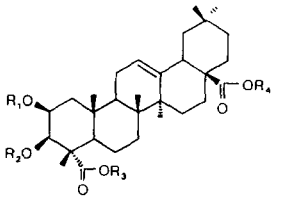
* If alfalfa roots are 4–6 % saponins by mass, this correlates to approximately 2 mg saponins per dish.
** Purified alfalfa root saponins isolated by JURZYSTA and WALLER (1989) were used as a standard. A concentration of 1 mg/dish inhibited wheat growth by 55 % and cheat growth by 65 %, and 0.5 mg/dish inhibited wheat growth by 30 % and cheat growth by 50 %.

means that crop rotation from alfalfa has to be practiced consistently because the concentration of residual saponins could be high enough to kill the succeeding crop. Sufficient time for the sugars to be hydrolyzed enzymatically should be allowed, and then no problem would be encountered.

In their investigation of pure alfalfa root saponins (cv. Cimarron), JURZYSTA and WALLER (1989) showed that the growth and development of weeds were inhibited: cheat and *Echinochloa crusgalli* L. (barnyard grass) (by 0.0002 %), *Amaranthus* spp. (pigweed) (0.0015 % for the root and 0.095 % for shoot), *Taraxacum officinale* (dandelion) (0.015 %), and *Sesbania exaltata* L. (coffeeweed) (0.025 %). The marked activity toward cheat was striking since it is a weed infesting wheat and alfalfa in the southwestern part of the United States. Pioneer 2157, the cultivar of wheat grown most commonly in Oklahoma, is inhibited by only 0.003 % of the root saponins. In another experiment, the saponin inhibition of cheat growth extended over a period of only about one month after the saponins were added, which is an indication that they inhibited germination and growth, but were less effective over time which is as described in Fig. 16.

F) Humic Acids from Wheat Soil as Allelochemicals

Humic acids were isolated (according to the procedure of HARTLEY and BUCHAN 1979) from wheat plots and shown to be inhibitory in the wheat germination and growth bioassay by 22 % when compared to a distilled water control. No differences



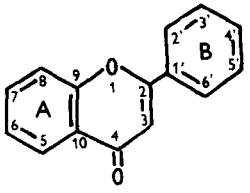
R_1	R^2	R_3	R_4	
H	H	H	H	Medicagenic Acid
H	β-D-Gluc-β-D-Arab-	H	β-D-Gluc-β-D-Rhamn-	
H	β-D-Gluc-β-D-Arab-	β-D-Gal-β-D-Gluc-	β-D-Gluc-β-D-Rham-	
H	β-D-Gluc-β-D-Arab-β-D-Gal-	β-D-Gal-β-D-Gluc-	β-D-Gluc-β-D-Rhamn-	
H	β-D-Gluc-β-D-Arab-β-D-Gal | β-D-Gluc-	β-D-Gal-β-D-Gluc- | β-D-Gluc-	H	

Fig. 16. Hypothetical structure of saponins from medicagenic acid that serve as allelochemicals until hydrolyzed.

between wheat growth in no-tillage *versus* conventional-tillage soils were observed. The preparation of purified humic acid by careful washing with water, methanol, ethyl ether and acetone, however, yielded a product that was slightly stimulatory in the wheat bioassay (5–10 %). Analysis of the washes showed that acetone and ether extracts were stimulatory, whereas the methanol one was inhibitory and the water extract had no effect. It is known that changes in composition occur upon washing, *e.g.* phenolic acids are washed out with methanol. Changes in the conformation of this biopolymer undoubtedly are a factor in causing the humic acid to go from inhibitory to stimulatory in the wheat bioassay. Analysis of the humic acids and the extracts are currently under investigation. There have been a few reports on the biological activity of humic acids (POSPÍŠIL 1980, PFLUG and ZIECHMANN 1982, and HASSETT *et al.* 1987), but to our knowledge, this is the first report of allelochemical effect (WALLER and TIMKO 1986).

G) Structure-Function Study of Flavonols Serving as Allelochemicals on Chloroplast-Mediated Electron Transport and Phosphorylation (MORELAND and NOVITSKY 1988)

Flavonoids are commonly found in and widely distributed among families of higher plants; they represent an important class of allelochemical compounds. The general structure of flavonols is shown in Fig. 17. These compounds contain two aromatic rings (A and B) linked by a pyrone ring that permits them to assume



GENERAL FORMULA

	Hydroxylation at ring position(s)						
Compound	3	5	7	2'	3'	4'	5'
Flavone							
Flavonol	x						
Fisetin	x		x		x	x	
Galangin	x	x	x				
Kaempferol	x	x	x			x	
Morin	x	x	x	x		x	
Quercetin	x	x	x		x	x	
Myricetin	x	x	x		x	x	x

Fig. 17. Structure of flavonols studied (MORELAND and NOVITZKY 1988).

TABLE 7
Inhibition by flavone and flavonols of noncyclic coupled electron transport and phosphorylation, cyclic phosphorylation, and uncoupled electron transport in spinach thylakoids.

Compound	Assay*			
	Noncyclic coupled		Cyclic	Uncoupled
	Phosphorylation	Oxygen uptake	phosphorylation	oxygen uptake
	I_{50} [μM]			
Flavone	99 ± 28	122 ± 12	118 ± 8	220 ± 20
Flavonol	27 ± 2	NE	64 ± 5	NE
Fisetin	79 ± 8	86 ± 10	92 ± 14	70 ± 11
Galangin	7 ± 2	11 ± 3	14 ± 1	123 ± 12
Kaempferol	34 ± 10	58 ± 2	57 ± 6	203 ± 15
Morin	178 ± 34	242 ± 18	227 ± 21	613 ± 81
Quercetin	63 ± 3	79 ± 4	62 ± 2	121 ± 29
Myricetin	81 ± 19	ND[1]	67 ± 6	ND[1]

Source: MORELAND and NOVITZKY (1988)
* H_2O served as the electron donor and methyl viologen as the electron acceptor. O_2 consumption was measured polarographically and phosphorylation was measured potentiometrically. Data are presented as I_{50} values ± SD obtained with three isolated samples of thylakoids. Average specific activities of the controls were: 136 ± μmol O_2 consumed and 389 ± 22 μmol Pi esterified $mg^{-1}(Chl)h^{-1}$ for the coupled reactions, 522 ± 3 μmol Pi esterified $mg^{-1}(Chl)h^{-1}$ for cyclic phosphorylation, and 173 ± 19 μmol O_2 consumed mg^{-1} $(Chl)h^{-1}$ for the uncoupled reaction. Assay conditions are detailed in the original article. NE = no effect. ND = not determined.
[1]Myricetin reacted chemically with constituents of the reaction medium.

a planar configuration because of the double bond between C–2 and C–3. MORELAND and NOVITSKY (1988) undertook to study the effect of flavone and seven flavonols (Fig. 17) on the light-induced electron transport and phosphorylation of isolated spinach (*Spinacea oleracea* L.) chloroplasts. With the exception of flavonol (3-hydroxyflavone) all of the compounds interacted with components of both the ATP-generating and electron transport pathways. Flavonol interacted only with the phosphorylation pathway and was the only compound that acted as an uncoupler. Phosphorylation pathway interference was shown by greater sensitivity of the phosphorylation reaction than of coupled whole-chain electron transport, inhibition of cyclic phosphorylation, inhibition of light-activated Mg^{2+}-ATPase, and inhibition of heat-activated Ca^{2+}-ATP are in association with CF_1 coupling factor. The order of decreasing effectiveness found for inhibition of cyclic phosphorylation was: galangin > quercetin = kaempferol = myricetin = flavonol > fisetin > flavone >morin, and for the inhibition of uncoupled electron transport it was: fisetin > quercetin = galangin > kaempferol > flavone > morin (Table 7).

The pattern of hydroxylation of the compounds affected the way the flavonoids interacted with the thylakoids. Flavone (nonhydroxylated) was moderately active in all of the assays. Flavonol (hydroxylated at C–3) caused loss of electron transport

inhibition and the appearance of uncoupling activity. Hydroxylation of the B–ring tended to reduce inhibitory effectiveness on phosphorylation. Kaempferol, with a 4′–OH, was less inhibitory than galangin, quercetin (3′, 4′) even less, and myricitin (3′, 4′, 5′) even less so. Morin (hydroxylated in the 2′ position) caused a drastic decrease in inhibitory effectiveness as shown by the marked difference between morin and quercetin in all the assays. Fisetin was less active against phosphorylation, but more active against electron transport, than quercetin. RACKER (1986) studied the structure/activity of bioflavonoids as inhibitors of glycolysis and concluded that tetra- and penta-hydroxy flavones with hydroxy groups on carbons 7′, 3′, 4′ and either carbon 3′ or 5′, or both, were most effective inhibitors, whereas a hydroxy at C–5 (myricetin) showed poor activity. Trihydroxy compounds were poor inhibitors and dihydroxy and mono-hydroxy compounds were inactive. The double bond between C–2 and C–3 was a requirement for inhibitory activity as was the free hydroxy on C–3. Other than these observations of RACKER (1986) it is difficult to develop general structure /activity correlations from the wide range of responses on the flavonols.

The interactions of flavonoids with chloroplasts could, in MORELAND and NOVITZKY's (1988) opinion, have two different effects: a) as allelochemicals, flavonoids that are released into the environment may inhibit photosynthesis in target organisms, and b) by a homeostatic control mechanism, they may be involved in the regulation of energy metabolism within the plant, where they are synthesized, by modulating photosynthetic electron transport and phosphorylation.

CONCLUSIONS

Of the many types of naturally occurring compounds identified in plants, soils and microorganisms, few have been studied for their potential as allelopathic compounds. This paper has cited seven examples; however, the complexities of biochemical interaction between species and among species still remain little understood. There is a wide array of reactions (it might be referred to as a network for the particular plant species) that must be considered during the ebb and flow of the life cycle of the plants and microorganisms. Some of the frontier areas of research that may be expected to clarify matters are:

1. Recombinant DNA techniques, which are useful in gene manipulation, such as identification, characterization, splicing, replication, regulation, and transfer, will be used in manipulating the DNA from crop plants to modify them for resistance to microorganisms, weeds and insects.
2. Resistance to allelochemical compounds will be selected from wild types of crop plants which have been grown in the presence of important weed species.
3. Screening of crop varieties and cultivars will be expanded to select those with the most number of allelochemical compounds or resistance to allelochemicals, or both.

4. Increased interest in allelochemical effects may arise from:
   a) bacterial stimulation of bacteria,
   b) growth stimulation of plants by microorganisms,
   c) growth stimulation of microorganisms by plants, and
   d) growth stimulation of plants by plants.
5. Biochemical and physiological bases for the action of allelopathic chemicals will be developed.
6. Increased understanding will be gained of allelopathic relationships in plant-soil-plant phenomena.
7. Naturally occurring allelochemicals will suggest ways for synthesis of herbicides, fungicides, and other pesticides.

An interactive working group of scientists from fields such as agronomy, animal science, biochemistry, botany, ecology, entomology, forestry, horticulture, plant pathology, and soil science is necessary for carrying out all aspects of allelopathic research. The allelopathic effect (the effect of naturally occurring organic chemicals) needs to become an integral part of the teaching of agriculture and ecology students; only by education can we stimulate the interest of the future generations of scientists that will put this into their research programs and come up with the answers to solve some of the agricultural and ecological problems of the world.

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