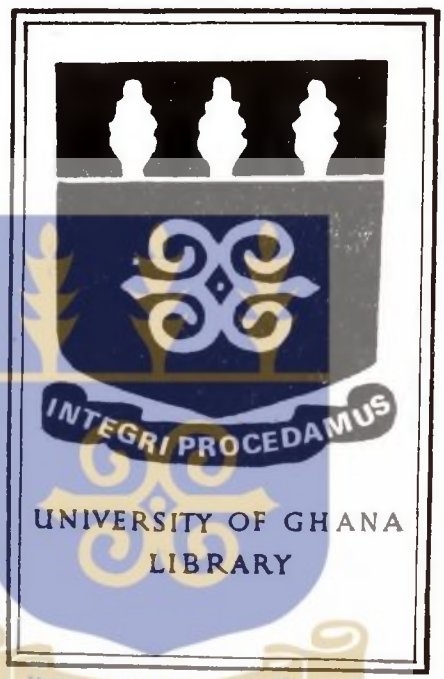

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ALLELOPATHY AS EXPRESSED BY SUGAR MAPLE ON YELLOW BIRCH

by

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of the requirements for the degree of
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Kweku O.A. Mensah

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INTRODUCTION

The Nature of the Problem

This study involves investigation of alleged inhibitory effects of leachate from sugar maple (Acer saccharum Marsh., donor plant) on yellow birch (Betula alleganiensis Britton., receiver plant). The term allelopathy, introduced by Molisch in 1937 refers to this phenomenon in which one plant produces a chemical which inhibits the growth of another plant.

The present investigation follows from that of Tubbs (1970) into competition between maple and birch for light and moisture. His results showed that there is a striking difference in birch growth between those grown in pure culture and those grown with maple, the latter being much lower. This reduced growth could not be accounted for by mere physical competition, and through further investigations, he was able to attribute some of this reduction in growth to allelopathic effects of maple leachate. Tubbs postulated that the active principle causing the inhibition was exuded from maple roots, that it was thermostable, water soluble and ephemeral.

The following questions which form the basis for the present investigations arise from Tubbs' results:

1. Is there a phenologic pattern to the inhibition of birch by maple?

2. Do maple organs, other than the roots, also exude inhibitor(s)?

3. How may the inhibition be expressed in birch?

4. What further can one learn about the chemical nature of the inhibitor(s)?

These questions are being investigated with a view to providing answers that may help better understanding of the phenomenon of allelopathy between sugar maple and yellow birch.

Literature Review

No less than sixteen reviews of biochemical inhibitio starting with one by Molisch (1937) to the recent review by Whittaker and Feeny (1971), have been published, not to mention also the many research papers which invariably contain partial reviews of the subject. It is intended here to reiterate various points in some of these reviews and papers, particularly with regard to their relevance to the present investigation.

The field of chemical inhibition traces back to 1828 when de Candolle drew the attention of scientists in his theory of crop rotation as a way of circumventing unfavorable effects of one species on another in soils (Schreiner and Reed 1907; Bonner 1950).

Early lack of interest was due principally to statements by eminent scientist of the day, as for example, Liebig in 1852 discounting any role of chemical inhibition, and attributing such inhibition to nutrient imbalances (Bonner 1950). In the early 20th century, Schreiner and his

colleagues (1907, 1908, 1909, 1911), after years of researc into soil fertility, not only showed that phytotoxic substances exist in soils as a result of decomposition of organic remains and of direct "excretion" from plant roots, but also isolated as toxic principles such substances as picolinic acid, salicylaldehyde, vanillin and dihydroxistea ic acid. They also pointed out the possibility of other organs of the plant yielding "poisonous" substances. Schreiner and Reed (1907) reported for the first time that maple was allelopathic to wheat. Another setback in the study of chemical inhibition between plants came when Clements, et al. (1929) insisted that physical competition was the only answer to differences in performance of plant associates.

Of the investigations carried out in the 1920's and 1930's, the most conclusive has been the allelopathic effects of black walnut (Juglans nigra) to various herbaceous plants (Cook 1921), with the active principle being juglone, a phenolic compound (Davis 1928). Juglans nigra was thus the first tree crop to be widely investigated for its toxicity.

However, in recent times, there have been conflicting reports to some of the earlier Juglans findings. McDaniels and Muenscher (1940) cited evidence from the California Walnut Growers Association which had made them "discard the idea that the walnut has any particular effect upon annual crops...and that in most instances [the] nitrogen fertili zer...will remedy the so-called toxic effect of walnuts on...crops." Further contradictory evidence cited from the

Northern Nut Growers Association was to the effect that tomato plants when planted with young black walnut trees grew so well that they nearly smothered the walnut trees.

Studies by Bonner (1946) and Gray and Bonner (1948) gave more credence to the existence of compounds in plants and soil which may be toxic to themselves or to other plants. They showed, inter alia, that Guayule and Encelia roots and leaves, respectively, produced inhibitors which in the case of Guayule was autotoxic. The active principle in Encelia was identified as 3-acetyl-6-methoxybenzaldehyde.

Went (1942) had previously suggested that allelopathy might be the reason for Encelia farinera not harboring annuals as did other shrubs. Mergen (1959) applied extracts of various organs of Ailanthus altissima to wounded surfaces of plants of the same species and 46 other species and observed wilting in all but one. He attributed limited succession in Ailanthus stands to allelopathic effects. Muller and his colleagues and students (1964, 1965, 1966) have shown that terpene production by shrubs, mainly Salvia spp. and Artemesia spp., facilitated invasion of these species into California grasslands.

Rice (1967), who regards allelopathy as "chemical warfare", sees the action in old fields of central Oklahoma as an indirect one, involving production by some plants of phenolic compounds that are inhibitory to nitrifying and nitrogen-fixing bacteria. Thus, via indirect effects on the soil microorganisms leading to nutrient imbalance, the growth of various plant species is inhibited.

Wilson (1968) concluded from his investigations that allelopathy by Helianthus annuus was of major importance in old field succession. He also isolated organic compounds which were believed to be the toxic principles in leaves and roots of H. annuus. DeBell (1969) observed in the field poor growth in seedlings of Quercus falcata and Q. michauxii under large trees of Q. falcata. He attributed this to the production of salysilic acid by the larger Q. falcata which was inhibitory to the two species of oak.

Sugar maple's detrimental effect on other vegetation, mentioned by McDaniels and Muenscher (1940), was not confirmed in observations by Buchenau and von Homeyer (cited by Tukey 1970). They found much improved growth of plants growing beneath the canopy of beech, maple and linden, as against poor growth under poplar, birch and willow; the improved growth was attributed to nutrients leached from the overhead canopy.

Voigt and Mergen (1962) showed, with Ailanthus, as had been done by Schreiner and Reed (1907) with Acer, pronounced inhibition on receptor plants during the summer months and a decline in inhibition with the approach of fall. Brown (1967) also observed seasonality in the inhibition of germination of jack pine (Pinus banksiana) by leaf extracts from its associated species.

In a review by Woods (1960), attention was drawn to the unstable condition of allelopathic compounds as a result of their rapid breakdown by soil microbial activity.

As mentioned earlier, this ephemeralness of allelopathic effects was one of the major findings of Tubbs'(1970) work with sugar maple and birch.

As a result of the introduction of modern techniques of plant physiology in investigations, such as bioassay techniques and chromatography (Rovira 1965; Muller 1966) and radioisotope techniques (Wardlaw 1968; Rovira 1969), allelopathic effects are now accepted as fact.

The foregoing review,together with findings in several other papers cited below,suggest some broad conclusions regarding allelopathy:

1. The phenomenon is extremely widespread. It occurs in tropical trees (Webb, et.al. 1967), as well as in many temperate (DeBell 1969) and desert plants (Went 1942).

2. Plant inhibitors seem to be mostly secondary metabolites; the strongest exponent of this conclusion is Muller (1966). (See also Whittaker and Feeny 1971; and Levin 1971).

3. The transmission of phytotoxins may be through varied routes: del Moral and Muller (1968) report fog drip from Eucalyptus sp.; as plant residues (Patrick 1955, McCalla, et.al. 1968); as root exudates (Bonner 1950; Woods 1960; Tukey 1970; Tubbs 1970); as crown leachates (Tukey 1970, DeBell 1969); as volatile substances

(Muller, et.al. 1964); through animal agents, such as ants (Janzen 1968); and through seed leachates (Ferenczy 1956).

4. The inhibition can be expressed in a variety of ways: a general reduction in growth, root injury, shoot wilting, poor root development (DeBell 1969); reduction in respiratory activity (Patrick, et al. 1958; Mergen 1959); poor uptake of minerals (Bucholtz 1968); and interference with enzyme systems or other biological activity (Grodzinsky 1968; Hare 1964; Rice 1964). In the field, these may take the form of "fairy rings," bare patches, merely stunted growth, or marked changes in species distribution (Wilson 1968).

These conclusions have tended to broaden for current usage the definition of allelopathy beyond what Molisch (1937) envisaged. Thus, instead of its being limited to the direct effect of one higher plant on another through the production of chemical retardants, the more inclusive definition incorporates both direct and indirect deleterious effects that one plant has on another plant through the production of chemical compounds. Whittaker and Feeny (1971) have suggested the term "allelochemics." They incorporate the broad based definition of allelopathy and also encompass the growth, health, behavior, or population biology of the receiver organism.

The absence of the sharing of a common factor between donor and receiver plants in the definition places allelopathy out of the realm of competition, although it can enhance the competitive performance of a donor plant against a receiver plant.

A striking feature of the literature on allelopathy is the preponderance of studies with herbaceous plants and grasses and the general paucity of those with woody plants, particularly tree species. To provide some groundwork for future studies, a summary of available literature on woody species is presented in Table 1. The list is largely limited to work done in the United States.

The indications in the literature were that allelopathy in woody plants was similar to that in herbaceous plants and grasses. The foregoing review is therefore applicable to herbaceous plants and grasses as well as woody plants.

Table 1. References to Phytotoxicity of Woody Plants Listed In Alphabetical Order by Common Names of Donor Plants

Donor Plant	Receiver Plant	Culture	Source of Toxin	Reference
1. Apple (*Malus* spp.)	Apple (*Malus* spp.)	Field, Water, Soil	Decomposed Root	Borner (1959)
	Pear (*Pyrus* spp.)			
2. *Araucaria* sp.	Hoop Pine (*A. cunninghamii*)	Soil	Root Leachate	Bevege (1968)
3. *Backhousia angustifolia*	Hoop Pine (*A. cunninghamii*)	Field	Leaf Litter	Cannon, *et. al.* (1962)
4. Black Locust (*Robinia pseudoacacia*)	Barley (*Hordeum vulgare*)	Water	Bark & Wood Extracts	Waks (1936)
5. Black Walnut (*Juglans nigra*)	Several Species	Field & Water	Root Excretion	Cook (1921)
		Field	Root, Leaves & Fruits	Massay (1925)
		Field	Root, Leaves & Fruits	Bode (1958)
6. Butternut (*J. cinera*)	Bush cinquefoil (*Potentilla fruticosa*)	Field	Through Soil	Jones & Morse (1902-03)
	Swiss Mountain Pine (*Pinus migomuglius*)	Field	Through Soil	Smith (1941)

Donor Plant	Receiver Plant	Culture	Source of Toxin	Reference
7. Cherry (*Prunus* spp.)	Cherry (*Prunus* spp.)	Field	Through Soil	Vogel & Weber (1931)
	Wheat (*Triticum* spp.)	Soil	Through Soil	Schreiner & Reed (1907)
8. Cherry (*P. pumila*)	Jack Pine (*P. banksiana*)	Bioassay	Leaf Extract	Brown (1967)
9. Cherry (*P. serotina*)	Jack Pine (*P. banksiana*)	Bioassay	Leaf Extract	Brown (1967)
10. Cherrybark Oak (*Quercus falcata*)	Cherrybark Oak (*Quercus falcata*)	Field & Bioassay	Leaf Extract	DeBell (1969)
	Swamp Chestnut Oak (*Q. michauxii*)			
11. Chestnut (*Castanea dentata*)	Wheat (*Triticum* spp.)	Water	Leaf & Bark Washings	Livingston, et. al. (1907)
12. Chinquapin (*Castanopsis sempervirens*)	*Ribes* spp.	Green-house	Water Extracts	Offord (1952)
13. Citrus (*Citrus* spp.)	*Citrus* spp.	Sand & Soil	Root Leachate	Martin (1950)
14. Dogwood (*Cornus* spp.)	Wheat (*Triticum* spp.)	Soil	Through Soil	Schreiner & Reed (1907)
15. *Encelia farinosa*	Tomato, Corn	Sand	Leaf Litter	Gray & Bonner (1948)

Donor Plant	Receiver Plant	Culture	Source of Toxin	Reference
16. Eucalyptus globulus	Various Herbaceous Species	Bioassay	Fog Drip From Foliage, Litter, Leachates, Volatile Terpenes	DelMoral & Muller
17. E. pilularis	Various Herbaceous Species	Field	Through Soil	Florence & Crocker (1965)
18. Flindersia sp.	A. cunninghamii	Soil	Root Leachate	Bevege (1968)
19. Guayule (Parthenium argentatum)	Guayule (P. argentatum)	Sand	Root Exudates	Bonner (1946)
20. Juniper (Juniperus osteoperma)	Blue Grama Bouteloua and Other Range Species	Field & Lab	Leaf Extract	Jameson (1970; 1966)
21. Kalmia angustifolia	Black Spruce (Picea mariana)	Lab	Leaf Extract	Peterson (1965)
22. Maple (Acer spp.)	Wheat (Triticum spp.)	Soil	Through Soil	Schreiner & Reed (1907)
23. Oak (Quercus spp.)	Wheat	Soil	Through Soil	Mellamby (1968)
	Oak			Hook & Stubbs (1967)
	Mustard (Brassica spp.)	Field	Through	Rumor & Yegorova (cited by DeBell 1969)
24. Peach (Prunus persica)	Peach	Field & Soil	Decomposed Plant Residues	Patrick (1955)

Donor Plant	Receiver Plant	Culture	Source of Toxin	Reference
25. Pine (*Pinus* spp.)	Wheat	Soil	Through Soil	Schreiner & Reed (1907)
26. Pine (*Pinus* spp.)	Hoop Pine (*A. cunninghamii*)	Soil	Root Leachate	Bevege (1968)
27. *Salvia* spp.	Several Species	Field	Volatile Terpenes	Muller, *et. al.* (1964) Grodzinsky (1968)
28. Silky Oak (*Grevillea robusta*)	Silky Oak (*G. robusta*)	Green-house	Canopy Drip & Root Leachates	Webb, *et. al.* (1967)
29. Sugar Maple (*Acer sacharrum*)	Yellow Birch (*Betula lutea*)	Field & Bioassay	Root Leachate	Tubbs (1970)
30. Tree of Heaven (*Ailanthus altissima*)	Slash Pine (*P. elliottii*) & Several Tree Species	Lab Assay	Leaf Extract	Mergen (1959)
31. Willow (*Salix pellita*)	Jack Pine (*P. banksiana*)	Lab Assay	Leaf Extract	Brown (1967)
32. Wormwood (*Artemesia absinthium*)	Several Species	Field & Sand	Leaf Excretion	Bode (1940) Funke (1943)
33. Yellow Poplar (*Liriodendron tulipifera*)	Wheat (*Triticum* spp.)	Soil	Through Soil	Schreiner & Reed (1907)

EXPERIMENTATION

Plant Material

The sugar maple plants used were 2-0 root pruned seedlings from an unknown seed source grown by a state nursery in Northern Ohio; seedlings were potted in seven inch pots in March 1970. These plants redeveloped their root systems and grew through a one-year establishment period to January 1971 when experimental work began. Maple seedlings used in the experiments ranged in size from 1-1/2 to 2 feet above ground. Yellow birch and sugar maple seeds used in the experiments were supplied from the laboratories of the Forest Service in Marquette, Michigan where they had previously been stratified. During the study,prior to use, the seeds were stored in a cold chamber at 5° to 10°C.

General Methodology

Through a combined use of biological assay, paper and thin layer chromatography, spectral analysis and radiosotope technique, it is possible to study not only exudation or leaching from plants and its seasonal variation, but also expression of allelopathy and some aspects of the nature of the inhibitory principle(s). These techniques have been used at varying levels in the present study and are discussed as they apply to specific experiments.

The terms leachate and extract have been used in the text to refer to specific processes in obtaining substances from plants. Leaching, producing leachate, as defined by

Tukey (1970), refers to the removal of substances from plants by the action of aqueous solutions such as rain, dew mist and fog, and soil water percolate without regard to the nature of the plant material. As used in the present investigation, leaching embodies not only Tukey's usage but also the proviso that the leachate is obtained from intact plants or parts thereof. This is opposed to extraction, yielding an extract, in which substances are removed generally from crushed and/or homogenized plant material either with water or other solvents.

Statistical comparisons used throughout the investigation tested simply means of treatments against those of controls. For this, the student t-test was considered adequate, using the five percent or one percent level of significance, as appropriate.

Experiment One: Phenologic Pattern of Inhibition

The purpose of this experiment was to establish seasonal periodicity in the expression of allelopathy by sugar maple; and also to investigate to what extent the periodicity, if any, relates to the different stages of development (phenophases) of the maple seedlings. Twenty-eight maple seedlings were sorted into seven groups of four each in outside cold frames, with plants being matched within groups for uniformity to reduce variation due to size. Beginning January 23, 1971 and ending May 10, 1971, the batches were periodically transferred onto a greenhouse bench following the schedule in Table 2.

Seedlings in each batch, after a day in the greenhouse, had their roots carefully and thoroughly washed free of soil and placed immediately, individually, into 800 ml glass beakers with a commercial nutrient solution (hyponex, Table 3). The four containers were then connected by means of tygon tubing to a four-watt vibrator for aeration. The open end of each line of tubing was fitted with an air stone. Each beaker was completely covered with aluminum foil to prevent algal growth in the solutions. Overhead lamps provided 24-hour photoperiod for the seedlings for the duration of the experiment.

At predetermined periods (i.e., two days before each Roman numeral in Table 2), the nutrient solution in each beaker was replaced with distilled water with a concurrent distilled water washing of the roots. Two days of growth

Table 2. Schedule for Handling the 7 Batches of Maple Seedlings[1/]

	Batches of Four Seedlings Each						
	1	2	3	4	5	6	7
1-29-71	T						
2-01-71	I						
2-20-71		T					
2-22-71	I	I					
2-24-71	I						
3-08-71		I					
3-13-71			T				
3-15-71	II	I	I				
3-20-71	II		I				
3-27-71	II		I				
4-03-71				T			
4-05-71				I			
4-07-71	III	II					
4-09-71			II				
4-11-71		II		I			
4-15-71				I			
4-21-71	III		II				
4-22-71		III		II			
4-24-71			III				
4-25-71					T		
4-26-71		III		II	I		
4-29-71	III				II		
5-01-71						T	
5-02-71		III				II	
5-10-71				III			T.II
5-14-71			III			II	
5-20-71		III			II		
5-30-71	III						II
6-05-71					III		III
6-10-71		IV				III	
6-12-71			IV	III			
6-14-71	IV				III		
7-21-71				IV			III
7-22-71	IV	IV					
7-31-71			IV		IV	IV	
8-18-71	IV	IV					IV
9-03-71				IV	IV		IV
9-18-71	IV	IV				IV	
10-02-71			V		V		IV
10-08-71	V					V	
10-12-71		V		V			
10-21-71			V		V		V

1/T - Time of transfer of maple to greenhouse.

Roman numerals show times of assay and correspond to the following phases:

I - Dormancy to first leaf flush.
II - First leaf flush to first full leaf expansion.
III - First full leaf expansion to final bud set.
IV - Final bud set to onset of leaf senescence.
V - Onset of winter dormancy.

Table 3. Formulation of Hyponex (Provided by Manufacturer)

Total Nitrogen (N)	7%
1.20% Ammonical Nitrogen	
5.80% Nitrate Nitrogen	
0.00% Other Water Soluble Nitrogen	
0.00% Water Insoluble Nitrogen	
Available Phosphoric Acid (P_2O_5)	6%
0.00% Insoluble Phosphoric Acid	
Soluble Potash (K_2O)	19%
Total Available Primary Plant Food	32%
Chloride, Not More Than	.10%

For water culture, the manufacturer recommends two teaspoonsful per gallon of distilled water, but this was diluted five times for use in the present study. This concentration used was equivalent to 2.4 gm per liter.

was allowed subsequent to this washing, after which the water in the beaker was harvested for assaying on newly germinated yellow birch seedlings. It was assumed that any current leachate from the maple roots would have accumulated in the beakers in the two-day period.

Before assaying, each solution harvested was first evaporated to one-tenth its volume under vacuum at 33°C. in a Buchler flash evaporator. This initial process took six to seven hours for each liter of solution. Three ml of this concentrated solution was used per assay per petri dish.

Yellow birch germinants for the assay were selected randomly from fairly large and uniform-sized newly germinated seeds. The seeds were germinated by soaking them in distilled water under continuous light (Tubbs 1970), and germination started after eight days. By the ninth day, radicle lengths of the birch germinants ranged from two to four mm, and their cotyledons were just emerging.

The assay procedure was similar to that used by Tubbs (1970). Three yellow birch germinants were grown in maple root leachate in a 5 cm petri dish for 24 hours in darkness and at the prevailing greenhouse temperatures of 95°F. during the day and 70°F. at night. The radicles of the germinants were measured for extension growth under a dissecting microscope before and after the 24-hour period of leachate treatment. The percent increase in elongation of radicles in the leachate compared with that in distilled water (control) indicated the presence or otherwise of

inhibition. A significant difference between the two growth percentages signified inhibition. Each assay was replicated three times.

Since roughly nine days were required for germination, the birch seeds were sown seven days before the change of maple seedlings from hydroponic solution to distilled water, so that the germinants were ready for the harvest of root leachate.

It was possible to distinguish five main phenophases in the development of sugar maple seedlings for the assay periods (as listed in the schedule of Table 2):

Phenophase I Covered the period of shoot dormancy up to the onset of the first leaf flush, after seedlings were brought into the greenhouse. The duration of this phase varied from batch to batch, and under the greenhouse conditions, lasted 16 days on the average (a range from 0 to 45 days). This period was so variable because buds on the latter batches, 5, 6 and 7, were ready to flush immediately after they were brought into the greenhouse. The outside early spring weather had already preconditioned them and they had all virtually passed through this phenophase prior to their being brought into the greenhouse. The earlier batches, 1 to 4, were invariably still frozen and had not had the benefit of this preconditioning at the time they were being transferred into the greenhouse. Sugar maple roots begin to grow several weeks prior

to bud opening. Except in seedling batches 1, 2 and 3, all other batches had growing roots at the time of their transfer into the greenhouse. Batches 1, 2 and 3 were frozen, and it was not until after 10, 11 and 6 days respectively, after their transfer, that they started producing roots. At the end of this phase, there was an average for all batches of 13 growing root tips per seedling.

Phenophase II Covered the period between the first leaf flush and the first full leaf expansion. This phenological phase in general was of much shorter duration than Phase I, lasting from 2 to 2-1/2 weeks within which all leaves on the initial flush would have fully expanded. As Tubbs (1970) pointed out, it is a characteristic of this species, sugar maple, to pass through this phase rather quickly. At the time of the first assay in this phase, the early leaves were partially expanded. Active roots, though more numerous, were not as elongated at tips as in Phase I. On the average, active roots numbered 15 per seedling, an increase of about 15 percent over the final average count in Phase I. The leaf count on the initial shoot flush averaged 16 per seedling, including those on all branches.

Phenophase III Covered the period after full leaf expansion on the first shoot flush to final bud set. It included the periodic new shoot flushes which resulted

in continued shoot and leaf expansion on all branches. The average counts of leaves and active roots at the beginning of this phase were 16 and 15, respectively, as in Phase II. The counts just before final bud set averaged 36 and 26, respectively, for leaves and active roots per seedling. The percent increases are 125 for leaves and 73 for roots. There were an average of two new shoot flushes per seedling during the average nine-week period (a range of seven to 11 weeks) before final bud set.

Phenophase IV - This covered the period after final bud set but before leaf senescence, characterized by the presence of mature leaves only and a decline in root growth. The phase lasted an average of 11 weeks. The counts of leaves and active roots at the end of this phase were 36 and 33, respectively. The respective percent increases over those of Phase III are 0 and 27. Most of the active root development in this phase took place within the first seven to eight weeks.

Phenophase V - This is the developmental period, following Phase IV, which reflected the onset of winter dormancy. The leaves were senescent and had started dropping. There was no new root development. The only active roots persisting were those carried over from Phase IV.

The results of the assays are presented under these

recognizable phenologic events.

Results

Phenophase I.

The assay results from the seven groups of maple seedlings are summarized in Table 4 for Phase I. There were no significant differences in elongation of birch radicles between those growing in maple root leachate and those in distilled water. This indicated that there was no inhibition expressed in this phase of dormancy.

Phenophase II.

The summary of assay results for Phase II is shown in Table 5. None of the differences were significant at the five percent level. The results, therefore, like those of Phase I, did not show any inhibition by maple root leachate on birch radicle elongation.

Phenophase III.

The bioassay results are summarized in Table 6 for Phase III. The first indications of inhibition came at the beginning of this phase and continued periodically to the end of it.

Where differences in birch radicle elongation between treatment and control were significant, it was always at the five percent level and occasionally at the one percent level. No particular pattern over time emerged as regards

Table 4. Bioassay of Leachate from Intact Maple Roots Before Leaf Flushing (Phase I) on Radicles of Yellow Birch Germinants

Maple Batch Number	Percent Increase in Growth of Birch Radicles[1/] Control	Leachate	
1	54	56	NS
2	55	52	NS
3	63	67	NS
4	60	57	NS
5	35	38	NS
6	69	71	NS
7	64	67	NS
Grand Average	57.3	58.3	NS
Percent of Control	100	101.7	NS

NS = Not significantly different from controls at 5% level.

1/Each figure is the average of three groups of three germinants each.

Table 5. Bioassay of Leachate From Intact Maple Roots After Leaf Flushing but Before Full Leaf Expansion (Phase II) on Radicles of Yellow Birch Germinants

Maple Batch	Percent Increase in Growth[1/]		
Number	Control	Leachate	
1	52	49	NS
2	52	54	NS
3	79	75	NS
4	57	53	NS
5	48	46	NS
6	58	52	NS
7	39	39	NS
Grand Average	55.0	52.6	NS
Percent of Control	100.0	95.6	NS

NS = Not significantly different from controls at 5% level.

1/Each figure is the average of three groups of three germinants each.

Table 6. Bioassay of Leachate from Intact Maple Roots During Periods of Full Leaf Expansion in Phase III, on Radicles of Yellow Birch Germinants

Maple Batch	Percent Increase in Growth[1/]	
Number	Control	Leachate
1	57	33 *
2	60	47 *
3	72	42 **
4	45	28 *
5	68	31 **
6	67	45 *
7	58	31 *
Grand Average	61.0	36.8 *
Percent of Control	100.0	60.3 *

*Significantly less than controls at 5% level.

**Significantly less than controls at 1% level.

1/Each figure is the average of three groups of three germinants each.

a gradation in the degree of inhibition. All these assays coincided with periods when most or all leaves on a new shoot flush were fully expanded, and all leaves on earlier shoot flushes were inactive.

Table 7 summarizes the results of another set of unscheduled assays carried out during Phase III, but only during periodic new shoot flushes with accompanying leaf expansion. The birch radicle elongation in maple leachate was not significantly different from that in distilled water. The results, therefore, showed no inhibition during these isolated periods in Phase III.

The general pattern reflected by Tables 6 and 7 was one of the inhibition in this phase except during the periodic new shoot flushes with their attendant leaf expansion.

Phenophase IV.

The results of assays for Phase IV were not separately tabulated. The results showed significant inhibition of birch radicle elongation by maple leachate in the early part of this phase, but no inhibition towards the end of it.

Phenophase V.

After Phase IV came a period during which the assay results were very much like those of Phase I. Table 8 summarizes the results of the bioassays of Phase V. There was no significant inhibition in any of the batches.

Table 7. Bioassay of Leachate from Intact Maple Roots During Periods of New Flushing and Leaf Expansion in (Phase III) on Radicles of Yellow Birch Germinants

Maple Batch No.	Percent Increase in Growth[1/]	
	Control	Leachate
1	57	61 NS
2	64	35 *
3	39	18 *
4	67	65 NS
5	52	49 NS
6	54	55 NS
7	43	45 NS
Grand Mean	53.7	46.8
Percent of Control	100	87.2

*Significantly lower than control at 5% level.

NS = Not significant from control at 5% level.

1/Each assay average is from three groups of three germinants each.

Table 8. Bioassay of Intact Maple Roots After Leaf Senescence and Leaf Abscision (Phase V) on Radicles of Yellow Birch Germinants

Maple Batch No.	Percent Increase in Growth[1/]	
	Control	Leachate
1	68	64 NS
2	61	61 NS
3	38	40 NS
4	56	55 NS
5	57	60 NS
6	52	55 NS
7	60	59 NS
Grand Average	56.0	56.3 NS
Percent of Control	100.0	100.5 NS

NS = Not significant at 5% level.

1/Each figure is the average of three groups of three germinants each.

Discussion of Experiment One

When the results from Phase I, II, III, IV and V were further summarized, a pattern emerged which correlated with the different phenophases of sugar maple development identified earlier and which also suggested a seasonality in the inhibition induced by the roots of sugar maple as detected by the birch radicle elongation assay. In Table 9, an X denotes no inhibition, an * shows inhibition, and the continuous lines demarcate the five phenophases from each other. Thus, in Phase I, when active sugar maple roots were present, but there were no leaves and shoot buds were unopened, there was no inhibition by root leachate on birch radicles. In Phase II both active roots and leaves were present, but the leaves were immature and still expanding. Here also there was no inhibition by root leachate. In Phase III, when roots were still actively growing, but most leaves were fully expanded and mature, inhibition was expressed by root leachate. The periods of no inhibition during Phase III were similar to the situation in Phase II, in that they were periods of new shoot flushing and leaf expansion. Inhibition in Phase IV by root leachate coincided with the early part of the phase when there was active root development and all foliage was mature, and the lack of inhibition occurred later when active root development was declining. The lack of inhibition by root leachate in Phase V seemed to complete a phenologic cycle which may form a basis for predicting the cessation of inhibitor(s) production.

Table 9. Schedule of Bioassays Showing the Periodicity in Inhibition by Leachates of Intact Maple Roots

Date of Assay	Batches of Four Seedlings Each						
	1	2	3	4	5	6	7
2-01-71	X						
2-22-71	X	X					
2-24-71	X						
3-08-71		X					
3-15-71	X	X	X	I			
3-20-71	X		X				
3-27-71	X		X				
4-05-71				X			
4-07-71	*	X					
4-09-71			X				
4-11-71		X		X			
4-15-71			II	X			
4-21-71	*		X				
4-22-71		*		X			
4-24-71			*				
4-26-71		*		X	X		
4-29-71	*				X		
5-02-71			*			X	
5-10-71		III		*			X
5-14-71			*			X	
5-20-71		*			X		
5-30-71	*						X
6-05-71					*	*	
6-10-71		*				*	
6-12-71			*	*			
6-14-71	*				*		
7-21-71				*			*
7-22-71	*	*					
7-31-71			*		*	*	
8-18-71	X	X					*
9-03-71			IV	X	X		X
9-18-71	X	X				X	
10-2-71			X		X		X
10-8-71	X			V		X	
10-12-71		X		X			
10-21-71			X		X		X

X = No Inhibition * = Inhibition

The correlation between inhibition by active roots and the presence of fully expanded and mature leaves suggested the role of current photosynthesis, translocation and source-sink relationships in the maple plants. Tubbs (1970) observed a close relation between inhibition and peak root activity, but he did not investigate a relation between inhibition and leaf development. Schreiner and Reed (1907) attributed inhibition from their test plants, including maple, to the active metabolism of the plants and implied absence of or decline in inhibition to a low metabolic activity, the latter occurring at the beginning of the growing season and at the end of the growing season. Smith (1969), writing on plant phenolics, mentioned that rapidly metabolizing leaf tissue would be expected to synthesize a large proportion of the phenolics which occur in various plant organs, as oak leaves are believed to do in the production of pyrogallol phenols; these are translocatable, but local synthesis in other organs also takes place and involves precursors which originate in the leaves.

Assuming that sugar maple transports inhibitors or their precursors from the leaves basipetally to the roots primarily when there is little competition within the plant from other organs where growth or storage is rapid, the role of source-sink relationships becomes more obvious.

Wardlaw (1968) reviewed the literature on movement of carbohydrates in plants and pointed out that very young leaves demand carbohydrates from older leaves for their growth and that it is not until a leaf has attained about

one-half of its final area that the photosynthates it produces are adequate to meet its growth requirements and later for export to other parts of the plant. In a more recent study, Donnelly (1970) showed that in Populus grandidentata there is appreciable transport of photosynthates from base leaves to less developed tip leaves, even when the former are approximately 50 to 60 percent of their maximum size. Thus, newly developing leaves during a major part of their expansion are strong sinks drawing assimilates from more mature leaves, and only later changing to become sources and to export assimilates.

It is postulated then that in Phases I and V, in the absence of leaves, the contribution of current photosynthate to the production of inhibitors in roots is lacking and therefore, inhibition by root leachate is not expressed even though roots may themselves be growing. In Phase II, the newly expanding leaves are strong sinks, and channel most currently produced photosynthate from earlier formed more mature leaves towards themselves, thus depriving the roots of assimilates that might have contributed to the production of the inhibitor(s). In Phase III, during those periods when all leaves are mature and no new leaves are flushing, large amounts of current photosynthate are available for translocation to root sinks, which may include inhibitors or their precursors. The active roots are therefore in a position to release to the surrounding solution what they receive either intact or after resynthesis.

During those periods in Phase III when recurring shoot flushes are producing new leaves again, it is suggested that the new shoots were stronger sinks than the roots and current photosynthate from mature leaves was diverted from roots, and no measurable inhibitors were released into the solution.

Experiment Two: Exposure of Maple Seedlings to $^{14}CO2$

This experiment provided supporting evidence to the assumption that there was, in fact, leaching of currently photosynthesized assimilates from maple seedling roots into the hydroponic solution. The purpose of this experiment was to demonstrate that leaching of photoassimilated carbon-14 from maple roots into the hydroponic solution is related to the phenophases of the plant.

The apparatus shown in Figure 1 and the treatments followed a modification of those used by Hale and Weaver (1962). The shoot of the maple seedling to be labelled was enclosed in the polyethylene bag which was connected by tygon tubing to the closed system containing ^{14}C-labelled barium carbonate ($BaCO_3$) (50 uc carbon-14 per 1 mg.) An excess of lactic acid was dropped onto the $Ba^{14}CO_3$ to yield ^{14}C-labelled carbon dioxide. The $^{14}CO_2$ was then pumped to circulate around the shoot at ten minute intervals for 90 minutes. Since the exposures were carried out on sunny days, the 90 minute exposure time was considered adequate for assimilation of a large fraction of the $^{14}CO_2$. The polyethylene bag was removed after the exposure, and the seedlings were then transferred into fresh hyponex solution in the greenhouse. The nutrient solution was then sampled every day for seven days.

Two samples of 50 ml each were removed daily from the flasks containing the roots of the labelled seedlings, and each sample was evaporated under vacuum to 1 ml. The 1 ml

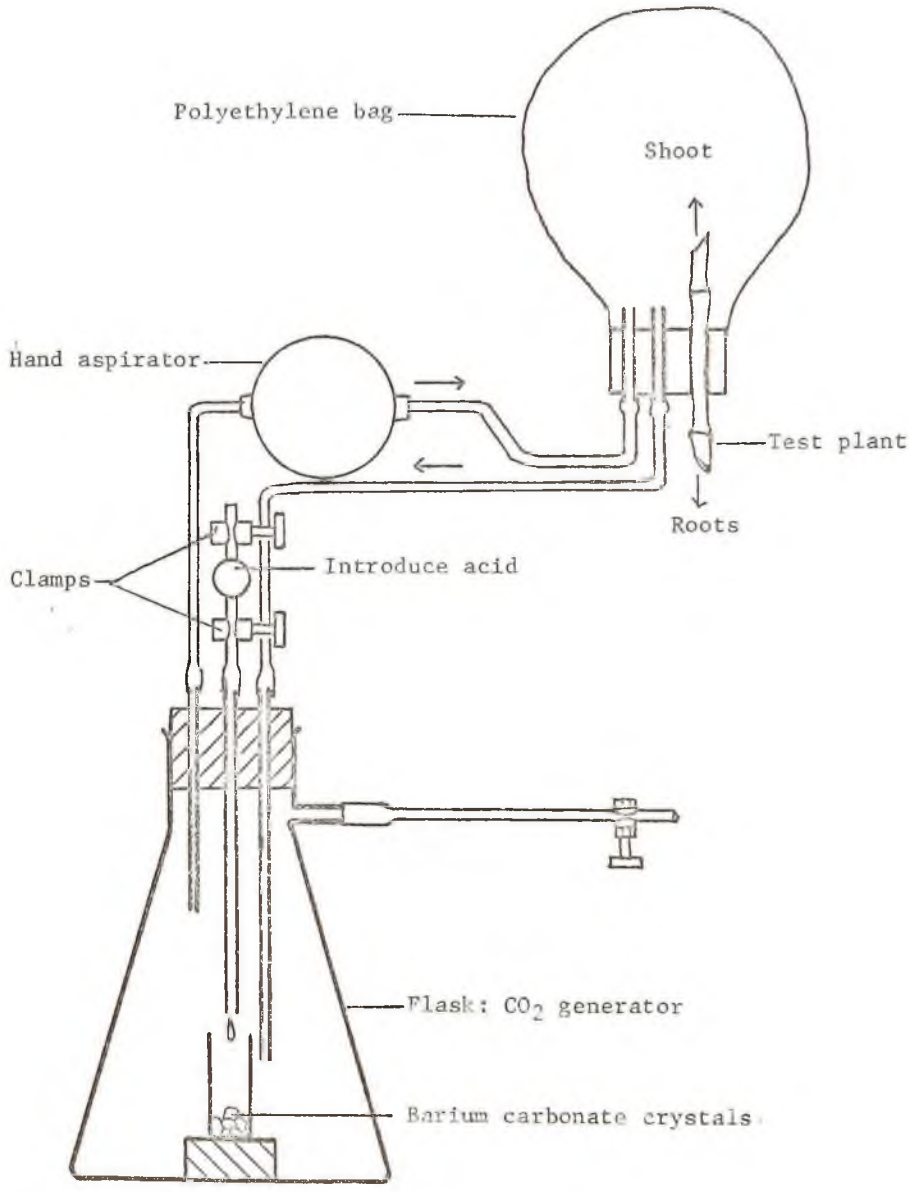


Figure 1. Diagram of system for exposing shoot of sugar maple seedling to $^{14}CO_2$.

concentrate was placed in a planchet, dried under infrared light, and the radioactivity measured with a Nuclear Chicago Model 470 gas flow detector (35% efficiency), for ten minute counts. The pH of the solutions at the times of sampling fluctuated between five and six. In this acid medium, there was a greater likelihood of any ^{14}C detected to be incorporated into assimilated compounds leaked from roots, and not due to accumulation of HCO_3^- and $CO_3^=$ from the CO_2 of root respiration. Nevertheless, in two trial runs samples were acidified with dilute sulphuric acid before the evaporation and drying. The counts were not significantly different from these in the regular samples; thus, one could attribute the radioactivity to ^{14}C in forms other than respired $^{14}CO_2$. Control sample solutions were collected from unlabelled plants.

There were three sets of maple test plants with two plants per set as follows:

A. Plants with growing roots but no leaves.

B. Plants with no active roots (elongating roots had been pruned off so only suberized roots were present) but fully expanded mature leaves.

C. Plants with growing roots and fully expanded mature leaves.

The summary of results are presented in Table 10. The departures from the background were so apparent that these results were not subjected to statistical analysis.

Table 10. Radioactivity of Solutions in Which Maple Seedlings Labelled with ^{14}C Have Been Growing. Background was 17 CPM

Day	Activity in Counts Per Minute (CPM)[1]								
	Control	A[2]		B		C		Acidified[3]	
1	17	17	NS	17	NS	52	**	-------	
2	19	36	*	17	NS	490	**	-------	
3	17	93	**	16	NS	2000	**	-------	
4	17	62	**	17	NS	2800	**	2115	**
5	17	17	NS	74	**	3201	**	-------	
6	17	17	NS	100	**	3255	**	-------	
7	17	17	NS	501	**	3400	**	3505	**
Average	17	37	*	106	**	2171	**	2810	**

*Significantly different from control at 5%.

**Significantly different from control at 1%.

NS = Not significant at 5%.

1/Each figure is the average of two separate counts.

2/Letters refer to test plants:

A. Plants with growing roots but no leaves.

B. Plants with no active roots but fully expanded mature leaves (elongating roots pruned so only subrized roots present).

C. Plants with growing roots and fully expanded mature leaves.

3/Acidified - Sample solution plus dilute H_2SO_4.

Only the leachate from Set C (i.e., plants with growing roots and fully expanded leaves) and Set B in the last two days of sampling gave a remarkably high count above the background. In B, the counts were probably due to several new roots which developed in the third day, and perhaps made transfer of ^{14}C into the nutrient solution possible. The slight increase in count in the leachate from A may be due to bark photosynthesis through which the $^{14}CO_2$ was assimilated.

The results show that there was leaching from the roots of maple when both the mature photosynthetic surface and active roots were present. This was the condition satisfied in Phase III and early Phase IV of Experiment One, when root leachate was so effective in inhibiting radicle elongation of birch germinants.

Evidence for leachate from roots of maple has also been established by Smith (1970) who identified several organic compounds, including amino acids, sugars and organic acid from the leachate of sugar maple seedlings and mature trees. Rovira (1969) also has reviewed several works in which exudation from root surfaces has been demonstrated. The present finding strengthens the hypothesis that it is only when both active roots and mature leaves are present on maple seedlings that substantial leaching occurs. This finding is discussed again later (see Page 49).

Figure 2 shows the depletion of ^{14}C from the leaves of sample trees in Set C relative to a build-up in the hydroponic solution. Leaf discs were extracted with ethyl alcohol,

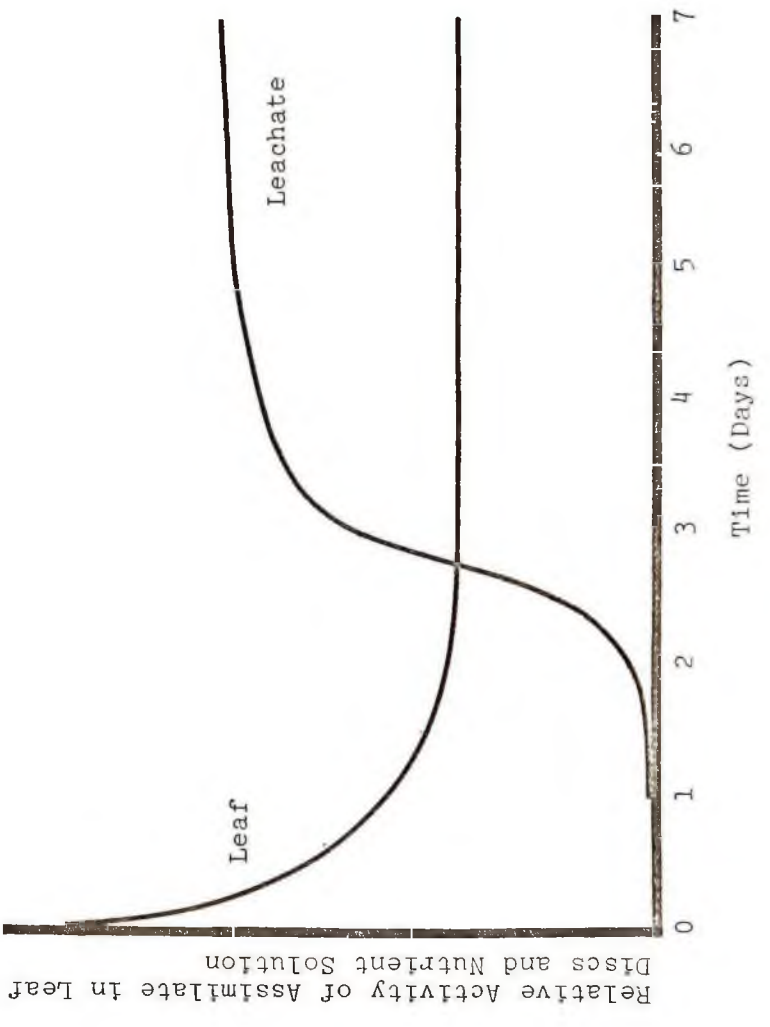


Figure 2. ^{14}C Depletion by Leaves and Leachate by Roots of Sugar Maple Seedlings with Active Roots and Fully Mature Leaves

an aliquot sample dried in a planchet and counted to follow the depletion.

The curves show that the ^{14}C was translocated from leaves rather rapidly during the first two days, and that ^{14}C accumulated rapidly in the solution on the third day.

Experiment Three: Effects of Reduced Light and Moisture Stress on Inhibition by Maple Seedling Leachate

The results of the study of the phenological pattern of inhibition suggested the probable role of current photosynthesis and translocation of currently photoassimilated materials to the roots in the production of inhibitor(s) by maple roots. The following experiment was designed to test the hypothesis that root-leached inhibitors originate from current photosynthate. A deliberate reduction in photosynthetic rate and in the rate of translocation should be reflected in a lower degree or an absence of inhibitor moderation.

To reduce current photosynthesis, without unduly affecting the photoperiod, an artificial shade was erected over several maple seedlings in hydroponic flasks with a double layer of brown paper. This cut off direct overhead light, but allowed a fair amount of reflected radiation laterally.

To slow down translocation, moisture stress was applied to test plants by growing them for five days in 10% (w/v) solution of polyethylene glycol 4000 (PEG) in hyponex solution. This mixture provided a solution water potential of approximately - 5 bars.

The treatment combinations were as follows:

Normal (N) Foliage under normal incident light roots in normal hyponex solution.

PEG	Foliage under normal light but roots growing in PEG-hyponex solution.
L	Foliage under reduced light, with roots in normal hyponex solution.
PEG/L	Foliage under reduced light and roots in PEG-hyponex solution.

Test plants were selected for size uniformity and were all in early Phenophase IV with all leaves fully expanded and mature.

The N and L plants were cultivated initially in hyponex for five days and then transferred into distilled water for two days prior to sampling. The PEG and PEG/L plants had five days in the PEG solution, were washed thoroughly to free the roots, as much as possible, of residual PEG, and then grown in distilled water for two days. Figure 2 shows that effective leaching from roots does not start until three days after treatment. The two days growth of maple seedlings in distilled water is therefore considered an optimum period for the previous PEG and light treatments to show their effects. The two-day old distilled water from each treatment was harvested, reduced to one-tenth its volume and assayed on birch germinants.

Each treatment was replicated three times, and there were three assays carried out for each treatment over the four weeks duration of the experiment. Each assay involved nine germinants. These treatment results were each compared

with the control results for significant (5% level) differences in birch radicle elongation. Table 11 summarizes the results of these PEG and light treatments.

Except for the normal plants, all other treatment results were not significantly different from the distilled water control results, which signifies that normal leachate was inhibitory and that leachate from the three other treatments selected to reduce the rate of photosynthesis and translocation were not inhibitory.

Discussion

The effects of reduced light and water stress on photosynthesis and translocation in sugar maple has not been reported in the literature, but the review by Wardlaw (1968) of carbohydrate distribution in plants covers relevant references on the subject which may explain the assay results.

The movement of photoassimilates from leaves via conducting tissue requires energy expenditure. It is known that a reduction in light intensity reduces not only the rate of photosynthesis but also the proportion of photoassimilates removed from leaves. Wardlaw (1968) offers the explanation that these decreases may be partly due to a drop in available energy for sugar transfer within the leaf, under conditions of low light. Transfer into the roots which in turn depends on the production and transfer of assimilate will therefore be decreased under this condition. It may be argued then that should an inhibitor or its precursor be produced in leaves as part of the general assimi-

Table 11. Bioassay of Leachates From Intact Roots of Maple Seedlings Grown Under Reduced Light and/or Under Water Stress, on Radicles of Yellow Birch Germinants

	Percent Increase in Growth[1/]				
Assay Number	Control[2/]	Normal (N)	PEG	L	PEG/L
1	29	17 *	30 NS	28 NS	33 NS
2	32	15 *	37 NS	31 NS	30 NS
3	26	17 *	24 NS	24 NS	21 NS
Grand Average	29	16 *	30 NS	28 NS	28 NS
Percent of Control	100	55 *	103 NS	96 NS	96 NS

*Significantly different from control at 5% level.

NS = Not significantly different from control at 5% level.

1/Each figure is averaged from three replications of three germinants each.

2/Control = Normal incident light and normal hyponex solution.

PEG = Normal light and PEG-hyponex solution.

L = Reduced light and normal hyponex solution.

PEG/L = Reduced light and PEG-hyponex solution.

late, its availability to roots will also be less under conditions of low light intensity. This result may explain why there was inhibition from the normal solution and none from those under reduced light in which inhibitor production may have been too low to effect significant reduction in root growth of birch.

As leaf water deficits increased from five percent to 20 percent, Roberts (1964) observed an 86% reduction in the translocation and rate of movement of ^{14}C in seedlings of yellow poplar. Applying Roberts' finding to the sugar maple seedlings of this experiment explains why with the application of PEG there was a reduction in translocation of inhibitor(s) or its precursors which may be in the photosynthetic assimilates.

Experiment Four: Studies with Fresh Germinants of Sugar Maple

The purpose of this experiment was to correlate the production of inhibitor(s) with current photosynthesis by investigating whether total lack of photosynthesis would contribute to birch inhibition, using leachate from sugar maple germinants whose cotyledons had not emerged.

Stratified sugar maple seeds were surface-sterilized by washing them with dilute solution of detergent for three to five minutes, rinsing them thoroughly with sterile distilled water and then soaking them for 15 minutes in two percent industrial chlorox solution. This treatment was to prevent growth of microorganisms on the seeds and in the germination substrate. The substrate consisted of sterile filter paper soaked in sterile distilled water. The petri dishes used had also been sterilized. Sterilization of dishes, filter paper and distilled water was accomplished in an autoclave at a pressure of 15 pounds per square inch and a temperature of 248°F. for 20 minutes. A parallel set of germinants was raised on non-sterilized media.

The leachate solutions were made under the following six conditions: (1) light and (2) dark treatments of control solution without germinants, (3) light and (4) dark treatments of leachate from sterilized germinants, and unsterilized germinants under (5) light and (6) dark conditions. To produce leachates, the maple germinants were suspended in distilled water in petri dishes for two days;

the distilled water was then harvested, concentrated by evaporation to one-tenth its volume and assayed on birch germinants. There were three germinants per treatment and two replications of each treatment. The assay results are presented in Table 12.

The treatment means of radicle elongation of birch germinants were not significantly different from the control means, implying that there was no inhibition by maple germinants from the treatment solutions, whether sterile or unsterile or in light or darkness.

Discussion

The condition of the maple germinants used for the foregoing experiment was similar to that of the older seedlings in Phase I, in that both had growing roots but no leaves and therefore no principal source of current photosynthesis. Unlike Phase I, there was neither bark photosynthesis nor a residual effect from a previous inhibitory state in the maple germinants.

The evidence provided by this experiment, though circumstantial in part, has shown again that in the absence of current photosynthesis, there was no inhibition; and also that the condition of sterility or nonsterility, the latter fostering development of bacteria, did not affect inhibitor production in the absence of current photosynthesis. The latter partly confirms Tubbs' (1970) finding that microorganisms do not play a primary role in the production of the inhibitor from sugar maple roots.

Table 12. Bioassay of Leachates From Intact Roots of Maple Germinants Before Emergence of Cotyledons On Radicles of Yellow Birch Germinants

Assay Number	Percent Increase in Growth[1/, 2/]				
	Control	Sterile Leachate		Nonsterile Leachate	
		Light	Dark	Light	Dark
1	68	62	62	67	70
2	42	50	47	45	41
3	83	79	88	88	81
Grand Average	64	63	66	67	64
Percent of Control	100	98	103	105	100

1/Each figure is an average of three replications of three germinants each.

2/No treatment result is significantly different from control at 5% level.

General Discussion of Experiments One to Four

The last three experiments were conducted to provide support for or against the hypothesis that the seasonal periodicity observed in inhibition by active roots in the presence of fully expanded mature leaves only is due to the role of current photosynthesis, translocation and source-sink relationships in sugar maple plants (Experiment One).

The ^{14}C study (Experiment Two) showed that root leaching of organic compounds indeed took place when there was root growth in the presence of fully expanded leaves; Experiment Three showed that under conditions of low current photosynthetic and translocation rates, inhibition was not expressed; and Experiment Four showed that leachate from five-day old maple germinants, collected before the emergence of cotyledons, did not inhibit birch radicle elongation. Microorganisms did not seem to affect inhibitor production in the fourth experiment.

These results provide the support needed: that the stage in which inhibition is expressed (Phase III and early Phase IV) is one in which leachate from roots is demonstrable; that without photosynthesis or with a lowering of it, or a lowering of translocation, inhibition is not expressed.

Parker and Houston (1971) working with sugar maple found a decline in root extractives if plants were artifi cially defoliated either in June or July which are major months in the growing season. These are also months which encompass Phase III and early Phase IV. There is no evidence

suggesting that there are residual effects of a previous inhibitory state that might operate through stored assimilates over a period of time. Assimilates from current bark photosynthesis may leach from roots in small quantities, but apparently they are of insufficient strength to affect the birch germinant bioassay.

Based on the evidence of the above experiments, the hypothesis for the periodicity in inhibition is explained. The different phases, in nature, would be associated with specific seasons; namely:

Phase I	-	Late Winter to Early Spring
Phase II	-	Late Spring to Early Summer
Phase III	-	Summer (Most of the Growing Season)
Phase IV	-	Late Summer to Early Fall
Phase V	-	Fall

While recognizing the dangers involved in extrapolation of laboratory data to field conditions or data on seedlings to mature trees (Smith 1970), one should be able to predict the onset of inhibition from maple leachates during the summer (Phases III and early Phase IV), and lack of inhibition at other times of the year.

Experiment Five: Effects of Extracts from Macerated Maple Tissue on Germination of Birch Seeds, on Radicle Growth of Birch Germinants and on Wounded Seedlings of Several Species

The literature on allelopathy indicated the occurrence of phytotoxins in several organs of plants. Species of Helianthus produce toxins from both their seeds and leaves (Curtis and Cottam 1950; Wilson 1968); extracts from leaves and flowers of Solidego juncea inhibited seed germination of jack pine (Brown 1967); walnut toxicity has been demonstrated in its fruits, leaves and roots (Massay 1925; Bode 1958). Griffiths in 1958 (cited by Smith 1969) reported detailed distribution of phenolics, including known inhibitors, in leaves, bark, wood, flowers, pods and beans of Theobroma cacao. The distribution of compounds throughout the latter plant was partly attributed to translocation from the leaves, either wholly or as precursors, a process similar to that postulated for sugar maple in this study.

On the premise that other organs may also induce inhibition, a study was undertaken to explore possible inhibitory effects of compounds from green leaves, litter and seeds (technically samara)[1/] of sugar maple on germination and root growth of birch; and also their effects on the growth of seedlings of other species. This experiment involved extracting substances from the organs in question and assaying them on appropriate receiver plant material. Receiver plant materials used were birch seed, lettuce seed,

1/A fruit, but for this project will be called seed.

birch germinants, and wounded seedlings of sugar maple, white ash, yellow birch and beech. The extracts were prepared by homogenizing 25 gms fresh weight each of sugar maple leaves, litter, or seeds with 250 ml of cold distilled water in a blender for two hours. The mixture was then vacuum filtered and the filtrate assayed on birch radicles. Extracts from roots of sugar maple were not included since their toxicity had been established by Tubbs (1970). For the germination assay, seeds of birch or lettuce were placed on filter paper in each petri dish. There were three replications of 20 seeds each. The filter paper was soaked with 5 ml of the extract and placed under light in the greenhouse. Control dishes containing distilled water were run with each extract. The control seeds germinated in 24 hours for lettuce and after about nine days for birch. The lettuce test was introduced to provide a more rapid assay that may complement both the birch germination and birch radicle elongation assays.

The wounded seedlings of the four species were used in an assay which is modified from one used by Mergen (1959) with Ailanthus leaf extracts. It involved exposing the tissues of the stem in a cut made to the pith, and applying the extract or distilled water (control) to the wound through a trough constructed with putty at the lower end of the wound. The reaction of the seedlings to the extract was observed over a 14-day period, during which the extract was periodically replenished. The seedlings used were two to three years old and were actively growing at the time of the

assay, a physiological state equivalent to Phase III. There were three plants of each species tested per treatment.

Results of these assays are presented in Tables 13 and 14. The extracts from macerated leaves or litter of sugar maple did not inhibit seed germination either of lettuce or birch. Extract from macerated sugar maple seeds did significantly reduce germination of both yellow birch and lettuce seeds. Extracts of macerated leaves and seeds of maple, but not of maple litter, significantly decreased birch radicle elongation. Maple seed extract was apparently the more inhibitory.

An explanation for maple litter extracts not showing inhibition of birch radicle elongation may be in the fact that there is a general transfer of assimilates and other substances from the leaves to storage tissues of the stem and buds with the onset of senescence and before absiscion. For inhibitors, this process has been demonstrated in Acer pseudoplatanus (Phillips and Wareing 1958). Thus, it is conceivable not to detect inhibitory properties in water extract of maple litter, but to find them in extracts of green leaves. This may also explain why Tubbs (1969) in a birch germination study did not detect any inhibition by maple litter used as a medium for the germination.

There were no visible toxic effects shown by seedlings in relation to the stem wound (Table 14) except to a limited extent by the seed extract of maple on yellow birch seedlings where there was a browning of the xylem surface

Table 13. Bioassay of Macerated Maple Leaf, Litter and Seed Extracts on Germination of Yellow Birch and Lettuce Seeds and on Yellow Birch Radicle Elongation

Assay Solution (Extracts)	Percent of Seed Germination				Radicle Elongation (Percent Increase)	
	Birch		Lettuce			
Control	43		100		65	
Leaf	39	NS	92	NS	35	*
Litter	44	NS	94	NS	60	NS
Seed	29	*	54	*	24	*

Each figure is averaged from three replications.

*Significantly different from control at 5% level.

NS = Not significantly different from control at 5% level.

Table 14. Effect of Extracts from Macerated Maple Leaf, Litter and Seed on Sugar Maple, White Ash, Yellow Birch and Beech Seedlings (Receiver Plants)

Receiver Plants	Effect of Extract From		
	Leaf	Litter	Seed
Sugar Maple	0	0	0
White Ash	0	0	0
Yellow Birch	0	0	1
Beech	0	0	0

0 = No Effect

1 = Some Effect (See Text For Detail)

above the level of the cut and a slight drooping of the leaves. This suggested either a low inhibitory or toxic effect of the extracts from the macerated maple seed, or that most of the species tested were resistant to any inhibitor that may have been in the maple extracts. Birch seedlings may be somewhat susceptible to extract from macerated maple seeds.

This experiment thus demonstrated a toxicity in extracts of macerated maple leaves and seeds, expressed as a lowering of germination percent in birch and lettuce seeds and as a reduction of birch radicle elongation. However, because the extraction procedure releases a wide range of metabolites from macerated tissue, this finding may be of little practical importance. In nature, there is no exact equivalent of the homogenizing, plus cold water extraction and vacuum filtering in the removing of extracts from fresh leaves and seeds. The results, however, do suggest the possible presence of inhibitors in sugar maple seeds and leaves that may be the sources of leachates into the soil. The experiments which follow pursue this matter further.

Experiment Six: Effects of Various Maple Leachates from Undisturbed Tissue on Germination of Birch Seed and on Radicle elongation of Birch

Because of the doubtful ecological significance of the results with macerated tissue, it was necessary to reexamine maple leaves and seeds in a way that would more nearly duplicate natural circumstances.

Leachates, as opposed to extracts, were therefore collected and assayed on seeds of birch and lettuce and radicles of birch germinants. The lettuce seed germination was used to provide a quick check on the birch germination in the leachates.

Three procedures were used for collecting the leachates: (1) 500 ml of distilled water were sprayed on the foliage of each of three maple seedlings used with a small DeVilbiss atomizer which gave a fine mist spray. The dripping water from the three seedlings was collected in a beaker pooled into one volume, vacuum filtered and evaporated down to one-tenth its volume. Five ml each of the concentrated solution and control were assayed on birch and lettuce. There were three replications of this procedure; (2) Mature and young maple leaves were detached from their stems and the petiole ends were sealed with parafin wax to prevent exudation from the out surfaces. One leaf per treatment was placed in a petri dish with 5 ml distilled water and with the assay material. Control dishes had 5 ml distilled water each with the assay material. This procedure was also

used to detect any seasonality in the leaching of inhibitors from the leaves by assaying detached leaves from maple plants growing in the greenhouse, at monthly intervals from April through September; and (3) Twenty-five maple seeds were soaked in 250 ml distilled water for 24 hours and the leachate was collected and assayed. In another trial, seeds were soaked for varying lengths of time (one day, three days, five days, seven days and 10 days) providing leachates for testing the effect of length of soaking on the inhibitory properties of maple seed leachate.

Each assay had three replications of three birch germinants each or 20 seeds each of birch or lettuce. The results of the assays under the above three leaching procedures are presented in Tables 15, 16, 17 and 18.

Table 15 shows that none of the leachates from either attached or detached leaves inhibited germination of birch seed. Maple leaf leachate was thus not inhibitory regardless of the age of the leaf or attachment to the plant. The inhibition due to maple seed leachate was significant in its effect on birch germination. It was also observed that the roots of the birch germinants coiled rather perculiarly in the leachate from maple seed (Figure 3), a condition which did not occur in leachates from other tissues, even from roots in earlier experiments.

The results for the lettuce seed assay (Table 16) agree generally with those for the birch seed germination. No inhibition in percent germination was noted except due to maple seed leachate. The root coiling observed with birch germi-

Table 15. Germination of Yellow Birch Seeds Soaked in Leachates of Intact Mature Leaves of Maple Seedlings (IML), Detached Mature Maple Leaves (DML), Detached Young Leaves (DYL) and Maple Seeds (MS)

Replicate Means	Percent Germination					
	Control	IML	DML	DYL	MS	
1	45	44	40	47	19	**
2	50	45	52	43	18	**
3	41	45	42	49	22	**
Grand Mean	45.3	44.7	44.7	46.3	19.7	**
Percent Control	100.0	98.7	98.7	102.2	43.4	**

**Significantly different from the control figure at 1% level. All other treatment figures are not significant.

Figure 3. Drawings of Yellow Birch Germinants Showing Their Root Development in Distilled Water and Maple Seed Leachate (Six Day Old Germinants)

Table 16. Germination of Lettuce Seeds Soaked in Leachates From Intact Mature Leaves of Maple Seedlings (IML), Detached Mature Maple Leaves (DML), and Young Leaves (DYL) and Maple Seeds (MS)

Replicate Means	Percent Germination				
	Control	IML	DML	DYL	MS
1	100	100	99	100	52 *
2	100	100	98	100	61 *
3	98	100	100	100	64 *
Grand Mean	99.3	100	99.3	100	59.0 *
Percent of Control	100	100.7	99.7	100.7	59.4 *

*Significantly different from control at 5% level; others are not significantly different.

Table 17. Bioassay of Leachate From Intact Maple Leaves at Monthly Intervals from April to September 1971, on Radicle Elongation of Yellow Birch Germinants

Replicate Means	April		May		June		July		Aug.		Sept.	
	*C	L	C	L	C	L	C	L	C	L	C	L
	Percent Radicle Elongation											
1	57	61	53	51	64	66	60	59	67	65	60	60
2	69	74	54	58	45	41	59	58	60	61	46	44
3	60	60	70	66	49	52	63	65	70	68	60	57
Grand Mean	62	65	59	58	53	53	61	61	66	65	55	54

*C = Control, L = Leachate

Table 18. Bioassay of Maple Seed Leachate of Different Ages on Radicles of Yellow Birch Germinants

Replicate Mean	Period of Soaking in Days					
	Control	1	3	5	7	10
		Percent Radicle Elongation*				
1	45	20	25	21	26	10
2	60	34	37	42	43	39
3	69	41	30	47	23	39
Grand Mean	58	33	29	37	31	36
Percent of Control	100	56	50	63	53	62

*All figures are significantly different from control at 5% level.

nants in maple seed leachate did not occur with the lettuce, which may be due to the greater sturdiness in roots of lettuce germinants.

Table 17 shows the results of tests of seasonality in the leaching of inhibitors from surfaces of detached leaves of sugar maple. As shown, leaf leachate did not inhibit birch radicle elongation at any time during the growing season, which in the greenhouse under experimental conditions spanned the period from late March to late September.

McPherson and Muller (1969) after a failure to demonstrate inhibition of Bromus rigidus seed germination by leaf leachates of Adenostoma fasciculatum, sought explanation in the possible previous earlier washing of toxins from the surfaces of leaves by rain. In the present study, the procedure for obtaining leachate from sugar maple precluded such losses due to previous washing of the leaf surface. The lack of inhibition therefore should be due to the fact that there is no significant leaching of inhibitor from maple leaf surfaces.

Table 18 shows the results of the bioassay of maple seed leachates obtained by soaking maple seeds for various lengths of time. All leachates significantly reduced birch radicle elongation. There did not seem to be any increase in degree of inhibition with soaking periods longer than one day, the shortest soaking period. The duration of soaking apparently then neither reduced nor enhanced the inhibitory property of the maple seed leachate.

The results of the bioassay of leaf and seed leachates

indicate first that although extract from macerated maple leaf tissue did inhibit birch radicle elongation, inhibitory substances did not leach out of intact foliage to be effective ecologically, and second, that maple seed leachate was an effective source of inhibition to birch germinants and may add appreciably to the source in soil from maple roots. This would make the allelopathic effects of maple, thus far determined, the combined effect of toxic principles which leak from active roots during mid-summer, and in or on the seeds.

From the results of Experiments Four and Five, in which the effects of extracts and leachates from maple tissue were studied, leaves and litter of maple were eliminated as sources of inhibitors and further experiments were concerned with maple seed and maple root leachates.

Experiment Seven: Effects of Maple Seed and Root Leachates on Birch Seedling Development

Various investigators have reported expression of inhibition in receiver plants to take the forms of a general reduction in growth, root injury, shoot wilting and poor root development (DeBell 1969); reduction in respiratory activity, as in peach (Patrick, et al. 1958); or interference with nitrogen fixation in several old field plants (Rice 1964).

This experiment investigated some aspects of the external expression of inhibition in yellow birch seedlings by seed and root leachates of sugar maple.

The leachates were obtained through procedures already outlined; that is, solutions harvested from flasks in which roots of sugar maple seedlings in early Phase IV were growing in water culture, and from solutions in which maple seeds were soaked for 24 hours. Birch germinants were transplanted into individual pockets in a styrofoam pot using vermiculite as the root medium. One set of 96 pockets in the pot was divided into three and assigned to three main treatments as follows: daily watering with 10 ml each of (1) distilled water, (2) maple seed leachate, or (3) maple root leachate. Each treatment was subdivided so that one-half of the pockets received good drainage and the other one-half had seals to provide poor drainage. There were thus 16 birch seedlings per subtreatment. The seedlings were allowed to grow under light for three weeks, were then harvested, and their lengths and over dry weights taken; a

description was made of their root morphology. The greenhouse temperatures during the period of the study were 95°F. during most of the day and 70°F. at night.

The results are presented in Table 19. Differences among the means were significant only between the control (distilled water) with good drainage and seed leachate with slow drainage. Although the other differences were not significant, the apparent effect of the treatments on the variance in the data is of interest. The averages and standard deviations of root lengths (cm) are as follows:

I.	Control With Good Drainage:	28.0±3.1
II.	Control With Slow Drainage:	26.5±3.7
III.	Seed Leachate With Good Drainage:	24.8±7.8
IV.	Seed Leachate With Poor Drainage:	22.3±4.0
V.	Root Leachate With Good Drainage:	25.6±5.4
VI.	Root Leachate With Poor Drainage:	25.1±2.8

In Treatments I, II, IV and VI, the variance was very narrow, whereas it was large in Treatments III and V. This may represent the effect of drainage of the leachates. Where good drainage was provided, there seemed to be both stimulation of some and inhibition of other individual seedlings, and this may have caused a range in size greater than in the control. Significant differences at 5% level occur, however, when the smallest eight seedlings in each of well drained

Table 19. Some Effects of Seed and Root Leachates on Yellow Birch Seedling Development[1/]

Treatment[2/]	Lengths (cm)		Root Length / Shoot Length	Oven Dry Weight (mg) of R+S
	Root (R)	Shoot (S)		
I (Control)	28.0	4.5	6.2	24
II	26.5	5.0	5.3	21
III	24.8	5.8	4.3	22
IV	22.3*	5.6	4.0	15*
V	25.6	5.2	4.9	21
VI	25.1	5.0	5.0	20

*Significantly different from control at 5% level. All other treatment figures are not significant.

1/The yellow birch seedlings were grown in a chambered styrofoam pot (seedling starter) commercially available from garden suppliers.

2/ I = Control with good drainage.

II = Control with poor drainage.

III = Seed leachate with good drainage.

IV = Seed leachate with poor drainage.

V = Root leachate with good drainage.

VI = Root leachate with poor drainage.

control (I) and of poorly drained seed (IV) and root (VI) leachates are compared. The small individuals represent those most likely to have been inhibited. The root-shoot ratios of those inhibited individuals reflect the relatively poorer growth of the roots in Treatments IV and VI than the smallest control plants.

In a further study, 300 birch seeds were pregerminated in each of three large petri dishes and then thinned out to leave only 150 uniform seedlings per dish with a shoot height averaging 5 mm. Different solutions were used in watering the seedlings in different dishes every fifth day as follows: (a) distilled water, (b) maple seed leachate, and (c) maple root leachate from maple seedlings in early Phase IV. There were three replications. The seedlings were maintained for 15 days to observe their mortality in each of the watering solutions. The results are shown in Figures 4 and 5.

The difference is very striking between mortality in leachates and in distilled water. Within two weeks of their being in the different solutions, 49% and 82% respectively of seedlings in root and seed leachates had died as against 1% in distilled water. A few seedlings growing in maple seed leachate showed the peculiar coiling which was encountered in Experiment Five (Figure 3). Some of these seedlings with coiled roots were transferred into distilled water after the third and eighth days. After 24 hours, and within 48 hours, the coils in the third day plants straightened, but those in the eighth day plants did not. This would suggest

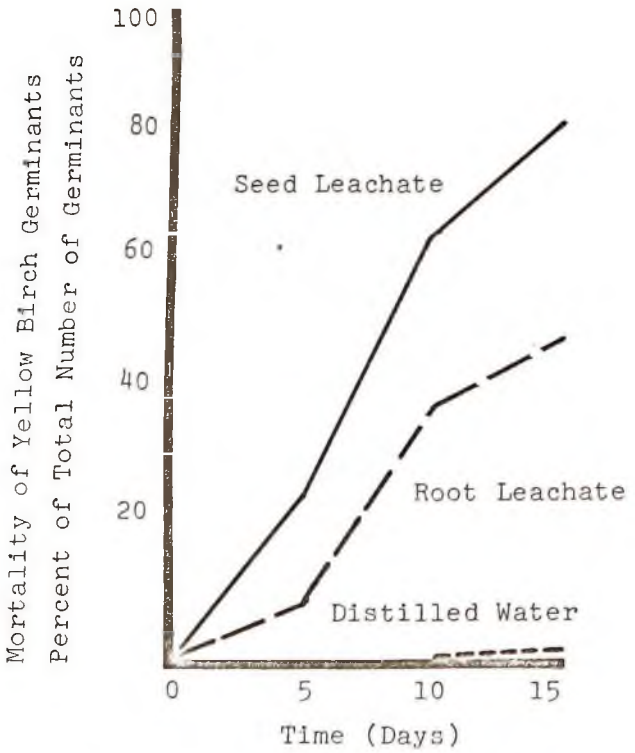


Figure 4. Graphs Showing Mortality of Yellow Birch Germinants in Different Leachates of Sugar Maple

A B

Figure 5. Photograph Showing Mortality of Yellow Birch Germinants in Seed Leachate of Maple and Distilled Water After 15 Days

A = Distilled Water

B = Seed Leachate

that the toxicity resulting in root coiling may not be harmful as long as it did not extend beyond a few days.

Discussion

The presence of inhibitors in leachates from roots and seeds of maple has already been demonstrated in previous experiments and therefore is presumed. The reduction in growth, the root coiling and mortality in birch caused by maple leachates may also be presumed due to inhibitory substances leached from maple tissue. An explanation is now sought for the results in well-drained and poorly drained sets of pockets in the styrofoam pots and for the mortality curves shown in Figure 4. Wilson (1968) noticed that he had greater inhibition from leachates in loam soil than in sand, and attributed this to the possible role of soil colloidal material in accumulating the inhibitor to a toxic level. In the present exercise, the slower drainage in Treatment IV in the styrofoam pot (and the lack of drainage in the petri dish) simulated, to some degree, a condition of toxin accumulation from maple leachate, and therefore caused either a marked reduction in growth or death of birch plants. Some microbial activity was observed over the 15 days, and may have contributed to the death of some seedlings in the petri dishes, but one still cannot discount the added role of toxins. Where there seemed to be both stimulation and inhibition of birch seedlings in the styrofoam pots, the free drainage treatments could have dispersed or leached substances such that, depending on their distribution in the

root media, and on the physiological condition of birch seedlings, either inhibitors or stimulators may have been absorbed by the birch seedlings.

The mortality curves for birch germinants shown in Figure 4 would suggest, as previous results from maple extract and leachate assays have, that the seed leachate exerts a stronger toxicity or inhibition than does the root leachate. This is examined in the next section, Experiment Eight, and further discussion will then be considered.

It is postulated that the expression of inhibition by maple leachate on birch seedling development, based on evidence reviewed thus far, takes the form of inhibited root development which, if extended, can result in poor seedling development and possibly death.

Experiment Eight: The Chemical Nature of the Root and Seed Leachates of Sugar Maple

Evidence from the preceding experiments and from those of Tubbs (1970) indicate the existence in leachates of maple seed and roots of a wider range of compounds than just an inhibitor. The reviews by Rovira (1965, 1969) and Tukey (1970) in general indicate this to be the case for most plants. Available literature on maple specifically shows that there can be in maple root leachates as many as five sugars, four organic acids and 12 amino acids (Smith 1970).

The objective of this experiment was to identify some general characteristics of the compounds in both root and seed leachates of sugar maple to form a basis for future detailed biochemical study.

The methods used were: (1) Fractionation, involving either extraction of inhibitor(s) from acid and basic solutions of maple root and seed leachates, using the effect of each fraction on inhibition of birch germinants as a bioassay; (2) Paper and thin layer chromatography of maple root and seed leachates to study the effect of specific Rf separates on inhibition of birch germinants, and (3) Boiling and freezing of maple root and seed leachates to study their effects on inhibition of birch germinants.

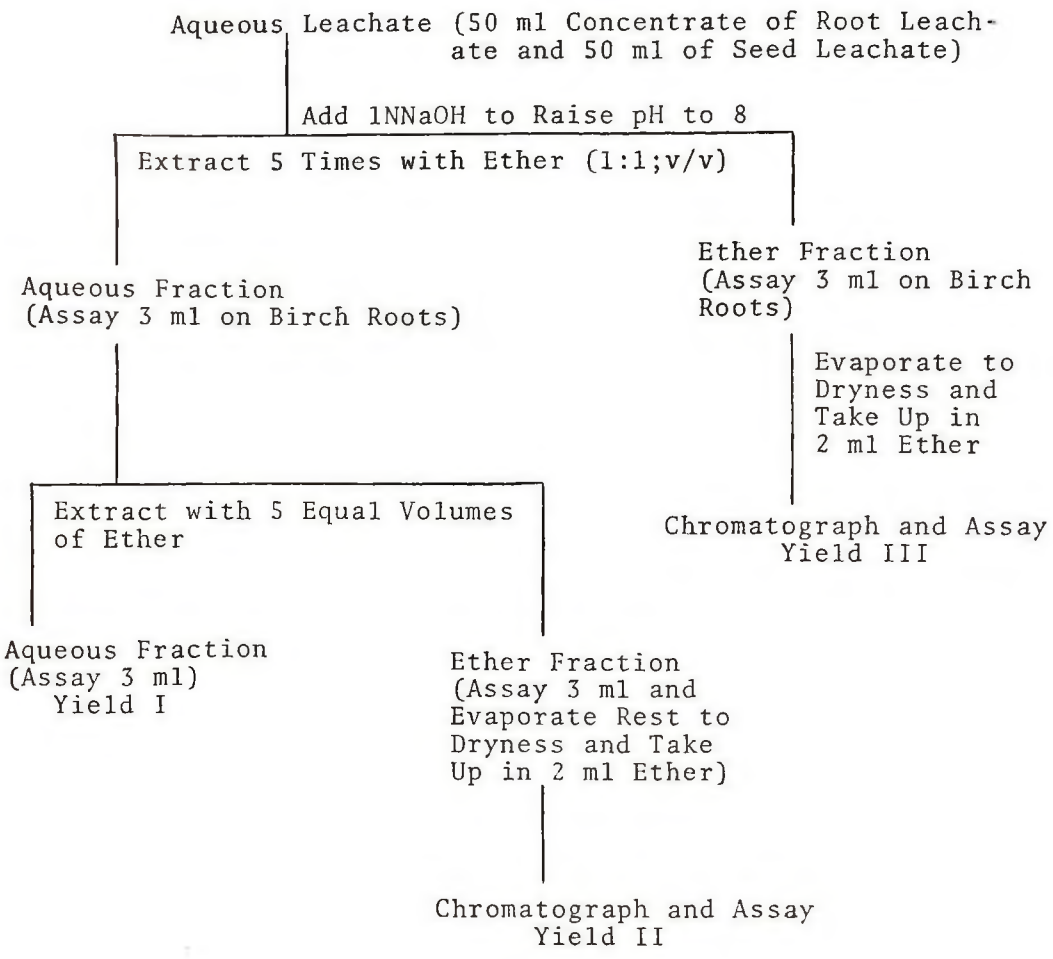
The fractionation procedure was arrived at initially in conslutation with Mr. James Weber of the Botany

Department, University of Michigan, and later confirmed by Professor Philip LeQuesne of the Chemistry Department, University of Michigan. Most of the chromatograms involving BAW (Butanol-acetic acid-water) and 2% acetic acid in water solvent systems on Whatman No. 3 Chromatographic paper were developed at Michigan State University under the advice and supervision of Professor James Hanover of that University, who suggested locating reagents along those in Smith (1969). I did all thin layer chromatography using the principles learned from the paper chromatography.

Maple root leachate was collected by growing maple seedlings in early Phase IV in distilled water for two days and harvesting the distilled water in which it was assumed that maple root leachate would have accumulated. The solution was then evaporated to one-tenth its volume under vacuum, as in Experiment One, for most of the studies. Leachate from maple seeds was obtained by soaking maple seeds in distilled water for three days and pouring the solution off the seeds, as in Experiment Six, for use in the experimental work which follows.

1. Fractionation

The following flow chart was used to separate various possible inhibitory fractions of the maple root and seed leachates:



At pH8 of the leachates, ether will extract all basic compounds (Yield III) and leave in solution all acidic and neutral compounds. At pH 2.6 of the residual aqueous solution, ether extracts the acidic compounds (Yield II) and leaves in solution all neutral compounds (Yield I). Aliquots of each of the yields were assayed by the birch radicle elongation test as follows: 3 ml of a yield was pipetted into a 5 cm glass petri dish and the ether was

blown off with a current of air. The residue was taken up in 3 ml of distilled water and assayed on radicles of three birch germinants. Below is a summary of the assays with Yields I, II and III from maple root and seed leachates expressed as percentages of radicle elongation of control yellow birch germinants. There were three replications of the assay for each yield.

Yield	Root Leachate	Seed Leachate
I - Neutral	85	90
II - Acidic	51[1]/	43[1]/
III Basic	91	89

1/Significantly lower than control at 5% level.

The results show that in both maple root and seed leachates, inhibition was induced on birch germinants by the acid ether fractions. This suggested that the inhibitors in the maple leachates were acidic in reaction.

2. Chromatography

The acidic and basic ether fractions (Yields II and III respectively) of the maple leachates were each chromatographed in a single descending direction on Whatman No. 3 Chromatographic paper using Isopropanol-Ammonia-Water (IAW) (10:1:1; v/v/v) as the solvent system. The papers were dried and cut up into the ten component Rf's. Each Rf was eluted by soaking it with 3 ml distilled water in a 5 cm petri dish. The eluates were assayed on the radicles of fresh birch germinants. Blank chromatograms were also run as controls.

The results of the assays with eluates from maple root and seed leachates are presented in Table 20. The results show that there was no inhibition in the basic fraction and confirmed the finding in the fractionation study. In the acid fraction of maple root leachate, significant inhibition occurred at Rf .5 and .6. In the acid fraction of the maple seed leachate, significant inhibition occurred at Rf .5 and .7.

For a second set of chromatograms, 100 ml and 20 ml respectively of fresh maple root and seed leachates were each concentrated to yield 2 ml of solution which was then spotted on Whatman No. 3 paper and developed in a single direction descending manner, using Butanol-acetic, acid-water (BAW) (4:1:5;v/v/v) as the solvent system. The papers were dried and viewed under ultra violet (UV) light to detect fluorescence.

The maple root leachate chromatogram gave no fluorescent spots, but the maple seed leachate chromatogram showed two bright blue spots at Rf .3 and .4 and bright blue streaks at Rf 0 to .2 and .55 to .9. Each of the two papers was exposed to ammonia fumes and viewed again under the UV light. There were no changes in their appearance from that before exposure to ammonia fumes.

Bioassay of eluates from this second set of chromatograms on birch radicles showed significant growth inhi bition at Rf .7 by maple root leachate and at Rf .15, .65, .75 and .95 by maple seed leachate.

Table 20. Bioassay of Eluates From Chromatograms of Acid and Basic Ether Fractions of Maple Root and Seed Leachates (Yields II and III) on Radicles of Yellow Birch Germinants[1/]

Rf	Root Leachate		Seed Leachate	
	Acid	Basic	Acid	Basic
.1	105	91	90	100
.2	98	91	102	98
.3	89	102	99	91
.4	92	100	97	103
.5	61 *	94	52 *	95
.6	58 *	98	81	89
.7	86	94	63 *	95
.8	95	87	89	100
.9	80	91	100	100
1.0	101	88	104	96

*Significantly lower than control at 5% level; all other treatment results not significantly different from control.

1/Solvent system used was IAW. Each figure is averaged from three replicates of three germinants each, and expressed as a percentage of birch radicle elongation of control.

To detect the general classes of compounds in the foregoing single dimensional chromatograms, four sets of two dimensional chromatograms were prepared from concentrated maple root and seed leachates. Whatman No. 3 paper was used with BAW and 2% acetic acid as the solvent systems in the first and second directions respectively. These chromatograms were first viewed under UV light, and then tested with four location reagents as follows (one set of chromatograms was used for each type of test):

Phenolic Compounds. The chromatogram was dipped into a solution of Ferric-Chloride-Potassium-Ferricyanide reagent ($FeCl_3$ -K_3 $Fe(CN)_6$), washed successively with dilute hydrochloric acid (HCl) and water and then air dried. The reagent locates phenolic compounds by the formation of Prussian blue color.

Organic Acids. The chromatogram was sprayed with a .1% solution of Bromocresol green reagent in 99.9% ethanol (w/v), whose color had been altered to blue-green by the addition of 1N NaOH solution, and then mixed with acetone in a ratio of 1:4. Organic acids appeared yellowish.

Amino Acids. The paper was sprayed with .2% solution of Ninhydrin in acetone. The paper was then allowed to air dry or heated for two to three minutes in an oven at 105°C. Amino acids reacted to give purple color.

Sugars. The paper was first sprayed with a mixture of silver nitrate saturated solution in water and acetone (1:20; v/v), and then sprayed with NaOH solution (.5 g dissolved in 5 ml water and diluted to 100 ml with ethanol). Sugars showed up as dark brown to black spots on a brown background.

The results of these tests with location reagents are tabulated below:

	Number of Spots Detected	
	Maple Root Leachate	Maple Seed Leachate
Phenolic Compounds	None	9 (See Table 21 for Rf Values)
Organic Acids	2(.10;.36)[1]/	1(.06)
Amino Acids	1(.14)	2(.14 and .17)
Sugars	1(.13)	None

Only the nine phenolic compounds fluoresced or were just visible under the UV light. The colors under the UV light are indicated in Table 21.

Using the information obtained by the locating reagents, duplicate chromatograms were assayed as follows: Areas of the paper corresponding to each of the spots for each of the four classes of compounds were cut out, eluted and tested with the yellow birch radicle elongation assay. Only the eluates from the regions of the nine phenolic

1/ figures in parentheses are Rf values.

Table 21. Results of Tests on Nine Phenolic Spots on Maple Seed Leachate Chromatograms

Spot No.	Rf BAW2%AA 1st	Rf BAW2%AA 2nd	Fluorescence[1] Longwave (UV)	UV Maxima (nm)	Reagent Color $FeCl_3$	Reagent Color $K_3Fe(CN)_6$	Inhibition[2]
1	.13	0	LB1	290	B		Yes
2	.52	.09	LB1	293	B		No
3	.91	0	LB	237,280	B		Yes
4	.55	.58	LB1	276#	B		No
5	.65	.61	LB1	240,278#	B		Yes
6	.76	.58	B1	280	B		Yes
7	.16	.84	YB	277,300,320	B		No
8	.74	.82	LB1	237,283	B		No
9	.70	.94	LY	243	B		No

1/L = Light; B1 = Blue; Y = Yellow; B = Brown

2/Inhibition of Birch Radicle Elongation

Yes = Inhibition; No = No Inhibition

= Minima

spots in the seed leachate chromatograms significantly reduced birch radicle elongation. None of the regions corresponding to the other spots from either the root leachate or seed leachate chromatograms inhibited growth of birch radicles.

The results of the assay with eluates from the nine phenolic spots in the seed leachate chromatograms are summarized in Table 21. Spots 1, 3, 5 and 6 corresponding to Rf values of .13, .91, .65 and .76, inhibited birch radicle elongation.

Finally, it was decided to determine the spectral properties of the nine phenolic compounds in maple seed leachate. For this, a two dimensional chromatogram was run on maple seed leachate with BAW in the first direction and 2% acetic acid in the second direction using precoated Gelman (SA) Instant Thin Layer Chromatographic Medium (ITLC) for ease of elution. Each of the nine phenolic spots was cut and eluted in absolute ethanol and its UV absorption maxima (minima in two cases) determined on a Beckman DK-2 ratio recording spectrophotometer. The reference cuvette contained absolute ethanol.

Table 21 summarizes the results of the observations made on the nine phenolic spots on the maple seed leachate on the two dimensional chromatograms. The UV absorption maxima are well within the range of maxima reported for several phenolic compounds (Hedin, et. al. 1965).

3. Boiling and Freezing. Batches of maple root leachate,

seed leachate, and distilled water were each either boiled for five minutes just before use, or frozen overnight, or kept at room temperature overnight. Each of these differently treated solutions was assayed on radicles of birch germinants. Assay of each solution was replicated three times with three birch germinants per assay.

The results are tabulated below as average percent increase in birch radicle lengths:

	Boiled	Room Temp.	Frozen
Root Leachate	60	63	57
Seed Leachate	57	61	58
Control (Distilled Water)	88	92	91

The differences between the treated solutions and the control (distilled water) are significant at the 5% level which indicated that the boiling and the freezing did not affect the inhibitory properties of the maple root and seed leachates.

Samples of these treated maple leachates were chromatographed on Gelman (SA) ITLC medium using BAW and 2% acetic acid as the solvent systems in the first and second directions respectively.

The chromatograms showed the same pattern of spots under UV light and with locating reagents as the chromatograms developed earlier under the section on chromatography, which means that the boiling and freezing did not alter the

pattern of spotting in the chromatograms.

Samples of the boiled and fresh leachates from maple roots and seeds were then stored at room temperature and under refrigeration. All samples of these maple leachates were periodically assayed on birch radicles to detect their loss of inhibitory properties with time, using distilled water as control assay solution.

Figure 6 summarizes the results of the bioassays to detect loss of inhibition in stored maple root and seed leachates.

For maple root leachate loss of inhibition was faster at room temperature (about six days) than under refrigeration (about 18 days). Fresh maple root leachate at room temperature lost its inhibitory property fastest (about six days) followed by the boiled maple root leachate (about 10 days) and slowest in the frozen root leachate (16 to 20 days). The apparent degradation of maple root leachate at room temperature could be attributed partly to the activity of microbes which were quite numerous after the fourth day. The boiling provided temporary sterile conditions which may have prolonged the life of the solutions for a few more days. Microbial degradation is known to occur to inhibitors in nature (Buxton, cited by Rovira 1965; Woods 1960).

In maple seed leachates inhibition of birch radicle elongation was detectable under all the storage treatments mentioned above during the 30-day storage period over which the experiment lasted. No significant differences were detected between treatment means at 5% level. Maple seed

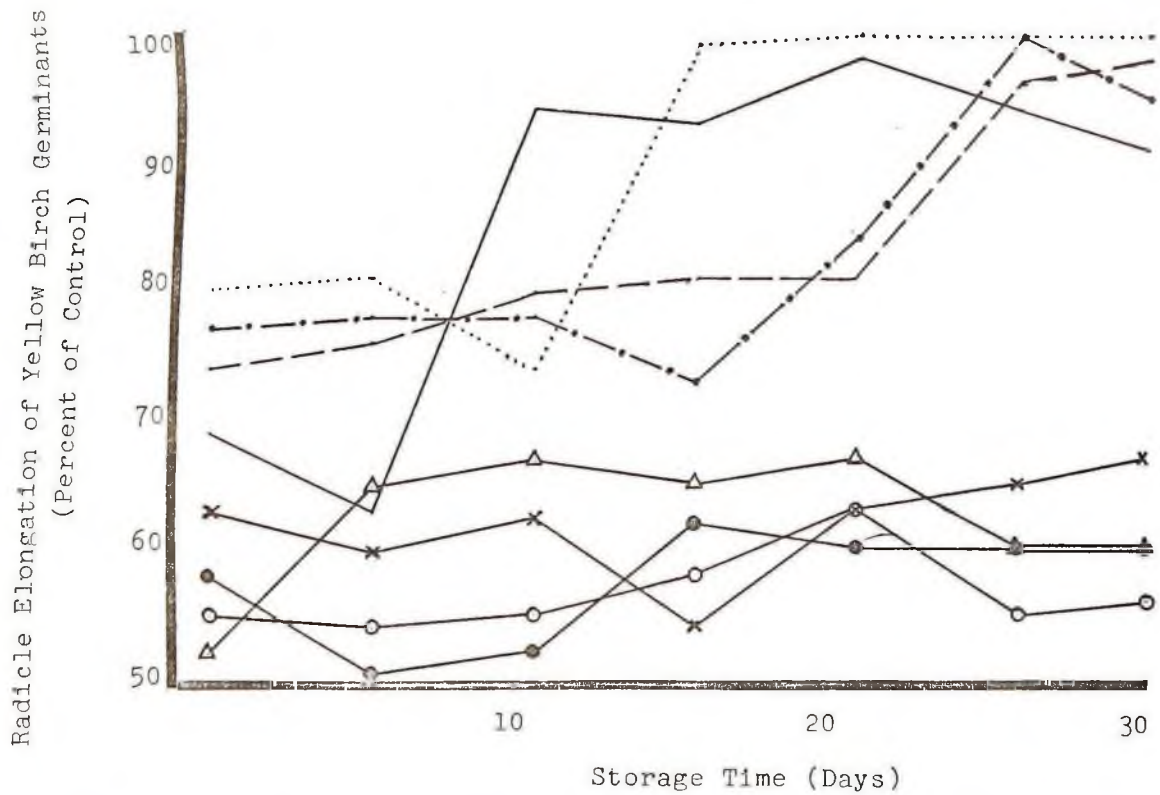


Figure 6. Periodic Bioassay of Boiled and Fresh Maple Root and Seed Leachate Stored at Room Temperature and Under Refrigeration on Radicles of Birch Germinants

- ·····= Boiled Root Leachate at Room Temp.
- — —= Boiled Root Leachate Refrigerated
- ——= Fresh Root Leachate at Room Temp.
- —•—= Fresh Root Leachate Refrigerated
- ×—×= Boiled Seed Leachate at Room Temp.
- •—•= Boiled Seed Leachate Refrigerated
- o—o= Fresh Seed Leachate at Room Temp.
- Δ—Δ= Fresh Seed Leachate Refrigerated

leachate seemed to be more stable therefore than maple root leachate.

Discussion of Experiment Eight

The foregoing tests yielded information on the nature of some of the inhibitory principles in maple root and seed leachates. The inhibitory principle(s) of maple root leachate was acidic and occurred at specific Rf values of chromatograms developed in IAW and BAW-acetic acid solvent systems. In maple seed leachate, on the other hand, the results indicated that the principles which inhibit birch radicle elongation are not only acidic and migrate to specific Rf values on the chromatograms, but that they are very likely phenolic compounds and that they are either more stable, perhaps more plentiful, or both, than the inhibitory principles in maple root leachate.

Phenolic compounds are hydroxyl derivatives of benzene, the latter having a six-carbon aromatic ring. Structurally, they range from the simple phenols such as phloroglucinol to the complex ones such as lignin. Levin (1971) pointed out in a review that phenolics are widespread in plants and that a number of them have been reported as inhibitory to plant growth.

That the active principles in the maple seed leachate are phenolic should not be surprising since it is well known that many plant phenolic compounds are inhibitory (toxic) to the growth of many plants. Rice (1964, 1965, 1968) had consistently pointed to phenolics as the in-

hibitory factors in the growth retardation of many old field plants in Oklahoma. DeBell (1969) identified salicylic acid, a phenolic acid, as the inhibitory principle in Q. falcata crown leachates. Wilson (1968) identified four inhibitory phenolic acids which successfully suppressed germination of Amaranthus retroflexus seeds. Smith (1969), in a review of plant phenolics in allelopathy, listed salicylic, chlorogenic, p-coumaric, fenilic, p-hydroxibenzoic and vanillic acids and scopoletin as some of the phenolic compounds which are known to be toxic to plant growth.

Only four out of the nine phenolic spots from the maple seed leachate chromatogram were inhibitory to birch radicle elongation (Table 21). The physical properties that all nine spots exhibited in the study; namely, the fluorescence under UV light, color reaction to location reagent and the UV maxima absorption values, suggested that they are all phenolic compounds. The combination of characteristics in the nine compounds, however, do not match directly those of published phenolic compounds that are known to be toxic (Smith 1969; Hedin, et. al. 1967). Further study is, therefore, necessary to identify the nine compounds from the maple seed leachate.

The two characteristics of the inhibitors in maple root leachate; namely, that they are acidic and in the IAW solvent system, occur at Rf values .5 and .6, are not sufficient for identification. It is necessary here also to carry out further studies to characterize the inhibitors in maple root leachate.

In Figure 6, a semblance of the ephemeralness of maple root leachate previously reported by Tubbs (1970) is seen in the boiled and fresh maple root leachates kept at room temperature. The other maple root leachates, under refrigeration, were inhibitory to birch radicle elongation at least up to 16 days of storage. The latter observation seemed contradictory to Tubbs' observation of a loss of inhibitory property of maple root leachate after five days storage at 5°C. This apparent contradiction may be due to difference in the concentrations of inhibitors in the maple root leachates at the time of the two studies.

Maple seed leachate showed marked stability in the present study. This may be attributed to the phenolic nature of the inhibitors in maple seed leachate, since plant phenolic compounds are among the most stable plant products (Levin 1971).

CONCLUSIONS

That sugar maple has allelopathic properties was first mentioned by Schreiner and Reed in 1907. This fact was not considered in interpretation of the natural dominance, in the field, of sugar maple over yellow birch until Tubbs (1970) undertook investigations that demonstrated a chemical interaction between the two species. In the series of experiments documented in this thesis, I have attempted to add to the knowledge gleaned by Tubbs for a better understanding of the ecology of northern hardwood forests in which both maple and birch occur.

The problems studied were identified following from Tubbs' work in 1970, in which he demonstrated that sugar maple root leachate was allelopathic to yellow birch. These problems have been investigated in greenhouse and laboratory experiments employing assays with sugar maple root, leaf, litter and seed extracts and leachates on radicle elongation of yellow birch to detect pattern and onset of inhibition. Radioactive tracer and light and moisture stress treatments were used to provide supporting evidence for explaining the pattern of inhibition that emerged. For broad characterization of the compounds in maple root and seed leachates, paper and thin layer chromatography, locating reagents and spectrophotometric analysis were employed.

Results may be summarized as follows:

1) That the results generally confirm the existence of allelopathic effects of sugar maple on yellow birch.

2) That allelopathy by maple root leachates exhibit a seasonal periodicity in their effect on radicle growth of birch germinants.

3) That the periodicity of maple root leachate is related to the stage of maple leaf development, current photosynthesis and translocation and the presence of active roots on maple plants. Both fully expanded, mature leaves and growing roots must be present on maple plants concurrently for the allelopathic effects of maple root leachate to be expressed in the reduction of birch radicle elongation. This stage of maple development occurs only in the summer, during the middle of the growing season.

4) That maple seeds produce leachate with strong al lelopathic properties on yellow birch germinants. That leachate from maple leaves at all stages of development do not exhibit inhibition on birch germinants.

5) That the expression of inhibition by maple root and seed leachate on yellow birch seedlings and

germinants takes the form of poor root development in birch, which if prolonged beyond a few days, can result in overall poor seedling development and possibly death. Maple seed leachate also inhibits birch seed germination and can prevent germination altogether.

6) That for maple root leachate, the inhibitory principle is acidic, and it occurs with organic and amino acids and sugars. For maple seed leachate, the inhibitory factors are acidic and all of them seem to be phenolic compounds. These maple seed leachate inhibitors are apparently stronger and more stable than the inhibitor(s) in maple root leachate. Organic and amino acids also occur with the inhibitors in maple seed leachate.

These findings when related to the timing of birch seed germination and root development of birch germinants, seem of doubtful ecological significance. Sugar maple seed mature in early fall, is dispersed during leaf drop, and germinates in early spring in the underlying litter. Yellow birch seed also matures in early fall, but is dispersed gradually in the winter months, become encrusted in the snow above the maple seed and then germinates during the cool moist conditions of the late spring (Tubbs 1965). Birch tends also to inhabit organic microsites (e.g., logs) and other sites where maple roots and seeds are not impor-

tant factors in initial seedling establishment. These temporal and spatial distributions of birch seeds and birch germinants in relation to inhibiting maple roots and seeds present little or no contact between maple root and seed leachates and birch seed and birch germinants. Thus, maple would not appear to prevent the early stages of birch seedling development in the field.

On the other hand, roots of established birch seedlings and older birch trees which may already have developed roots in soil with maple roots and which may be growing under maple seeds, are quite likely to make contact with maple root and seed leachates. Such a maple leachate-birch root contact can prove detrimental to the growth of the birch. Maple seed leachate is likely to exert a great influence on birch seedlings because of the large quantities of seeds that maple produces, and the stability of maple seed leachate. The limited seasonal production and the less stable nature of maple root leachate renders the major influence of root leachate during the mid-summer growing season. This can be very important since the inherent pattern of growth in yellow birch is one of major shoot and root extension during mid-season, after most of the foliage of sugar maple has expanded fully and is mature (Jacobs 1965).

Tinnin and Muller (1971) attributed dominance of Avena fatua in parts of California to allelopathic influence in a system where the greater the density of inhibiting A. fatua the greater the allelopathic influence on the environment.

This system favors A. fatua but results in exclusion of many other species to sites where their interaction with A. fatua is reduced. The allelopathic effects of sugar maple on yellow birch may be similarly seen as contributing to an evolutionary process which results in differences in the spatial and temporal distribution between the two species. Thus, the process favors sugar maple, the dominant species, to the exclusion of yellow birch from microsites occupied by inhibiting sugar maple.

Our understanding of the allelopathic phenomenon by maple is still quite incomplete. We still do not know whether roots of mature maple trees actually produce leachates which are inhibitory; we do not know how maple leachates may react to microbes or to chemical reactions in soil; and we do not know the identity of the inhibitors produced. The ecological importance of the allelopathic relationship between sugar maple and yellow birch cannot be fully appreciated until we have such information.

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