1979

The Allelopathic Potential of *Rhododendron macrophyllum* in a Western Cascades Clearcut

Ivan W. Clark
Portland State University

AN ABSTRACT OF THE THESIS OF Ivan W. Clark for the Master of Science in Biology presented January 19, 1979.

Title: The Allelopathic Potential of Rhododendron macrophyllum in a Western Cascades Clearcut.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

[redacted]

Robert O. Tinnin, Chairman

[redacted]

Richard B. Forbes

[redacted]

Richard D. Tocher

[redacted]

Larry W. Price

Rhododendron macrophyllum is a dominant species in the shrub stage of secondary succession on burned and logged sites in the Tsuga heterophylla zone of the western Cascades of Oregon. A study was undertaken to determine if R. macrophyllum has the potential to inhibit the germination and growth of surrounding vegetation through water-soluble toxins which are produced in its leaves and leached out of its litter by rainfall.

Aqueous extracts of R. macrophyllum leaf litter significantly reduced germination and radicle growth in Bromus tectorum, Epilobium angustifolium, Picea sitchensis, Pseudotsuga menziesii, and Tsuga heterophylla. Osmotic pressure of the leaf extract solution was determined not to be a significant causative factor in the inhibition observed.

Field studies showed four plant species to increase in frequency of presence with increasing distance from R. macrophyllum drip lines. Two species, both ericaceous, decreased in frequency of presence. Five species increased in density with increasing distance from R. macrophyllum drip lines, while only one species, which was ericaceous, decreased. It is postulated that these patterns result from the allelopathic influence of R. macrophyllum. Members of the Ericaceae are thought to either have a common response to environmental conditions or to be able to tolerate the toxins produced by members of their family.

THE ALLELOPATHIC POTENTIAL OF RHODODENDRON MACROPHYLLUM IN A WESTERN CASCADES CLEARCUT

by

IVAN W. CLARK

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University

1979

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of Ivan W. Clark presented January 19, 1979.

[redacted]

Robert O. Tinnin, Chairman

[redacted]

Richard B. Forbes

[redacted]

Richard D. Tocher

[redacted]

Larry W. Price

APPROVED:

[redacted]

W. Herman Taylor, Jr., Head, Department of Biology

[redacted]

Stanley E. Rauch, Dean of Graduate Studies and Research

ACKNOWLEDGEMENTS

The author would like to extend his appreciation to his major advisor, Dr. Robert O. Tinnin, for his help and advice during the course of this investigation and for his helpful suggestions and criticisms in the preparation of this manuscript. Appreciation is likewise extended to the other members of my committee, Dr. Richard D. Tocher and Dr. Richard B. Forbes, for their advice and criticisms and for the use of their laboratories and equipment during portions of this study.

Appreciation is given also to Dr. Mary L. Taylor for use of her laboratory and equipment during a portion of this study.

Appreciation is given to Dr. Quintin D. Clarkson for his advice concerning the statistical analyses of my data.

Thanks are given to Jim Sweet for his helpful laboratory measurements.

Gratitude is also expressed to the Crown Zellerbach Nursery in Aurora, Oregon for the donation of conifer seeds for use in this study.

Finally, sincere appreciation is extended to Kathryn A. Farr, who had to put up with me throughout the entire process.

TABLE OF CONTENTS

PAGE

ACKNOWLEDGEMENTS iii

LIST OF TABLES vi

LIST OF FIGURES ix

INTRODUCTION 1

METHODS AND MATERIALS

BIOASSAYS 4

Treatments for Individual Species 7

Determination of Concentration of Leaf Extract Solution 8

FIELD STUDY 10

Site Description 10

Methods 10

RESULTS AND DISCUSSION

BIOASSAYS

Effect of Aqueous Rhododendron macrophyllum Leaf Litter Extract on Germination Rates 14

Effect of Aqueous Rhododendron macrophyllum Leaf Litter Extract on Radicle Growth 21

Discussion 24

FIELD STUDY - VEGETATION PATTERNS AROUND RHODODENDRON MACROPHYLLUM PLANTS IN A WESTERN CASCADES CLEARCUT . . 27

Effect of Rhododendron macrophyllum on Species Presence 27

PAGE

Effect of Rhododendron macrophyllum on Individual Species Densities and Total Plant Density . . . 37

SUMMARY AND CONCLUSIONS 49

REFERENCES CITED 51

APPENDIX A - DETERMINATION OF LEAF EXTRACT MOLAR CONCENTRATION FROM FREEZING POINT DEPRESSION 53

APPENDIX B - GERMINATION RATES AND MEAN RADICLE LENGTHS OF TEST SPECIES IN BIOASSAYS 65

APPENDIX C - LITERATURE REVIEW 70

LIST OF TABLES

TABLE		PAGE
I	Germination Rates of Test Species when Treated with Aqueous Rhododendron macrophyllum Leaf Litter Extract	15
II	Germination Rates of Test Species when Treated with Aqueous Solutions of Sucrose or Mannitol	16
III	Germination Rates of Picea sitchensis and Tsuga heterophylla when Treated with Aqueous Solutions of Rhododendron macrophyllum Leaf Litter Extract and Aqueous Solutions of Mannitol	19
IV	Chi-square Analysis of Germination Rates in Tsuga heterophylla and Picea sitchensis bioassays . . .	20
V	Radicle Growth of Test Species when Treated with Aqueous Rhododendron macrophyllum Leaf Extract . .	22
VI	Radicle Growth in Bromus tectorum and Pseudotsuga menziesii when Treated with Aqueous Solutions of Sucrose and Mannitol	23
VII	Radicle Growth in Picea sitchensis and Tsuga heterophylla when treated with Aqueous Rhododendron macrophyllum Leaf Extract and Aqeuous Solutions of Mannitol	25

TABLE		PAGE
VIII	Analysis of Mean Radicle Lengths in *Picea sitchensis* and *Tsuga heterophylla* Bioassays by Student-Newman-Keuls Test	26
IX	Absolute Frequency of Presence of Plant Species Within 97 0.5 M Wide Belt Transects Leading from *Rhododendron macrophyllum* Drip Lines	29
X	Absolute Frequency of Plants Within 97 0.5 M Wide Belt Transects Leading from *Rhododendron macrophyllum* Drip Lines	40
XI	Solutions Used in Freezing Point Depression Experiments	55
XII	Temperature Readings over Time in Freezing Point Depression Experiments	56
XIII	Analysis of Freezing Point Depression by Linear Regression	59
XIV	Calculations of Leaf Extract Molar Concentrations . .	60
XV	Germination Rates and Mean Radicle Lengths of *Bromus tectorum*, *Epilobium angustifolium*, and *Pseudotsuga menziesii* in Leaf Extract Bioassays	66
XVI	Germination Rates and Mean Radicle Lengths of *Bromus tectorum* in Sucrose Bioassays	67
XVII	Germination Rates and Mean Radicle Lengths of *Epilobium angustifolium* and *Pseudotsuga menziesii* in Mannitol Bioassays	68

TABLE PAGE

XVIII Germination Rates and Mean Radicle Lengths of *Picea sitchensis* and *Tsuga heterophylla* in Bioassays with Leaf Extract and Mannitol 69

LIST OF FIGURES

FIGURE		PAGE
1.	Method for Selection of Appropriate Belt Transects in Field Study	12
2.	Frequency of Presence of Plant Species within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines	31
3.	Frequency of Presence of Plant Species within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines	33
4.	Frequency of Presence of Plant Species within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines	35
5.	Frequency of Presence of Plant Species within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines	36
6.	Mean Number of Species Present Per Quadrat within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines	38
7.	Frequency of Quadrats with at Least One Plant Present	29
8.	Mean Density of Plant Species within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines	42

FIGURE PAGE

9. Mean Density of Plant Species within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines 45

10. Mean Density Per Quadrat of All Species Present, Excluding Gaultheria shallon, within 0.5 M Wide Belt Transects Leading from R. macrophyllum Drip Lines 47

11. Cooling Curves for Solutions without Supercooling and with Supercooling 54

12. Cooling Curves for Aqueous Solutions of Extract Used in Bromus tectorum bioassays 61

13. Cooling Curves for Aqueous Solutions of Extract Used in Pseudotsuga menziesii bioassays 62

14. Cooling Curves for Aqueous Solutions of Extract Used in a Preliminary Bioassay 63

15. Cooling Curve for an Aqueous Solution of Mannitol 64

INTRODUCTION

Allelopathy, the chemical inhibition of one plant by another, has been suggested to have an important role in determining the species composition of plant communities (Muller, 1966; Whittaker, 1970). Whittaker and Feeny (1971) have suggested that in plant succession a dominant species may, by allelopathic suppression, delay its replacement by invaders and limit the number of species able to occur with it. This has been shown by many workers to be a major mechanism affecting species composition and plant succession in shrublands in semi-arid environments (Muller, Hanawalt, and McPherson, 1968; McPherson and Muller, 1969; Hanawalt, 1971; Chou and Muller, 1972; Halligan, 1973, 1976; Christianson and Muller, 1975a, 1975b). These studies have shown both volatile and water-soluble inhibitors to be effective in controlling vegetation in semi-arid environments. Less evidence is available for the allelopathic control of vegetation by dominant shrub species in humid environments. Although volatile inhibitors are known to be present in plants from humid environments (del Moral and Cates, 1971), their effect in the field in these environments has been questioned (Muller and del Moral, 1966; Muller, 1970). Muller (1970) and Whittaker (1970) have suggested that phytotoxins produced by plants are waste products and that their method of release has evolved as a response to environmental conditions. Whereas volatile phytotoxins are more common in arid environments, water-soluble toxins (released by leaching) are more prevalent in humid environments.

Several Ericaceous shrubs from both arid and humid environments have been shown to be inhibitory to a number of species. Del Moral and Cates (1971) showed aqueous leaf and litter extracts of Arbutus menziesii and Rhododendron albiflorum to be inhibitory to Bromus tectorum and Pseudotsuga menziesii seeds in laboratory bioassays. In associated field studies these two inhibitory plants were also shown to reduce subordinate vegetation. Muller, Hanawalt, and McPherson (1968) showed that water-soluble toxins released from the crowns and leaf litter of Arctostaphylos glandulosa and A. glauca were inhibitory to a number of chaparral species in southern California. Fire or artificial removal of the shrubs resulted in increased growth of underlying herbs and shrub seedlings until shrub dominance was re-established (Muller, Hanawalt, and McPherson, 1968; Chou and Muller, 1972).

Rhododendron macrophyllum G. Don (Ericaceae) is a common understory shrub on moderately dry sites in Tsuga heterophylla and Pseudotsuga menziesii forests in the Tsuga heterophylla zone (Franklin and Dyrness, 1973) of the western Cascades of Oregon. It is commonly the dominant species in the shrub stage of secondary succession on burned-over and logged sites (Franklin and Dyrness, 1973; Dyrness, 1973). It may often be present in these cleared areas as a residual species which has regrown from surviving roots (Dyrness, 1973). As such it has the potential to have a large impact, through both competetion and allelopathy, on slower growing or later arriving species and ultimately on the rate of regrowth of conifer species and re-establishment of the forest.

The purposes of this study were to determine if Rhododendron

macrophyllum has the potential to inhibit the growth of other species through the production of water-soluble toxins which are leached out of its litter by rainfall, and to determine if this potential is realized in the field. The study was therefore composed of two part: 1) a series of bioassays to determine the presence and activity of water-soluble phytotoxins in R. macrophyllum leaf litter, and 2) a field study to describe vegetational patterns associated with R. macrophyllum in a western Cascades clearcut.

METHODS AND MATERIALS

BIOASSAYS

Five species were tested for inhibition of germination and reduction in radicle growth when treated with aqueous Rhododendron macrophyllum leaf extract: Bromus tectorum L. (cheatgrass), Epilobium angustifolium L. (fireweed), Picea sitchensis (Bong.) Carr. (Sitka spruce), Pseudotsuga menziesii (Mirbel) Franko (Douglas-fir), and Tsuga heterophylla (Raf.) Sarg. (western hemlock). (Nomenclature used here follows Hitchcock and Cronquist (1973).) E. angustifolium, P. menziesii, and T. heterophylla are found on western Cascades sites where R. macrophyllum occurs. P. sitchensis is found in a narrow strip along the Oregon and Washington coast where R. macrophyllum is also found. B. tectorum is a very common grass found east of the Cascades, not typically in association with R. macrophyllum. It was chosen for use in bioassays as a consequence of its high germination rate and convenient seed size.

B. tectorum seeds were obtained from the collection of Dr. Robert Tinnin at Portland State University. I collected E. angustifolium seeds in October 1976 from my field study site in the Clackamas River Drainage (described and discussed later in this manuscript). Conifer seeds were donated by the Crown Zellerbach nursery in Aurora, Oregon, where they had been stratified for several months. The conifer seeds were kept in a freezer at Portland State University until use.

Recently fallen R. macrophyllum leaves were collected in October 1975 before the winter rains had begun in the Mt. Hood National Forest near Rhododendron, Oregon. The leaves were allowed to air-dry in the laboratory for several days before use.

In preparation for each bioassay, dried leaves were ground to a powder in a Waring blender. The extract was prepared in two batches. In each batch 50.0 g of ground leaf material was mixed with 500 ml of distilled water and shaken for 30 minutes. The two batches were then combined and filtered through Whatman #1 filter paper. The yield after filtration was approximately 800 ml.

The bioassay seed bed consisted of steam-washed white sand which was rinsed three times in distilled water and dried in an oven at 100° C for 24 hours prior to use. A weighed amount of sand (see individual species treatments for exact amounts) was placed in each of several 9 cm diameter glass petri dishes and moistened with a measured volume of leaf extract solution. Sand in control dishes was moistened with distilled water. Seeds were placed on the sand or on filter paper (Whatman #1) placed on the sand. In the E. angustifolium, P. sitchensis, and T. heterophylla bioassays, sand in a third set of dishes was moistened with R. macrophyllum leaf extract which had been diluted 1:1 with distilled water. This solution is hereafter referred to as half-strength extract. Except for E. angustifolium, seeds were soaked for two hours in their respective test solutions prior to being placed on the sand or filter paper. Dishes were sealed with Parafilm and placed in a growth chamber for various lengths of time, depending on the species. Similarly, temperature and photoperiod were varied according

to the germination requirements of each species. Temperatures and photoperiods used in the conifer species bioassays were taken from those cited in U.S.D.A. Forest Service Agricultural Handbook No. 450 (1974).

Since inhibition of germination and growth could be caused by osmotic pressure effects of the leaf extract solutions, additional bioassays were conducted in which the sand in a series of dishes was moistened with aqueous solutions of sucrose (in the case of Bromus tectorum) or mannitol (in the cases of the other test species) of various concentrations, in order to determine the effects of concentration on germination and radicle growth. Concentrations of sucrose and mannitol used were 0.01, 0.02, 0.05, 0.10, 0.15, and 0.20 moles-liter^{-1} and 0.25 moles-liter^{-1} in the case of E. angustifolium. Sand in control dishes was moistened with distilled water. Dishes and seeds in these bioassays were prepared exactly like those in the leaf extract bioassays. The mannitol bioassays were run concurrently with the leaf extract bioassays in the tests conducted on P. sitchensis and T. heterophylla. In the cases of B. tectorum, E. angustifolium and P. menziesii, the sucrose and mannitol bioassays were conducted subsequent to the leaf extract bioassays.

At the end of each test period, the dishes were removed from the growth chamber for counting germinated seeds and measuring radicle lengths. Germination rates were analyzed by the chi-square test. Mean radicle lengths were analyzed by the t-test or by the Student-Newman-Keuls test, depending on the number of means being compared (Sokal and Rohlf, 1969).

Treatments for Individual Species

Bromus tectorum. Each dish contained 40.0 g of sand moistened with 11.0 ml of solution. Ten seeds were evenly placed on the sand in each dish. Six dishes were used for each sucrose treatment. Dishes were placed in a growth chamber for 48 hours at 25° C in complete darkness.

Epilobium angustifolium. Each dish contained 40.0 g of sand moistened with 13.0 ml of solution. Whatman #1 filter paper was placed on the sand. Forty seeds, from which the pappi had been removed, were then placed on the moist filter paper in each dish. The pappi were removed by rubbing the seeds on a No. 20 U.S.T.A. Standard Testing Sieve. Ten dishes were used for each of the water and extract treatments and four dishes were used for each of the mannitol treatments. The dishes were placed in a growth chamber for 72 hours at 25.5° C in complete darkness.

Picea sitchensis. Each dish contained 50.0 g of sand moistened with 15.0 ml of solution. Whatman #1 filter paper was placed on the sand. Twenty-five seeds were placed on the filter paper in each dish. Ten dishes were used for each of the water and extract treatments and four dishes were used for each of the mannitol treatments. Dishes were placed in a growth chamber for 14 days with a photoperiod of 8 hours light at 30° C and 16 hours darkness at 20° C. Both incandescent and fluorescent lights were used.

Pseudotsuga menziesii. Each dish contained 40.0 g of sand moistened with 12.0 ml of solution. Twenty seeds were placed on the sand in each dish. Ten dishes were used for each of the water and

extract treatments and four dishes were used for each of the mannitol treatments. Dishes were placed in a growth chamber for 12 days with a photoperiod of 8 hours light at 30° C and 16 hours darkness at 20° C. Both incandescent and fluorescent lights were used in the growth chamber.

Tsuga heterophylla. Each dish contained 50.0 g of sand moistened with 15.0 ml of solution. Whatman #1 filter paper was placed on the sand. Twenty-five seeds were uniformly placed on the filter paper in each dish. Ten dishes were used for each of the water and extract treatments and four dishes were used for each of the mannitol treatments. Dishes were placed in a growth chamber for 18 days with a photoperiod of 16 hours light at 20° C and 8 hours darkness at 20° C. Fluorescent lights only were used.

Determination of Concentration of Leaf Extract Solution

In order to determine the effects of the osmotic pressure of the leaf extract solution on germination and radicle growth, the concentration of the aqueous extract solution was determined. Freezing point depresssion is a colligitive property of solutions. The amount by which the freezing point of a solution is lowered is approximately proportional to the number of moles of solute dissolved in a given amount of solvent (Shoemaker and Garland, 1967). The chemical composition of the aqueous Rhododendron macrophyllum leaf extract used in the bioassays was not known, but since it was most likely a mixture of substances, it was assumed that the freezing point depression caused by dissolving a weighed amount of dry solute in a measured amount of water

would be a reflection of the average molecular weight of the substances present in the solute.

A measured volume of leaf extract solution was first flash-evaporated until it had the consistency of a thin syrup, then freeze-dried to dryness. The amount of dry solute obtained was weighed. This dry solute was then used in the freezing point depression experiments.

The procedure used for determining molecular weight from freezing point depression was adapted from Shoemaker and Garland (1967). A Dewar flask was used for the cooling bath containing an ice-salt mixture. Placed in the ice-salt bath was a large test tube (25 x 195 mm) held in place by a fitted piece of cellulose sponge. Held inside this test tube was another, smaller test tube (18 x 150 mm) into which was introduced the aqueous leaf extract solution. Fiberglass insulation was added to the air space between the test tubes to provide additional insulation to insure a low and uniform rate of cooling of the solution. A Beckman thermometer with graduations of 0.01° C was used. The thermometer was calibrated by establishing cooling curves for distilled water before and after each test was made.

A weighed amount of dry solute was added to a measured volume of distilled water (see Appendix A for exact amounts). When the temperature of the mixture approached 0° C, temperature readings were taken every 30 seconds while the extract solution was constantly stirred with a stainless steel stirrer. From the cooling curves so obtained, the freezing point of the mixture was determined. As a control, additional freezing point determinations were made, using a known solute (mannitol) of a known molecular weight. Once the freezing point depression was

known, molecular weight could be calculated from the following formula (Shoemaker and Garland, 1967):

$$\text{Molecular weight} = 1000 \times \frac{\text{g solute}}{\text{g solvent}} \times \frac{K_f}{\Delta T_f}$$

K_f = molal freezing point depression constant (1.855 deg-mole^{-1} for water)

ΔT_f = Difference between freezing point of pure solvent and depressed freezing point of test solution

FIELD STUDY

Site Description

The study site selected was clearcut in 1967 and was located in the Clackamas River drainage in the Mt. Hood National Forest (U.S. Forest Service, Tri-compartment 3203, Sale No. 42C2). It was located on a north facing slope at an elevation of 975 m in the western Cascades (Section 11, R7E, 6S, Willamette Meridian). The site was logged by tractor and subsequently burned. It was replanted in May 1971 with 13,000 trees, approximately half of which were three year old Pseudotsuga menziesii and half of which were four year old Abies procera. The adjacent forest is predominantly Tsuga heterophylla and Pseudotsuga menziesii. Rhododendron macrophyllum is the dominant shrub species in both the clearcut area and in the adjacent forest.

Methods

The study was conducted over a two month period - June 15, 1977 to August 15, 1977. The site was divided into 900 10 m x 10 m quadrats, from which 20 random quadrats were selected. Within each quadrat, density of each species present was measured as a function of distance

from the drip line of the nearest Rhododendron macrophyllum plant.

A 0.5 m x 1.5 m belt transect was laid out in four directions (corresponding to the grid lines of the study site) from the drip lines of all R. macrophyllum plants within the quadrat. In order to avoid the additive effects of two R. macrophyllum plants, a belt transect was judged suitable only if no other R. macrophyllum plant was located within 3.0 meters of the drip line of the R. macrophyllum plant in question in the semicircle bisected by the midline of the transect (see Figure 1). A total of 97 such belt transects were analyzed.

Each belt transect was divided along its length into fifteen 10 cm x 50 cm quadrats. Within each of these smaller quadrats, all plants of every species were counted. Where it was impossible to determine the exact number of individual plants of a particular species which were present, either the number of aerial shoots was counted (Epilobium angustifolium and Anaphalis margaritacea) or merely presence or absence was noted (Gaultheria shallon). The several Rubus species and Vaccinium species were classified simply as Rubus spp. and Vaccinium spp.. Since three-year-old Pseudotsuga menziesii and four-year-old Abies procera were planted at this site in 1971, it was arbitrarily decided to count only those individuals of these two species which were less than one meter tall. Results were tabulated to see if individual species densities increased with increasing distance from R. macrophyllum drip lines and if the number of species present increased similarly. In analyzing the results, linear regression was used to see if there was a significant linear relationship between distance from drip line and the following variables: species presence or absence, mean number

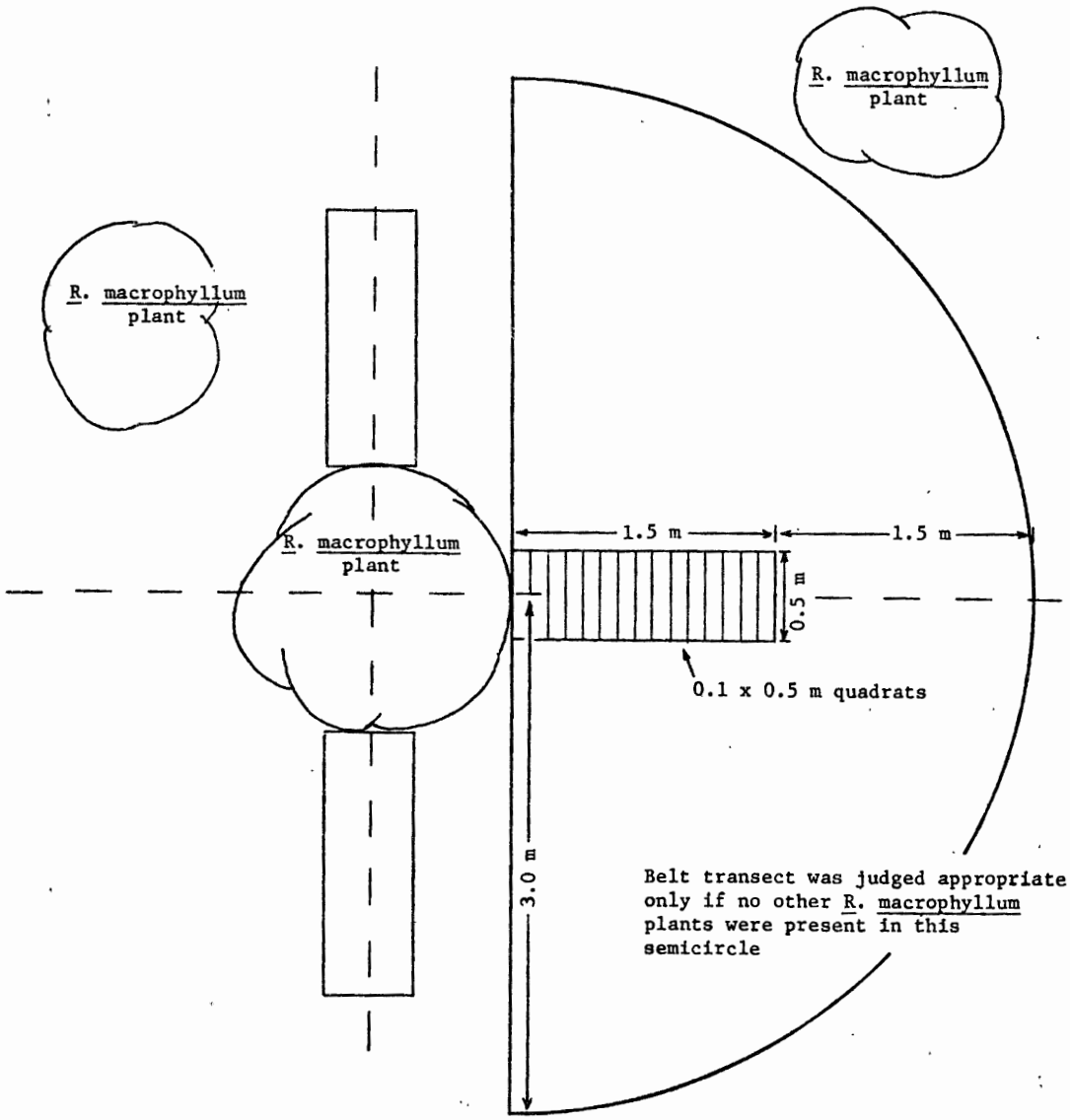

Figure 1. Method for selection of appropriate belt transects in field study.

of species present, vegetation presence or absence, individual species densities, and total plant density. Since distance from drip line was not measured as a continuous variable but in discrete units, the distance used for each quadrat was the farthest point of that quadrat from the drip line (e.g., the distance used for the 0.1 to 0.2 m quadrat was 0.2 m). Regressions were based on total observations, or their means, for the 97 belt transects.

RESULTS AND DISCUSSION

BIOASSAYS

Effect of Aqueous Rhododendron macrophyllum Leaf Litter Extract on Germination Rates

Aqueous extracts of dried Rhododendron macrophyllum leaf litter, when applied to the sand in the germination dishes, significantly reduced germination rates (0.05 confidence level) in Bromus tectorum, Epilobium angustifolium, Picea sitchenis, Pseudotsuga menziesii, and Tsuga heterophylla when compared with the effects of water (Table I). When diluted to half-strength with distilled water, the aqueous extract significantly reduced germination in E. angustifolium and P. sitchensis when compared with water.

The concentration of the leaf litter extract was determined for three preparations: those used for the B. tectorum and P. menziesii bioassays, and a third which was used in a preliminary bioassay not reported here. In all three cases the concentration was determined to be approximately 0.05 Molar (see Appendix A). In all other bioassays the concentration of the extract solution was assumed to be approximately this same concentration, i.e., 0.05 M.

When sucrose was added to the Petri dishes in the B. tectorum bioassays, concentrations up to 0.20 M had no significant effect on germination (Table II). Similarly, when mannitol was added to the P. menziesii dishes, concentrations up to 0.20 M did not significantly

TABLE I

GERMINATION RATES OF TEST SPECIES WHEN TREATED WITH AQUEOUS *RHODODENDRON MACROPHYLLUM* LEAF LITTER EXTRACT, EXPRESSED AS PERCENT OF CONTROL

Species	Treatment	
	Extract	½ Strength Extract
Bromus tectorum	67[a]	. . .[b]
Epilobium angustifolium	77[a]	83[a]
Picea sitchensis	60[a]	80[a]
Pseudotsuga menziesii	3[a]	. . .[b]
Tsuga heterophylla	56[a]	90[a]

[a]Significantly different from controls at 0.05 confidence level or better as determined by the chi-square test.

[b]Not tested.

TABLE II

GERMINATION RATES OF TEST SPECIES WHEN TREATED WITH AQUEOUS SOLUTIONS OF SUCROSE[a] OR MANNITOL[b], EXPRESSED AS PERCENT OF CONTROL

Species	Concentration, moles-liter^{-1}						
	0.01	0.02	0.05	0.10	0.15	0.20	0.25
Bromus tectorum[a]	103	101	103	103	96	98	. . .[c]
Epilobium angustifolium b	110	105	90	94	77[d]	62[d]	27[d]
Pseudotsuga menziesii[b]	102	100	100	98	98	82	. . .[c]

[a]*Bromus tectorum* seeds were treated with sucrose.

[b]*E. angustifolium* and *P. menziesii* seeds were treated with mannitol.

[c]Not tested.

[d]Significantly different from controls at 0.05 confidence level or better as determined by the chi-square test.

reduce germination when analyzed by the chi-square test in a 2 x 7 contingency table (Table II). It should be noted, however, that 0.20 M mannitol may have an inhibitory effect on germination of P. menziesii. When compared with all other concentrations lumped together in a 2 x 2 contingency table, 0.20 M mannitol did significantly reduce germination in P. menziesii. When compared with water alone, there was no significant difference.

Although mannitol concentrations of 0.15 M, 0.20 M, and 0.25 M reduced germination in E. angustifolium, concentrations below 0.15 M did not significantly reduce germination (Table II). As with B. tectorum and P. menziesii, the leaf litter extract bioassays were not run concurrently with the mannitol (or sucrose) bioassays, so direct analyses of results cannot be made. However, certain conclusions can be drawn from a comparison of the two experiments. The 0.05 M mannitol reduced germination of E. angustifolium slightly (90% of control), but not significantly at the 0.05 confidence level. On the other hand, the leaf litter extract, which was assumed to be approximately 0.05 M, reduced germination significantly at the 0.001 confidence level (77% of control). When the extract was diluted with distilled water to half-strength, the reduced germination rate (83% of control) was also significantly different from controls at the 0.001 confidence level. From the above results it appears that although germination of E. angustifolium can be inhibited by concentration effects at concentrations above 0.10 M, the inhibition observed in the extract treated seeds of B. tectorum, P. menziesii, and E. angustifolium can be attributed to phytotoxic effects of the extract rather than to concentration effects.

Increasing concentrations of mannitol tended to reduce germination in Tsuga heterophylla, but there was a significant reduction (0.05 confidence level) only at concentrations of 0.15 m or greater (Table III). When compared with the 0.05 M mannitol and 0.02 M mannitol, the leaf litter extract and half-strength extract, respectively, reduced germination significantly (0.05 confidence level or better; Table IV). Correspondingly, germination rates of seeds treated with 0.02 M and 0.05 M mannitol did not differ significantly from germination rates of seeds treated with water.

In the Picea sitchensis bioassays, mannitol concentrations of 0.05 M or greater significantly reduced germination when compared with water (Table III). However, the leaf litter extract significantly reduced germination both when compared with water (0.001 confidence level) and when compared with 0.05 M mannitol (0.01 confidence level; Table IV). Although the half-strength extract reduced germination significantly when compared to water, when compared with 0.02 M mannitol (of presumed slightly lower concentration than the half-strength extract), there was no significant difference between germination rates (at the 0.05 confidence level). However, these effects can be arranged on a continuum, so exact interpretation may be difficult, i.e., germination with 0.02 M mannitol was not significantly different from that with water or half-strength extract, but germination with water was significantly different from that with half-strength extract (0.001 confidence level). It appears that at lower concentrations (0.025 M) the inhibitory effects of the extract on germination of P. sitchensis cannot be separated from the concentration effects, but at concentrations

TABLE III

GERMINATION RATES OF *PICEA SITCHENSIS* AND *TSUGA HETEROPHYLLA* WHEN TREATED WITH AQUEOUS SOLUTIONS OF *RHODODENDRON MACROPHYLLUM* LEAF LITTER EXTRACT AND AQUEOUS SOLUTIONS OF MANNITOL, EXPRESSED AS PERCENT OF CONTROL

Species	Treatment							
	Extract	½ Strength Extract	Mannitol, moles-liter^{-1}					
			0.01	0.02	0.05	0.10	0.15	0.20
Picea sitchensis	60[a]	80[a]	93	90	80[a]	69[a]	78[a]	43[a]
Tsuga heterophylla	56[a]	90	95	106	87	87	57[a]	61[a]

[a]Significantly different from controls at 0.05 confidence level or better as determined by the chi-square test.

TABLE IV

CHI-SQUARE ANALYSIS OF GERMINATION RATES IN *TSUGA HETEROPHYLLA* AND *PICEA SITCHENSIS* BIOASSAYS

Treatment Contrast	Confidence level for difference observed	
	T. heterophylla	*P. sitchensis*
Extract vs. water	0.001	0.001
Extract vs. 0.05 M mannitol	0.001	0.01
Water vs. 0.05 M mannitol	ns	0.005
½ Strength Extract vs. water	ns	0.001
½ Strength Extract vs. 0.02 M mannitol	0.05	ns
Water vs. 0.02 M mannitol	ns	ns

of 0.05 M, the toxic effects of the extract can be seen to be significantly greater than the concentration effects.

Effect of Aqueous Rhododendron macrophyllum Leaf Litter Extract on Radicle Growth

Aqueous extracts of dried Rhododendron macroplhyllum leaf litter, when added to the germination dishes, significantly reduced radicle growth (0.05 confidence level) in Bromus tectorum, Picea sitchensis, Pseudotsuga menziesii, and Tsuga heterophylla when compared with controls (Table V). Similarly, when diluted to half-strength with distilled water, the aqueous extract significantly reduced radicle growth in P. sitchensis and T. heterophylla when compared with controls. Radicles of Epilobium angustifolium were not measured due to their minute size.

When sucrose was added to the B. tectorum dishes, mean radicle length tended to decrease with increasing concentration (Table VI). Radicles from seeds treated with sucrose concentrations of 0.05 M or greater were significantly smaller than those from seeds treated with water. However, the reduction in radicle growth caused by the 0.05 M sucrose in the concentration bioassays (radicle length 72% of controls) was not as great as the reduction in radicle growth caused by the 0.05 M extract solution (radicle length 33% of controls).

When mannitol was added to the P. menziesii dishes, mean radicle length tended to decrease with increasing mannitol concentration (Table VI), although only those seeds which were grown in mannitol concentrations of 0.15 M or greater produced radicles significantly smaller than those from seeds grown in water. Here also the reduction in radicle

TABLE V

RADICLE GROWTH OF TEST SPECIES WHEN TREATED WITH AQUEOUS *RHODODENDRON MACROPHYLLUM* LEAF EXTRACT, EXPRESSED AS PERCENT OF CONTROL

Species	Treatment	
	Extract	½ Strength Extract
Bromus tectorum	33[a]	. . .[c]
Picea sitchensis	31[b]	48[b]
Pseudotsuga menziesii	11[a]	. . .[c]
Tsuga heterophylla	56[b]	85[b]

[a]Significantly different from controls at 0.001 confidence level as determined by the t-test.

[b]Significantly different from controls at the 0.05 confidence level as determined by the Student-Newman-Keuls test.

[c]Not tested.

TABLE VI

RADICLE GROWTH IN *BROMUS TECTORUM* AND *PSEUDOTSUGA MENZIESII* WHEN TREATED WITH AQUEOUS SOLUTIONS OF SUCROSE[a] AND MANNITOL,[b] EXPRESSED AS PERCENT OF CONTROL

Species	Concentration, moles-liter^{-1}					
	0.01	0.02	0.05	0.10	0.15	0.20
Bromus tectorum[a]	84	93	72[c]	65[c]	51[c]	22[c]
Pseudotsuga menziesii[b]	116	100	96	81	75[c]	55[c]

[a]*Bromus tectorum* seeds were treated with sucrose.

[b]*Pseudotsuga menziesii* seeds were treated with mannitol.

[c]Significantly different from controls at the 0.05 confidence level as determined by the Student-Newman-Keuls test.

growth caused by the 0.05 M mannitol (radicle length 96% of controls) was not as great as the reduction caused by the 0.05 M extract solution (radicle length 11% of controls), suggesting that most, if not all, of the inhibition observed in the extract treated seeds of B. tectorum and P. menziesii was due to a toxic component of the extract rather than to concentration effects.

In the T. heterophylla and P. sitchensis bioassays, in which the mannitol and leaf litter extract bioassays were conducted simultaneously, mean radicle lengths tended to decrease with increasing mannitol concentrations (Table VII). Radicles from T. heterophylla and P. sitchensis seeds treated with mannitol concentrations of 0.05 M or greater were significantly smaller than those from seeds treated with water (Tables VII, VIII). Radicles from extract treated seeds, however, were significantly smaller than those from 0.05 M mannitol treated seeds. These results indicate that although concentration effects can reduce radicle growth in T. heterophylla and P. sitchensis, the reduction in radicle growth seen in the seeds treated with the leaf litter extract and half-strength extract must be largely attributed to toxic components of the extract

Discussion

Analysis of germination rates and mean radicle lengths of all five test species suggests that although concentration can have inhibitory effects on both germination and radicle growth in laboratory bioassays, concentration alone cannot account for the reduction in germination and radicle growth seen in the seeds treated with aqueous

TABLE VII

RADICLE GROWTH IN PICEA SITCHENSIS AND TSUGA HETEROPHYLLA WHEN TREATED WITH AQUEOUS RHODODENDRON MACROPHYLLUM LEAF EXTRACT AND AQUEOUS SOLUTIONS OF MANNITOL, EXPRESSED AS PERCENT OF CONTROL

Species	Treatment							
	Extract	½ Strength Extract	Mannitol, moles-liter^{-1}					
			0.01	0.02	0.05	0.10	0.15	0.20
Picea sitchensis	31[a]	48[a]	96	93	60[a]	58[a]	43[a]	28[a]
Tsuga heterophylla	56[a]	85[a]	122[a]	98	73[a]	75[a]	42[a]	43[a]

[a]Significantly different from controls at the 0.05 confidence level as determined by the Student-Newman-Keuls test.

TABLE VIII

ANALYSIS OF MEAN RADICLE LENGTHS IN *PICEA SITCHENSIS* AND *TSUGA HETEROPHYLLA* BIOASSAYS BY STUDENT-NEWMAN-KEULS TEST

Species	Ranked Treatment Means		Student-Newman-Keuls Analysis[a]
	Treatment	Mean, mm	
Tsuga heterophylla	0.01 M mannitol	11.3	
	Water	9.3	
	0.02 M mannitol	9.1	
	½ strength extract	7.9	
	0.10 M mannitol	7.0	
	0.05 M mannitol	6.8	
	Extract	5.2	
	0.20 M mannitol	4.0	
	0.15 M mannitol	3.9	
Picea sitchensis	Water	30.1	
	0.01 M mannitol	28.8	
	0.02 M mannitol	28.1	
	0.05 M mannitol	18.2	
	0.10 M mannitol	17.6	
	½ strength extract	14.3	
	0.15 M mannitol	12.8	
	Extract	9.3	
	0.20 M mannitol	8.4	

[a]Any two means which do not fall within the same range indicated by a vertical bar to the right of the treatment means were determined to be significantly different at the 0.05 confidence level by the Student-Newman-Keuls test.

extracts of Rhododendron macrophyllum leaf litter. This would indicate the existence of a toxic component in the extract which is responsible for the inhibition seen.

The mechanism of reduction in germination and radicle growth was not studied. However, it was observed that in those seeds that did germinate in the extract treatments, the root tips were often necrotic and with few root hairs, compared to control seedlings. In addition, many of the non-germinated seeds in the extract treated dishes supported heavy fungal growths. Fungal growths were seldom found in the control, mannitol, or sucrose treated dishes. It is possible then that, rather than having a direct allelopathic effect, the extract could serve as a nutrient source for fungi or other microorganisms which could attack the seeds or produce phytotoxic substances themselves.

FIELD STUDY - VEGETATION PATTERNS AROUND RHODODENDRON MACROPHYLLUM PLANTS IN A WESTERN CASCADES CLEARCUT

In the 97 belt transects which were analyzed, 25 plant species (some not specifically identified), or species-groups, were encountered. The effect of Rhododendron macrophyllum on the distribution of these species was broken down into two components: a) the effect on species presence or absence, and b) the effect on individual species densities and on total plant density.

Effect of Rhododendron macrophyllum on Species Presence

Several species appeared to show definite patterns of distribution when frequency of presence was measured as a function of distance

from R. macrophyllum drip lines. When analyzed by linear regression, four species showed significant positive correlation coefficients (0.05 confidence level) when total frequency per quadrat was regressed on distance from R. macrophyllum drip line (Table IX): Anaphalis margaritacea, Epilobium angustifolium, Rubus spp., and grass species 'B' (Figure 2). Two species had significant negative correlation coefficients: Arctostaphylos uva-ursi and Gaultheria shallon (Figure 3). The remaining species encountered either had non-significant correlation coefficients (11 species) or occurred too infrequently to reasonably subject to statistical analysis (8 species).

The correlation coefficient of grass species 'B' may be misleading, as this species occurred only in two belt transects, located in the same somewhat moist area. The grass was quite abundant here and may have been responding more to moisture than to any relationship with R. macrophyllum. Grass species 'B' did, however, increase greatly in density with increasing distance from R. macrophyllum drip lines within these two transects (see Figure 8e in next section). A. margaritacea, E. angustifolium, and Rubus spp. each showed a significant linear relationship between frequency of presence and distance from R. macrophyllum drip lines, suggesting the existence of some sort of interaction, be it competition or allelopathy, with R. macrophyllum.

Linear regression, of course, only shows whether there is a linear relationship between two variables. The absence of a significant linear relationship does not necessarily rule out a non-linear interaction between variables. Three species - Gnaphalium microcephalum, Hieracium albiflorum, and grass species 'A' - increased in frequency

TABLE IX

ABSOLUTE FREQUENCY OF PRESENCE OF PLANT SPECIES WITHIN 97 0.5 M WIDE BELT TRANSECTS LEADING FROM *RHODODENDRON MACROPHYLLUM* DRIP LINES

Species	Distance from drip line (m)															r^a
	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	
Abies procera	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	-0.2700
Anaphalis margaritacea	3	4	3	6	8	7	7	7	9	7	4	8	6	9	10	0.6680[b]
Arctostaphylos uva-ursi	2	1	0	0	1	1	1	1	1	1	0	0	0	0	0	-0.5808[b]
Berberis nervosa	2	1	3	3	1	2	3	1	2	1	2	1	1	2	1	-0.3799
Castanopsis chrysophylla	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	. . .
Chimaphila umbellata	0	1	1	1	2	0	1	1	1	1	1	1	2	1	0	0.1076
Epilobium angustifolium	17	17	16	20	15	15	18	21	18	23	21	19	19	26	21	0.6684[b]
Epilobium minutum	2	2	1	2	2	1	2	1	2	2	3	2	1	2	1	-0.0538
Fern sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	. . .
Gaultheria shallon	15	10	14	13	16	11	11	9	7	7	8	8	10	10	11	-0.5944[b]
Gnaphalium microcephalum	0	1	2	3	1	1	3	4	1	0	1	2	3	3	2	0.3043
Hieracium albiflorum	7	3	9	7	10	7	7	10	11	5	7	6	8	5	9	0.0590
Linnaea borealis	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	. . .
Pachistima myrsinites	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	. . .

TABLE IX--Continued

Species	Distance from drip line (m)															r^a
	0-0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0	1.0-1.1	1.1-1.2	1.2-1.3	1.3-1.4	1.4-1.5	
Pseudotsuga menziesii	0	0	2	0	4	1	0	1	1	2	0	0	0	2	1	0.0
Rubus spp.	1	0	0	1	1	1	0	2	2	1	1	2	4	1	2	0.6031[b]
Senecio sp.	1	1	0	1	2	0	0	2	0	0	0	0	1	0	1	-0.2385
Tsuga heterophylla	1	1	0	1	0	0	0	1	1	0	0	0	1	2	0	0.0250
Vaccinium spp.	6	2	4	3	2	3	4	6	5	3	3	5	3	3	5	0.0726
Xerophyllum tenax	1	1	2	2	0	0	0	1	1	0	0	0	0	2	0	-0.3521
Unidentified grass sp. 'A'	2	3	3	4	3	4	6	3	3	3	3	4	3	4	4	0.2617
Unidentified grass sp. 'B'	1	1	0	1	2	2	2	2	2	2	2	2	2	2	2	. . .
Unidentified grass sp. 'C'	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.6987[b]
Unidentified herb sp. 'A'	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	. . .
Unidentified herb sp. 'B'	0	0	1	0	0	0	0	0	0	1	0	2	1	0	0	. . .
Mean number of species per quadrat	0.660	0.526	0.639	0.701	0.722	0.577	0.680	0.763	0.701	0.619	0.577	0.649	0.670	0.763	0.753	0.3800

[a]Correlation coefficient for regression of absolute frequency of presence on distance from drip line.

[b]Significant at 0.05 confidence level.

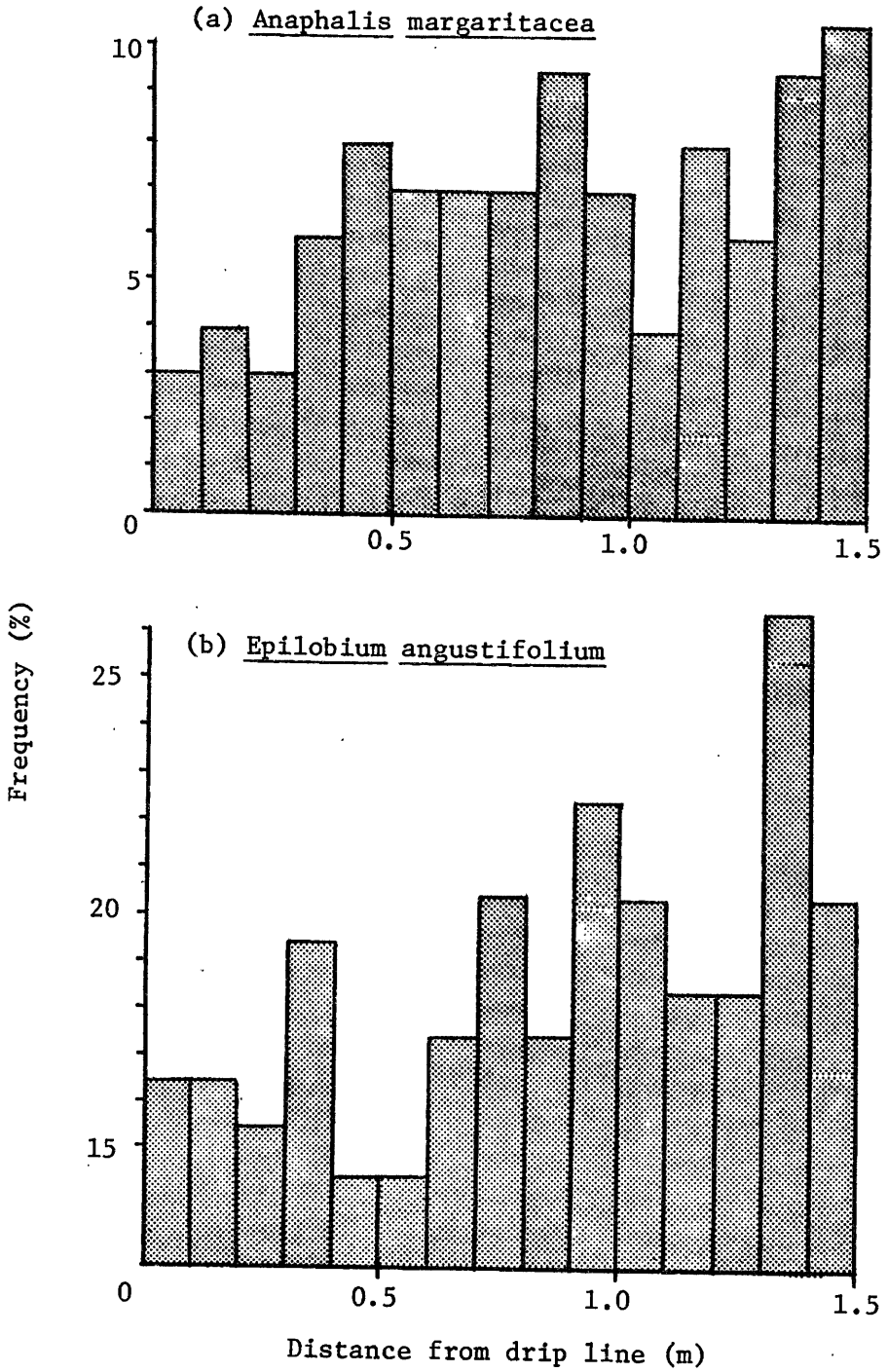

Figure 2. Frequency of presence of plant species within 0.5 m wide belt transects leading from R. macrophyllum drip lines. Expressed as the percent of quadrats in which the species was present.

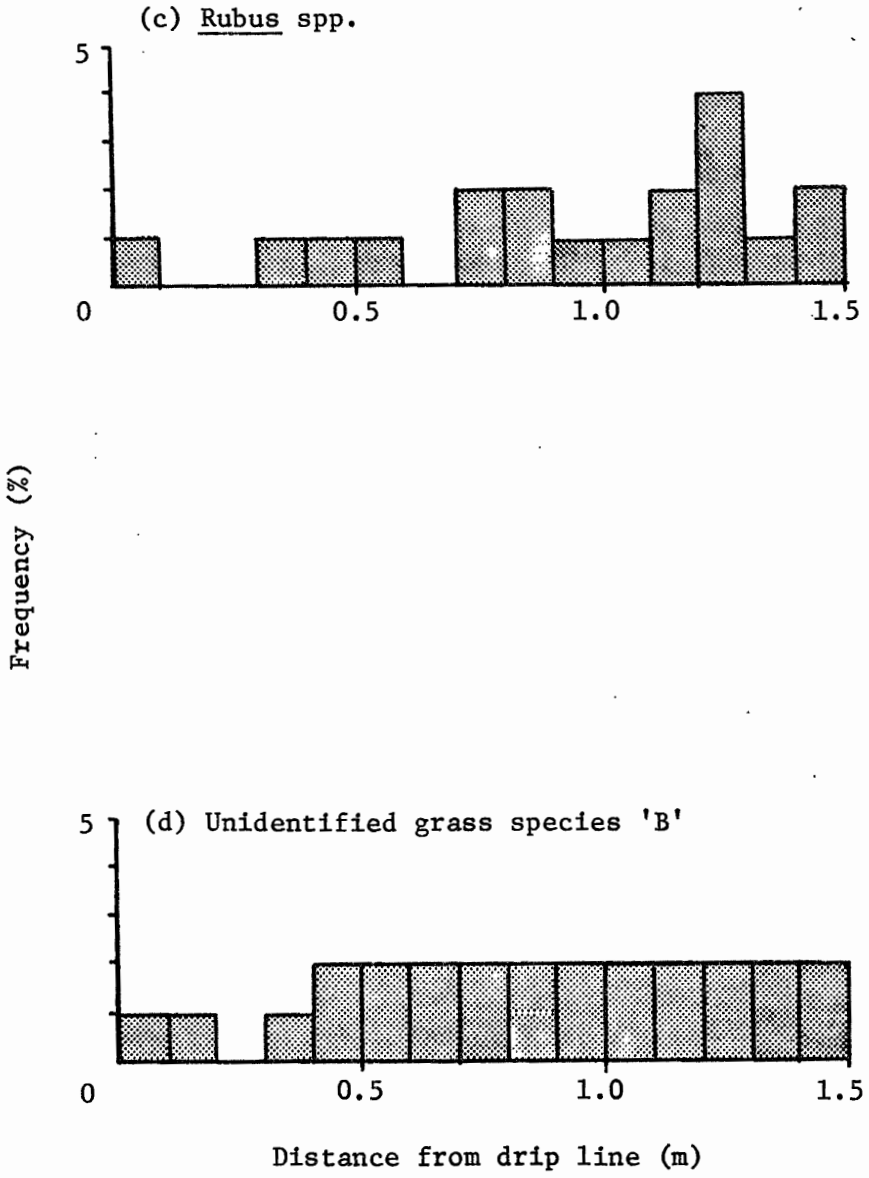

Figure 2.--Continued

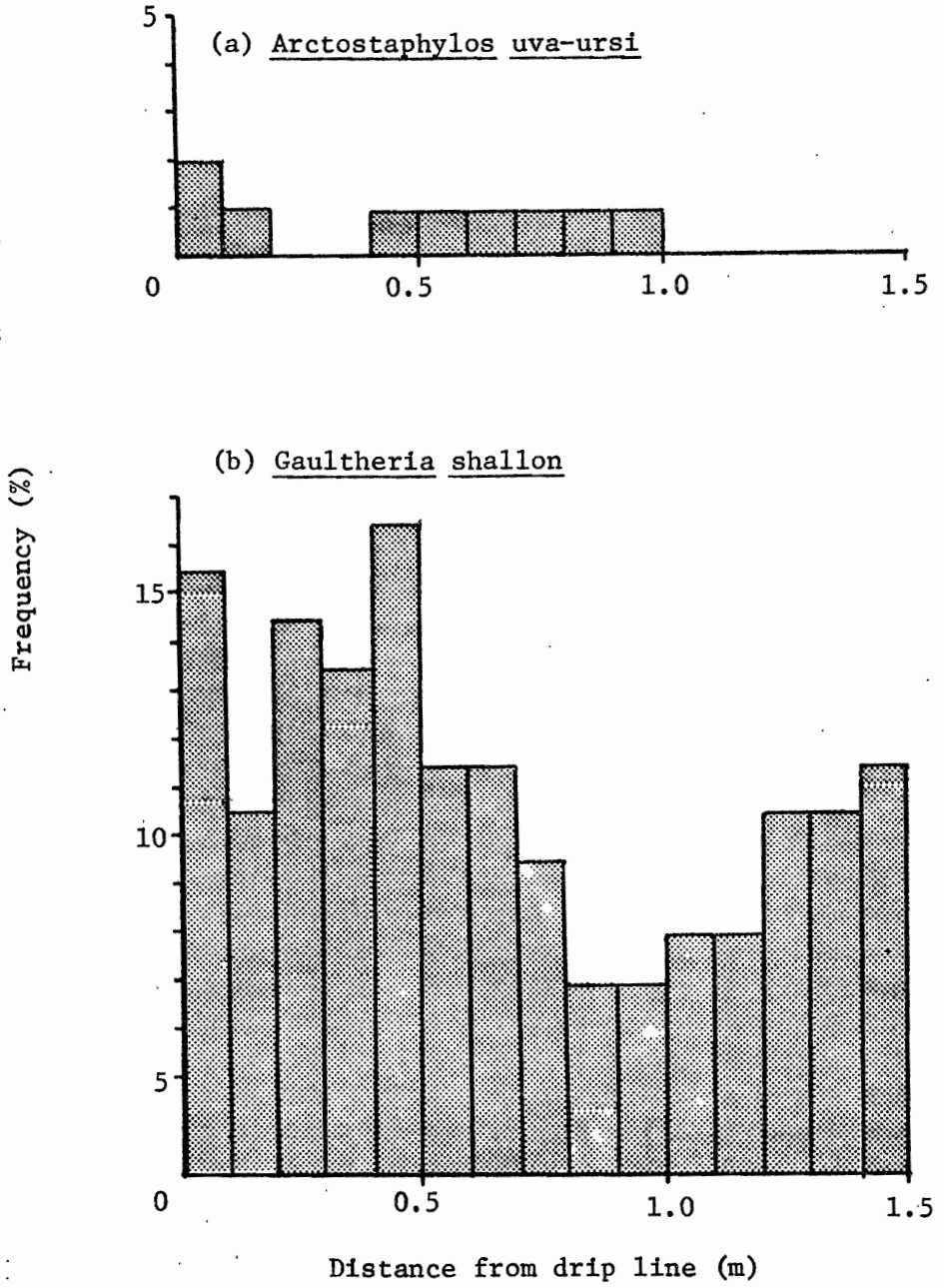

Figure 3. Frequency of presence of plant species within 0.5 m wide belt transects leading from R. macrophyllum drip lines. Expressed as the percent of quadrats in which the species was present.

of presence out to 0.8, 0.8, and 0.7 m respectively, after which their frequencies did not increase any further or actually decreased (Figure 4). This may indicate that competition or allelopathy by R. macrophyllum may not extend out to 1.5 m for these species. Clearcut sites in the western Cascades can be very harsh environments. The soil at the study site was shallow and rocky. In late summer, when this study was conducted, soil surface temperatures can be very high. R. macrophyllum, however, often forms relatively dense stands in this environment. (One reason may be its existence as a residual species with already well-developed root systems.) By limiting the placement of my belt transects to those areas where no two R. macrophyllum plants were within 3.0 m of each other, I may have been selecting areas where little vegetation, of any type, could be supported.

The two species with significant negative correlation coefficients, Arctostaphylos uva-ursi and Gaultheria shallon, are both members of the family Ericaceae, as is R. macrophyllum. This would suggest a resistance to phytotoxins produced by R. macrophyllum due to common metabolic pathways. These two species are then able to exist in those areas left less populated by other species excluded by R. macrophyllum. Other ericaceous species encountered in the belt transects were Chimaphila umbellata and Vaccinium spp., both with only slightly positive correlation coefficients (Figure 5a, b).

Berberis nervosa, a low shrub occupying habitats similar to those occupied by G. shallon, like G. shallon showed a tendency to decrease in frequency of presence with increasing distance from R. macrophyllum drip lines (Figure 5c).

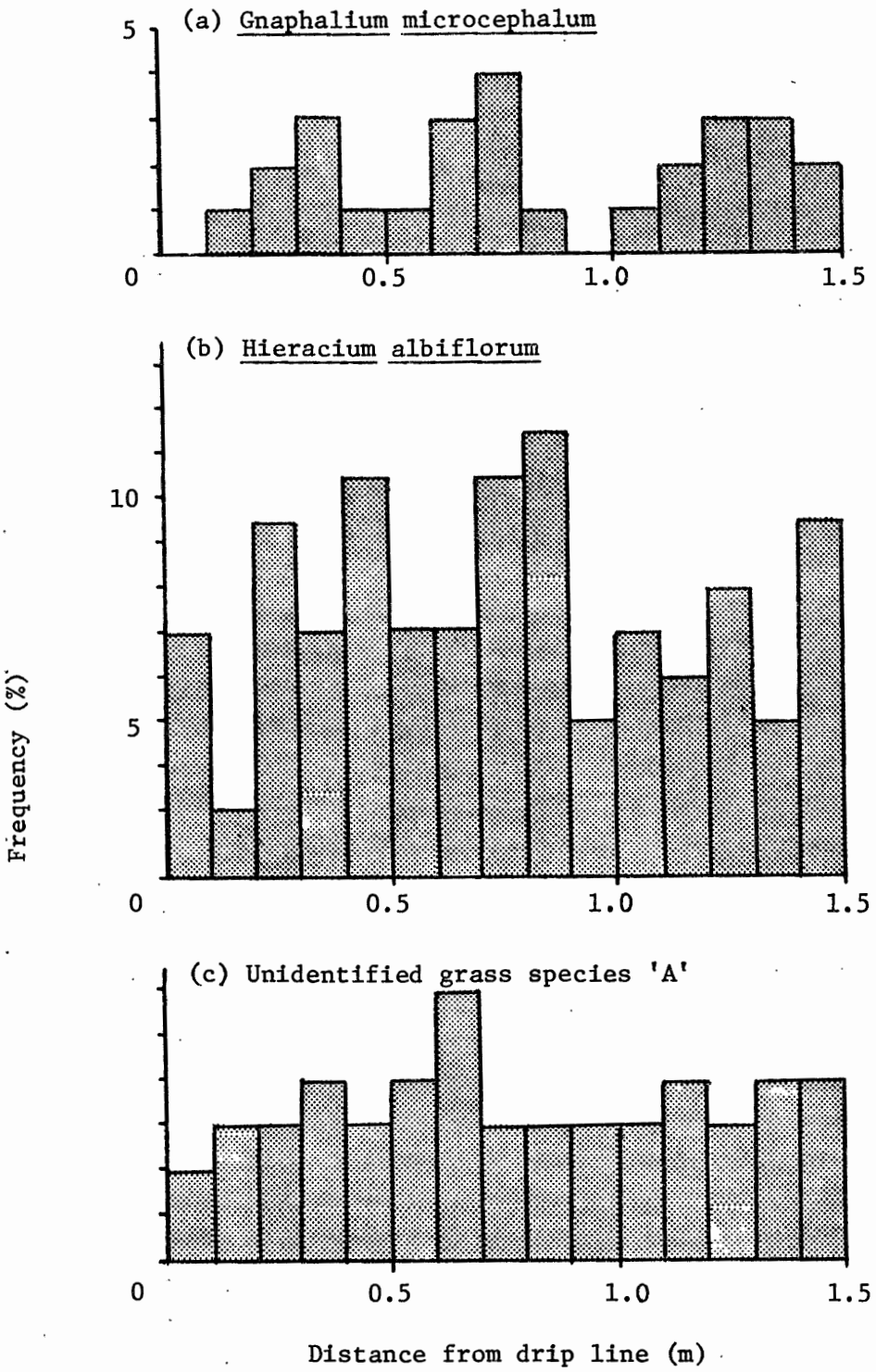

Figure 4. Frequency of presence of plant species within 0.5 m wide belt transects leading from R. macrophyllum drip lines. Expressed as the percent of quadrats in which the species was present.

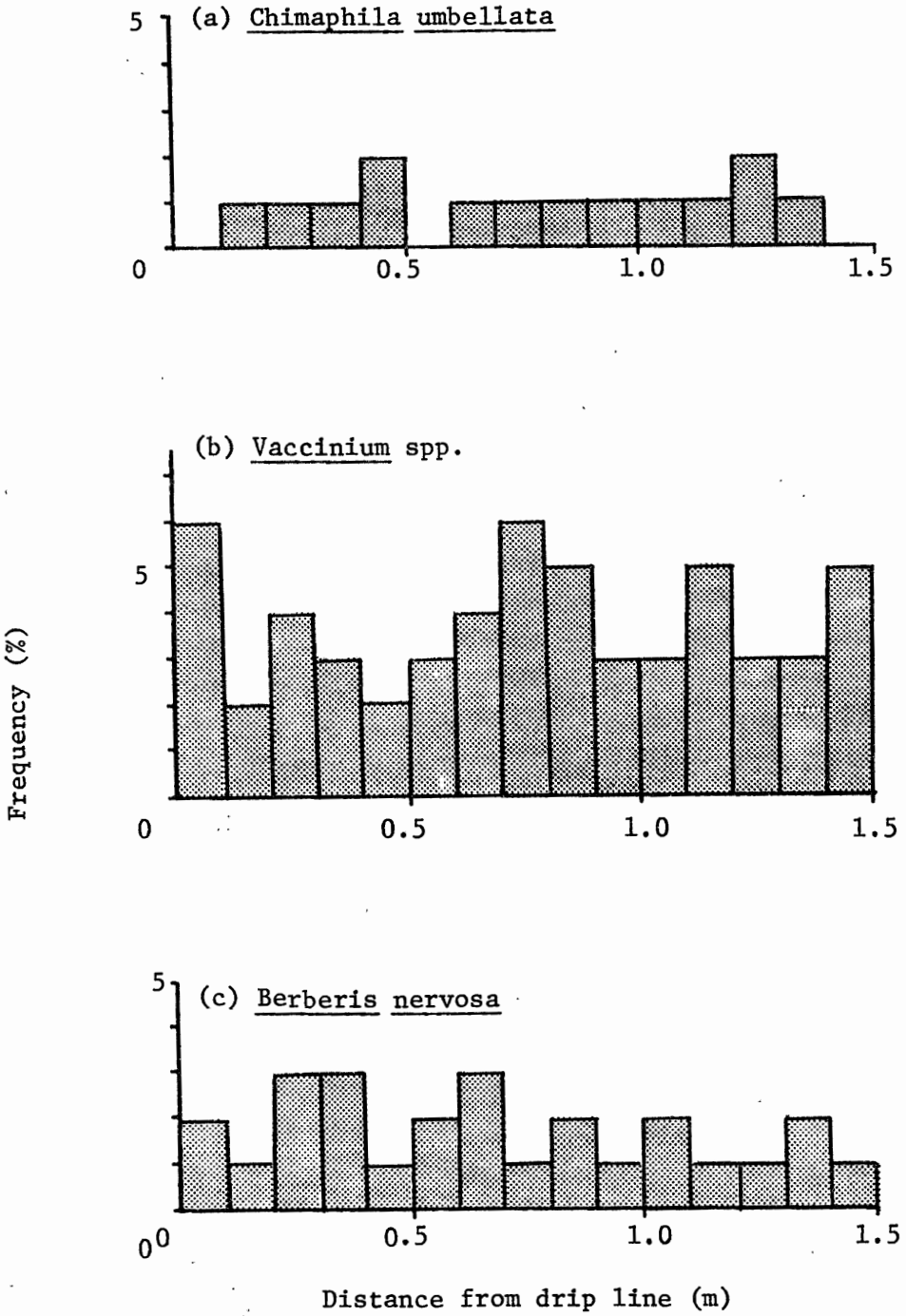

Figure 5. Frequency of presence of plant species within 0.5 m wide belt transects leading from R. macrophyllum drip lines. Expressed as the percent of quadrats in which the species was present.

When mean number of species present per quadrat was plotted against distance from R. macrophyllum drip lines (Figure 6), there was seen a general trend of increasing number of species with increasing distance from R. macrophyllum drip lines. When analyzed by linear regression, however, the correlation was not significant ($r = 0.3800$).

The number of quadrats with at least one shoot of any species present increased slightly, but not significantly, with increasing distance from R. macrophyllum drip lines (Figure 7). There was a large increase in the two farthest quadrats (1.3 - 1.4 and 1.4 - 1.5 m), but the explanation for this remains unclear. It may be due to sampling error.

Effect of Rhododendron macrophyllum on Individual Species Densities and total plant density

When the density of each species present was measured as a function of distance from Rhododendron macrophyllum drip lines, five species showed significant positive correlation coefficients when analyzed by linear regression (Table X): Anaphalis margaritacea, Chimaphila umbellata, Epilobium angustifolium, Rubus spp., and grass species 'B' (Figure 8a-e). Only one species, Arctostaphylos uva-ursi, had a significant negative correlation coefficient (Figure 8f). However, Gaultheria shallon was not included in the individual species density counts due to difficulty in determining what was an individual plant.

As noted, grass species 'B' data is from two belt transects only. It also should be noted that the C. umbellata regression line is significantly positive on the basis of zero plants in the 0.1 - 0.2 m

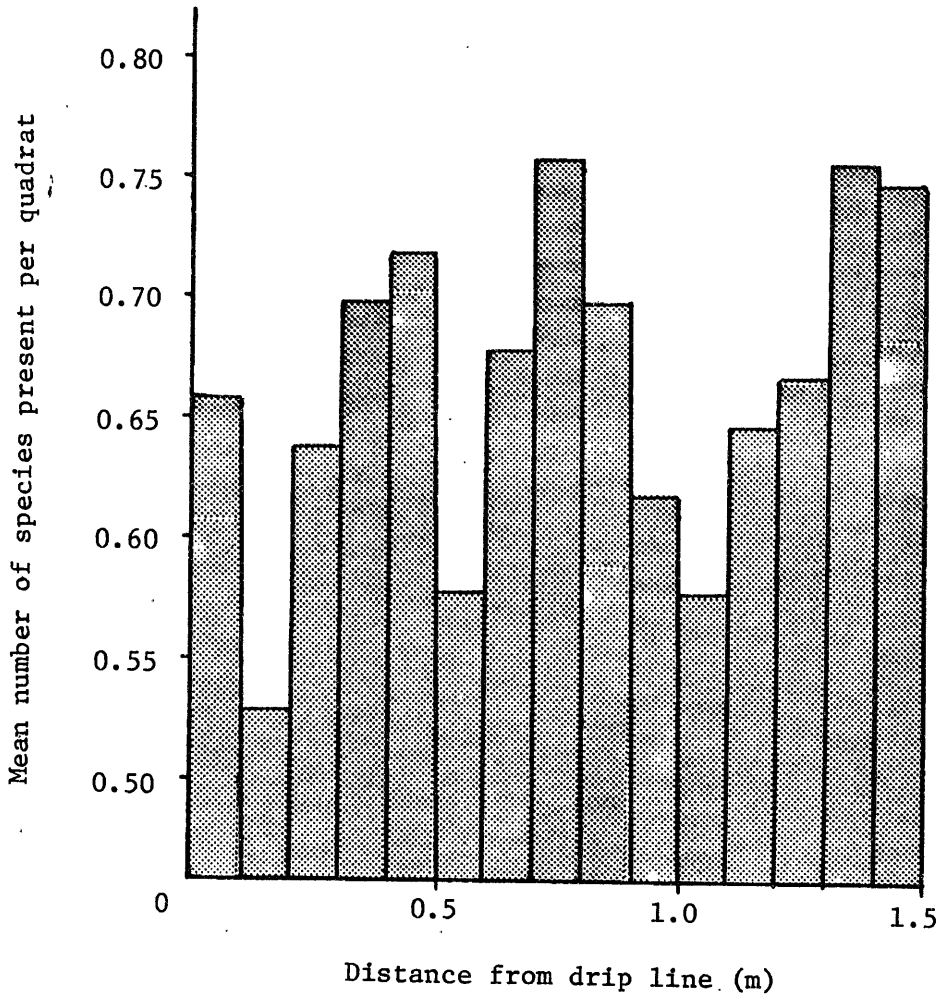

Figure 6. Mean number of species present per quadrat within 0.5 m wide belt transects leading from R. macrophyllum drip lines.

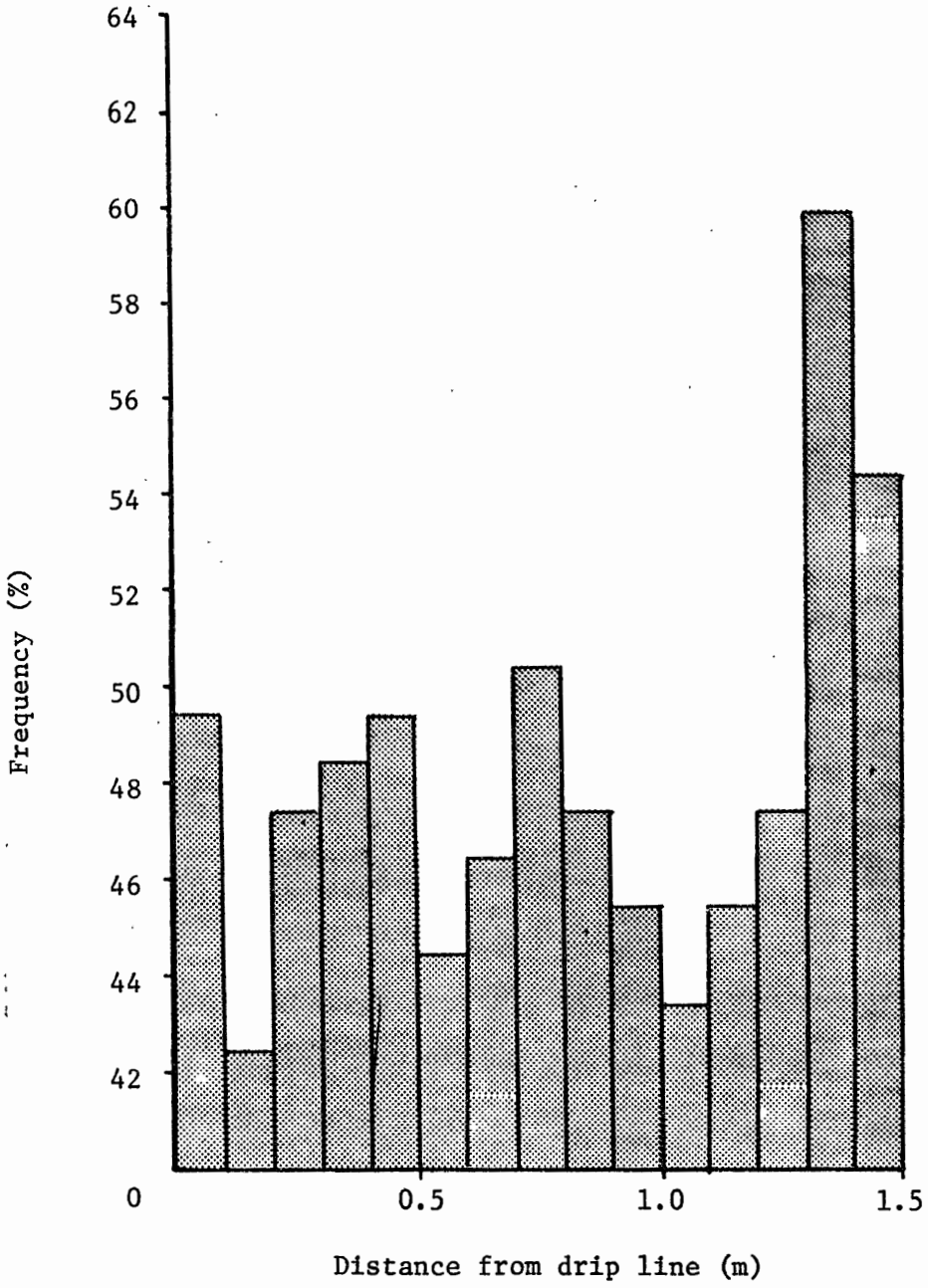

Figure 7. Frequency of quadrats with at least one plant present.

TABLE X

ABSOLUTE FREQUENCY OF PLANTS[a] WITHIN 97 0.5 M WIDE BELT TRANSECTS LEADING FROM *RHODODENDRON MACROPHYLLUM* DRIP LINES

Species	Distance from drip line (m)															r^b
	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	
Abies procera	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	-0.2700
Anaphalis margaritacea	6	18	10	13	21	17	10	9	20	18	21	24	17	38	40	0.7407[c]
Arctostaphylos uva-ursi	2	1	0	0	1	1	1	1	1	1	0	0	0	0	0	-0.5808[c]
Berberis nervosa	2	1	4	3	1	4	4	2	3	1	3	1	1	2	1	-0.3308
Castanopsis chrysophylla	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.0908
Chimaphila umbellata	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	0.5494[c]
Epilobium angustifolium	27	25	26	25	19	22	23	34	26	33	31	32	37	41	31	0.7131[c]
Epilobium minutum	2	3	6	9	6	2	4	1	3	9	4	7	2	3	3	-0.0877
Fern sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	. . .
Gnaphalium microcephalum	0	1	4	3	1	1	3	6	0	1	1	3	3	3	3	0.2411
Hieracium albiflorum	5	7	10	15	18	15	8	15	14	13	9	10	14	12	18	0.3918
Linnaea borealis	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-0.1362
Pachistima myrsinites	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	-0.4629
Pseudotsuga menziesii	0	0	2	0	4	1	0	1	1	2	0	0	0	1	2	0.0137

TABLE X--Continued

Species	Distance from drip line (m)															r^b
	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	
Rubus spp.	1	0	0	1	1	1	0	2	2	1	1	1	4	1	2	0.5512[c]
Senecio sp.	1	2	0	7	5	0	0	2	0	0	1	0	1	0	1	-0.3646
Tsuga heterophylla	1	1	0	1	0	0	0	1	1	0	0	0	1	2	0	0.0250
Vaccinium spp.	7	3	4	3	2	4	4	6	7	6	3	5	4	5	5	0.1697
Xerophyllum tenax	1	1	3	2	0	0	0	1	1	0	0	0	0	2	0	-0.3822
Unidentified grass sp. 'A'	2	3	3	5	3	4	7	4	3	3	4	5	2	4	7	0.3437
Unidentified grass sp. 'B'	1	1	0	3	4	8	6	6	6	8	7	13	14	13	9	0.8924[c]
Unidentified grass sp. 'C'	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	. . .
Unidentified herb sp. 'A'	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	. . .
Unidentified herb sp. 'B'	0	0	1	0	0	0	0	0	0	0	0	2	1	0	0	0.2152
Mean number of plants per quadrat	0.629	0.722	0.773	0.938	0.897	0.835	0.742	0.959	0.928	1.010	0.887	1.082	1.052	1.330	1.300	0.8746

[a]Figures for A. margaritacea and E. angustifolium represent number of shoots present.

[b]Correlation coefficient for regression of absolute frequency on distance from drip line.

[c]Significant at 0.05 confidence level.

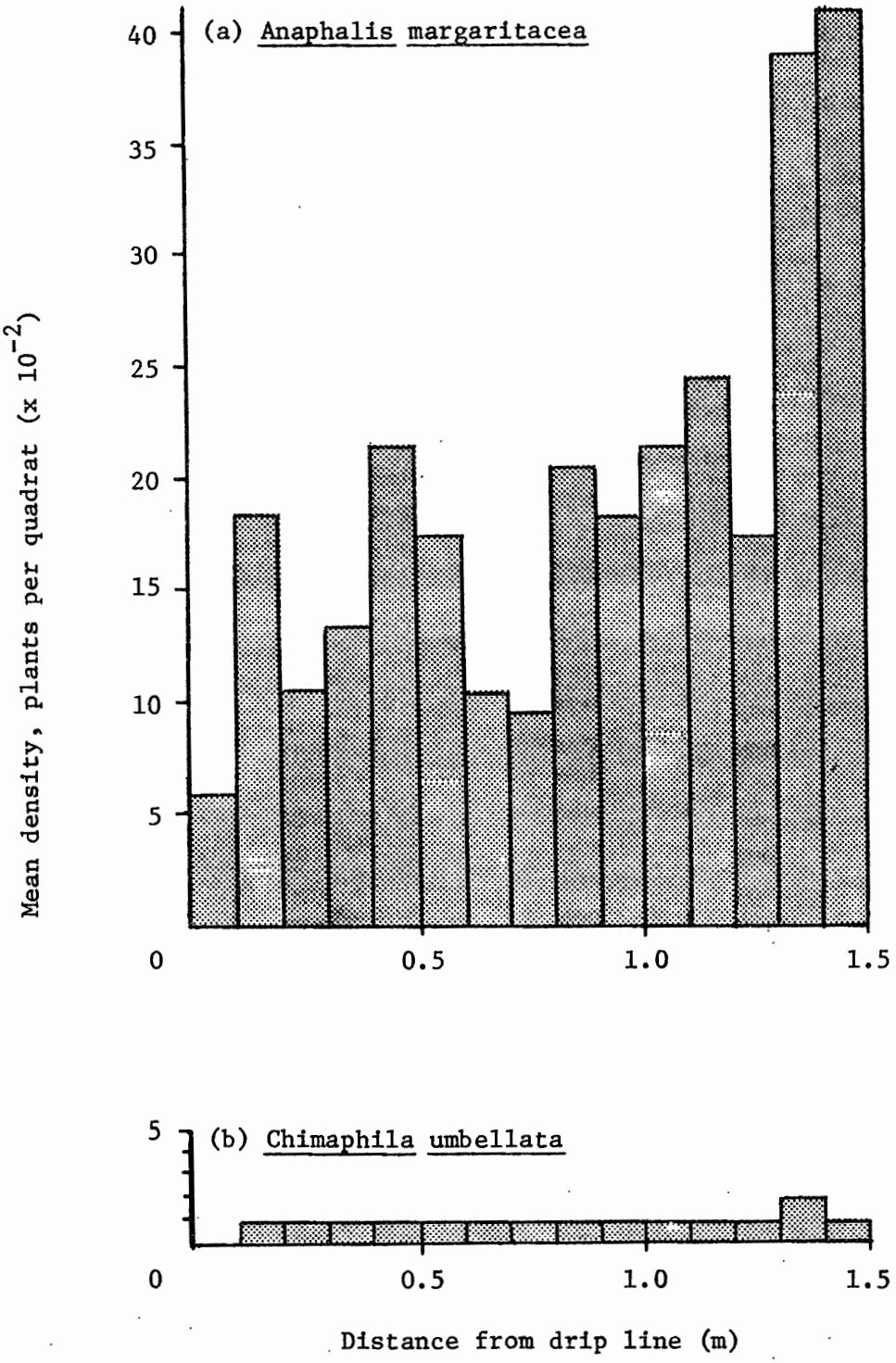

Figure 8. Mean density of plant species within 0.5 m wide belt transects leading from R. macrophyllum drip lines.

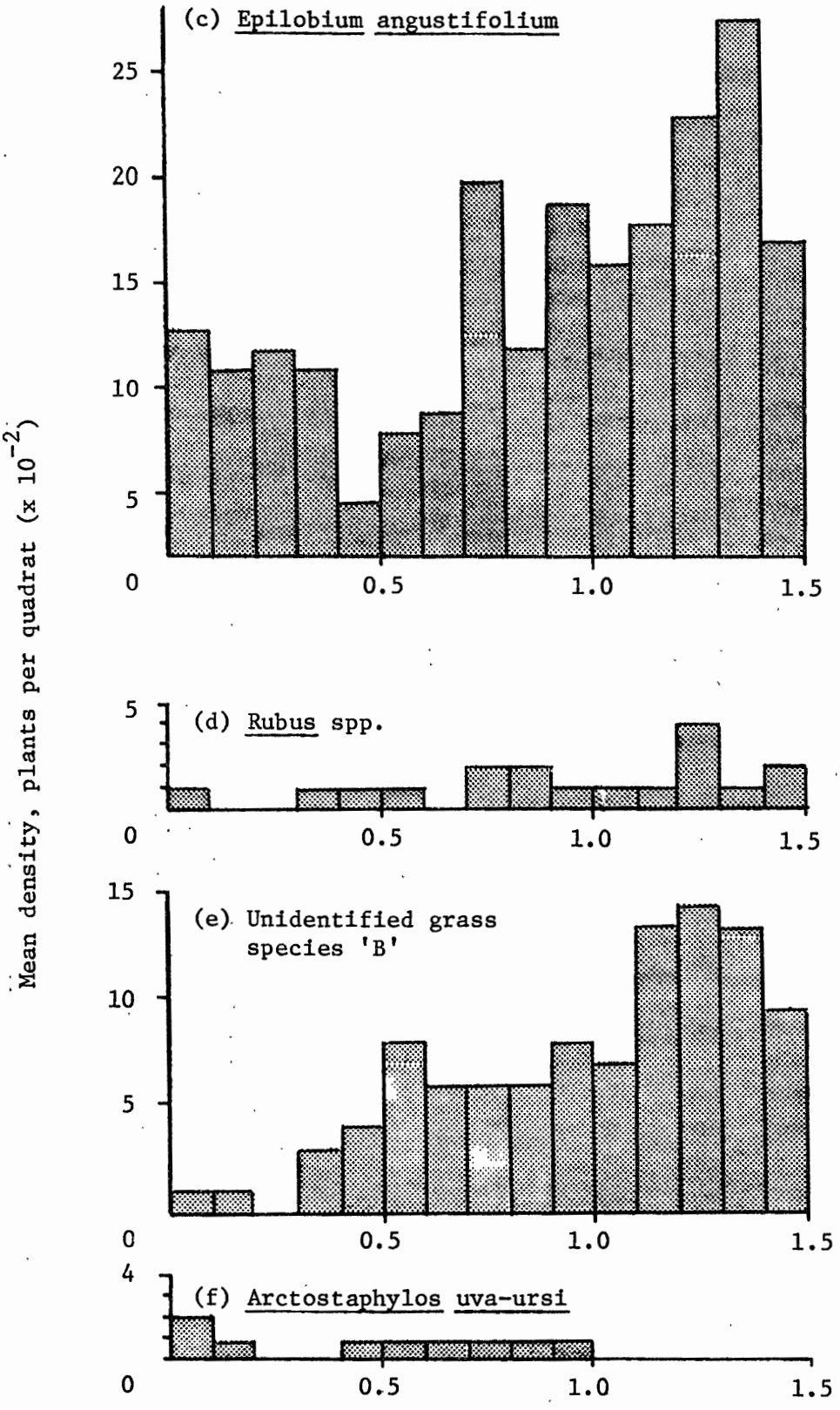

Figure 8.--Continued

quadrat and two plants in the 1.3 - 1.4 m quadrat, with one plant in each of the other quadrats (Figure 8b).

Of those species without significant correlation coefficients, Hieracium albiflorum (Figure 9d) showed a sharp increase in density out to 0.5 m, after which density increased no further. Similarly, Epilobium minutum (Figure 9b) and grass species 'A' (Figure 9e) increased in density out to 0.4 m and 0.7 m, respectively, after which points density did not increase any further. Gnaphalium microcephalum (Figure 9c) and Berberis nervosa (Figure 9a) showed general tendencies to increase in density out to 0.8 m and 0.7 m, respectively, but with considrable fluctuations in these trends out to those points. Density of B. nervosa steadily decreased after 0.7 m.

Of the ericaceous species measured, Vaccinium spp. (Figure 9f) showed little correlation of density with distance from R. macrophyllum drip lines. Vaccinium spp., however, did actually decrease in density out to 0.5 m, after which it increased back to initial levels. As noted earlier, density of A. uva-ursi (Figure 8f) decreased significantly with increasing distance from R. macrophyllum drip lines and density of C. umbellata (Figure 8b) increased significantly (but with some question as to the biological significance). G. shallon was not included in the individual species density counts.

When total plant (shoot) density per quadrat of all species (excluding G. shallon) was plotted against distance from R. macrophyllum drip lines, there was a significant increase in plant density with increasing distance ($r = 0.8746$; Figure 10). It should be remembered, however, that frequency of G. shallon presence decreased significantly

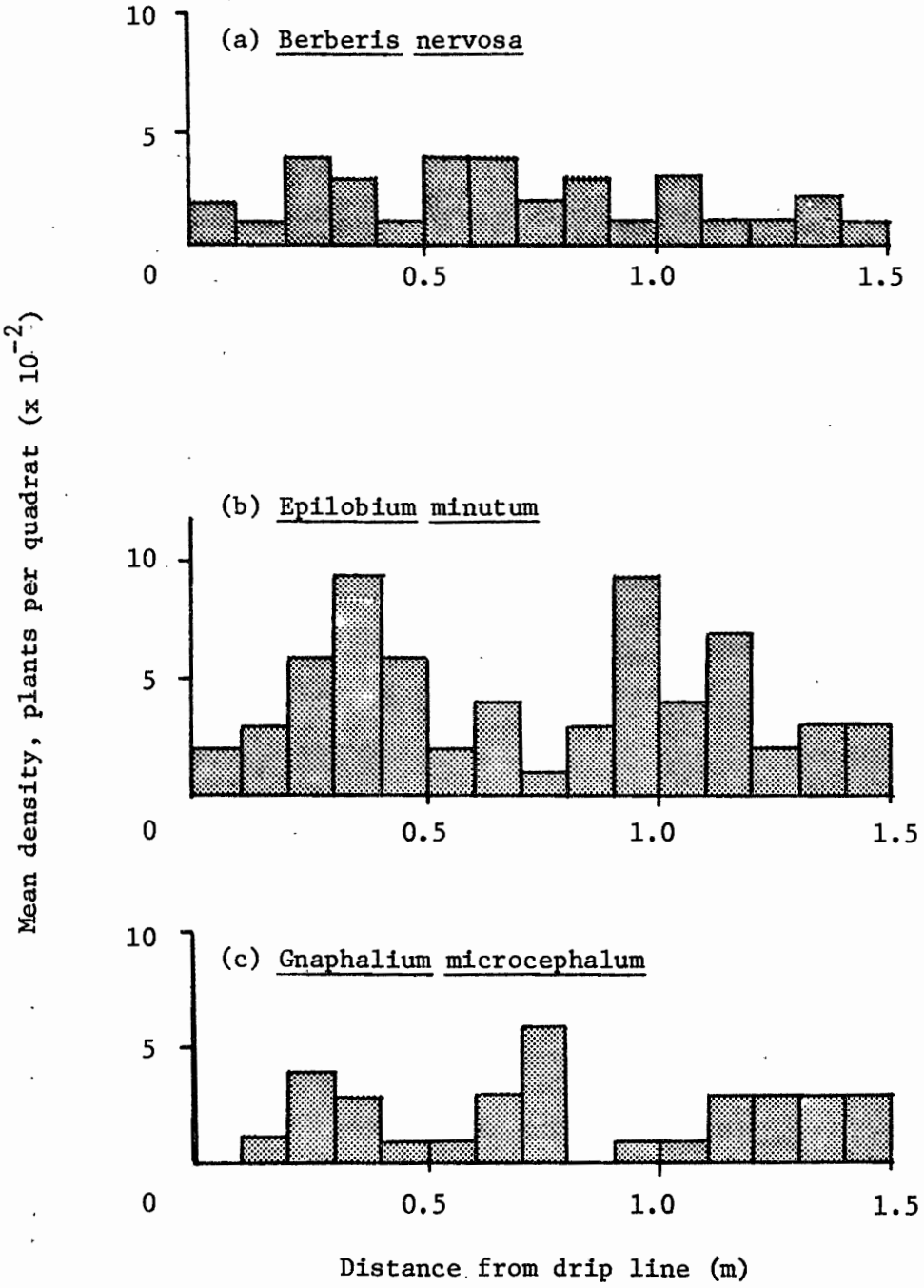

Figure 9. Mean density of plant species within 0.5 m wide belt transects leading from R. macrophyllum drip lines.

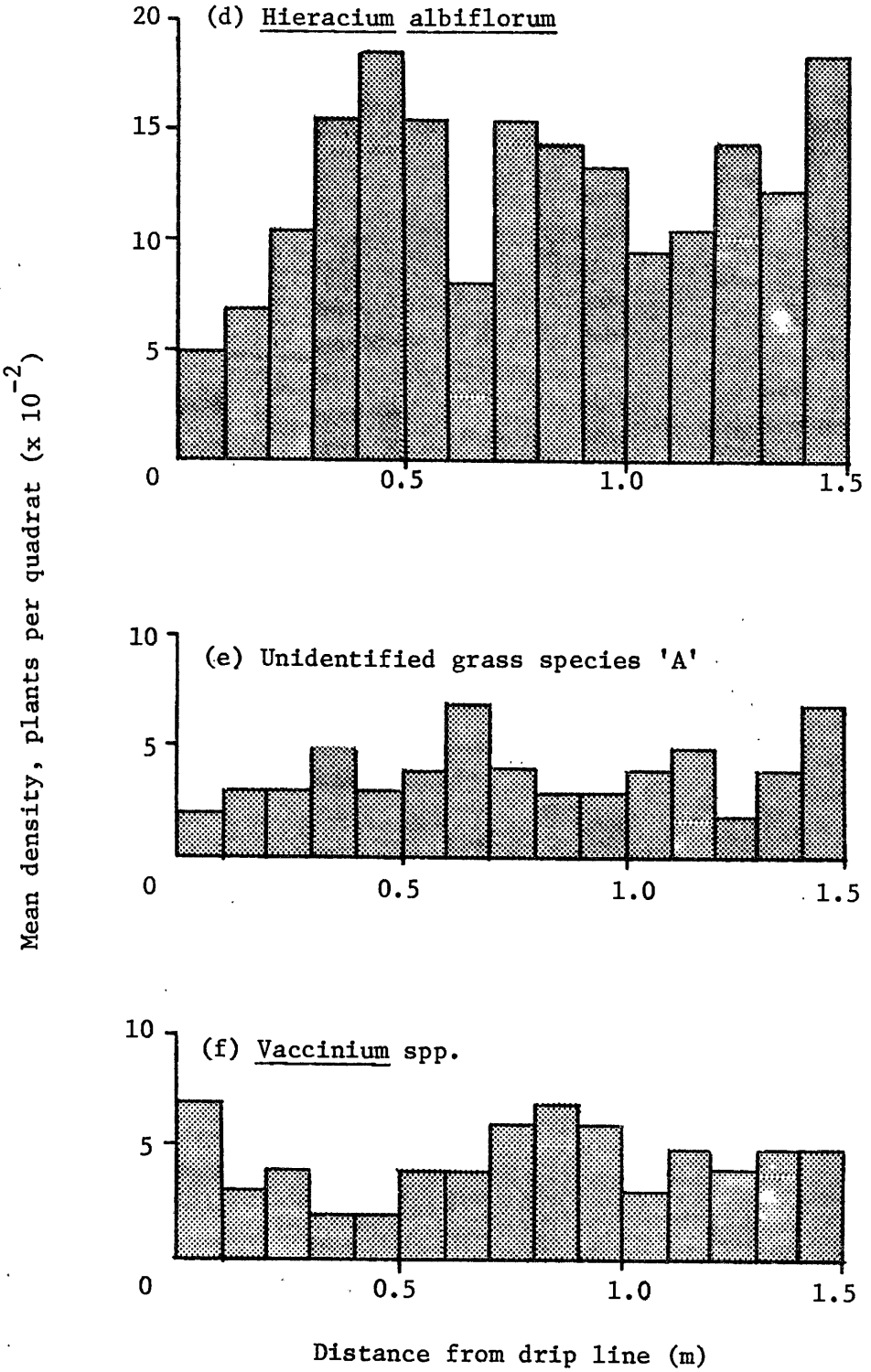

Figure 9.--Continued

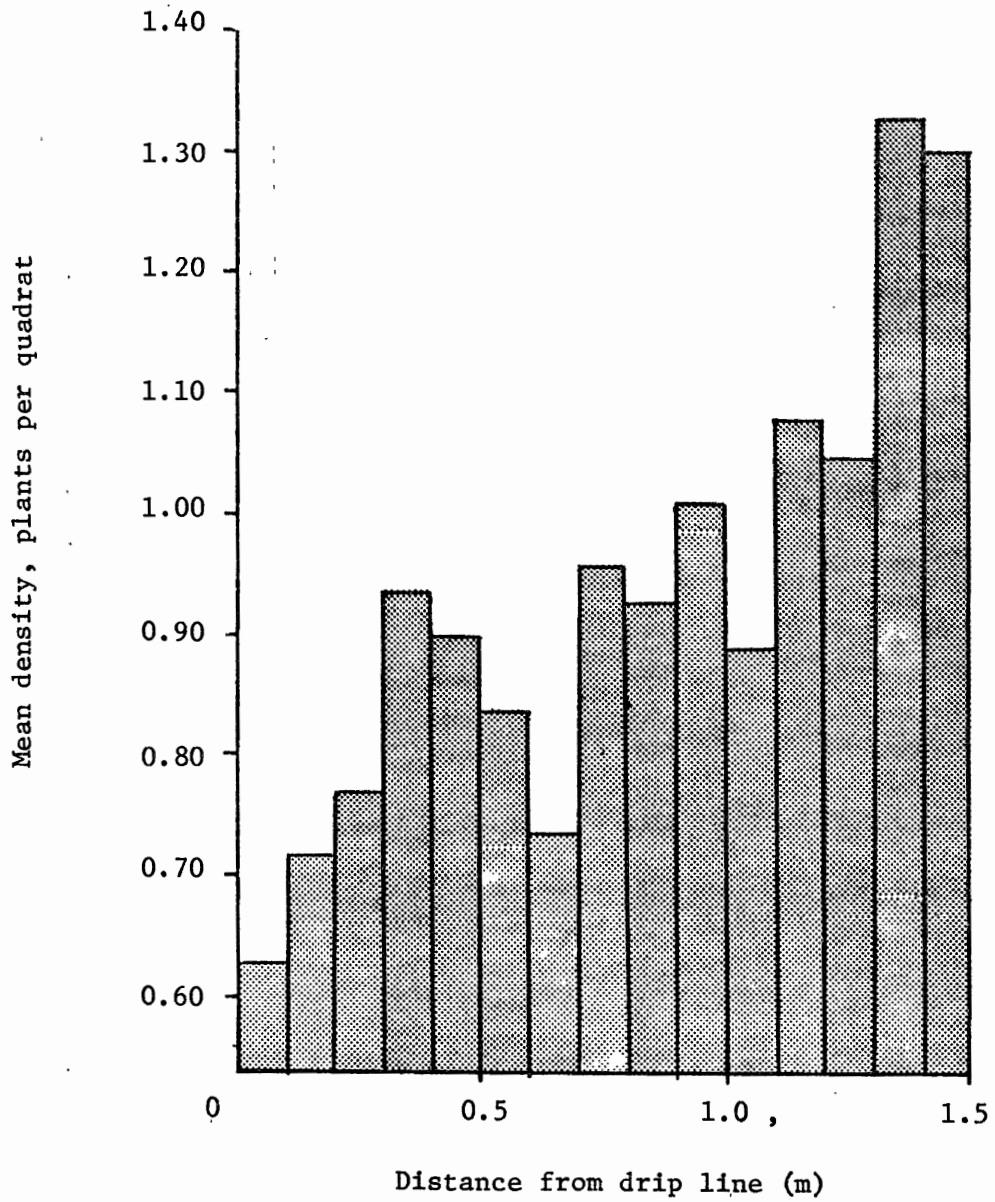

Figure 10. Mean density per quadrat of all species present, excluding Gaultheria shallon, within 0.5 m wide belt transects leading from R. macrophyllum drip lines.

with increasing distance from R. macrophyllum drip lines (Figure 3b), so that plant densities could also be correlated with presence or absence of G. shallon.

SUMMARY AND CONCLUSIONS

Laboratory bioassays have shown that leaf litter of Rhododendron macrophyllum contains water soluble substances which, when in aqueous solutions as low as 0.05 or 0.025 molar concentration, were shown to inhibit germination and radicle growth of certain species which are often found in association with R. macrophyllum. It was shown that these concentrations were not great enough to cause, through osmotic pressure effects, the inhibition seen in the leaf extract bioassays. Whether these concentrations exist in the field at the appropriate times or whether the phytotoxic substances are stable for a sufficiently long time in the soil is not known. Nevertheless, the allelopathic potential was demonstrated in these experiments. In addition, the above experiments dealt only with the effects of aqueous effects of leaf litter. No attempt was made to assess the added effects of substances leached from living R. macrophyllum crowns in the laboratory, although extracts from intact crowns of shrub species have been shown to reduce germination and growth of subordinate vegetation (McPherson and Muller, 1969).

Field studies attempted to measure the effects of R. macrophyllum on the surrounding vegetation in a western Cascades clearcut. It was seen that certain species form discernable patterns around R. macrophyllum plants. Four species declined in frequency of occurrence with increasing proximity to individual R. macrophyllum shrubs, while two species, both ericaceous, increased. Five species increased in density

with increasing distance from R. macrophyllum shrubs. The single species which became more dense nearer to R. macrophyllum shrubs was also a member of the Ericaceae. Although not overwhelming, the evidence suggests that the slight depression of the surrounding vegetation is due to the release of allelochemics from the crowns and/or litter of R. macrophyllum. That certain members of the Ericaceae show increased growth in the vicinity of R. macrophyllum plants points to either a common response to environmental conditions or to the existence in this family of a resistance to the toxins produced by its members and an ability to survive in those areas left less populated by other species more susceptible to the toxins.

Shrubs can influence the surrounding vegetation in a number of ways: competition for light, nutrients, and moisture, along with interference via allelochemics released from crowns, roots, and litter. It is therefore difficult to separate the effects of competition and allelopathy. However, the overall results of this study - inhibition by aqueous R. macrophyllum leaf litter extracts in the laboratory along with reduced vegetation surrounding R. macrophyllum plants in the field - strongly suggest that allelopathic control of vegetation by R. macrophyllum has a role in determining community structure in western Cascades clearcuts.

REFERENCES CITED

Chou, C., and C.H. Muller. 1972. Allelopathic mechanisms of Arctostaphylos glandulosa var. zacaensis. Am. Midl. Nat. 88: 324-347.

Christiansen, N.L., and C.H. Muller. 1975a. Relative importance of factors controlling germination and seedling survival in Adenostoma chaparral. Am. Midl. Nat. 93: 71-78.

Christiansen, N.L., and C.H. Muller. 1975b. Effects of fire on factors controlling plant growth in Adenostoma chaparral. Ecol. Monogr. 45: 29-55.

del Moral, R., and R.G. Cates. 1971. Allelopathic potential of the dominant vegetation of Western Washington. Ecology 52: 1030-1037.

Dyrness, C.T. 1973. Early stages of plant succession following logging and burning in the Western Cascades of Oregon. Ecology 54: 57-69.

Franklin, J.F., and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. USDA Forest Service General Technical Report PNW-8. 417 p.

Halligan, J.P. 1973. Bare areas associated with shrub stands in grasslands: the case of Artemesia californica. Bioscience 23: 429-439.

Halligan, J.P. 1976. Toxicity of Artemesia californica to four associated herb species. Am. Midl. Nat. 95: 406-421.

Hanawalt, R.B. 1971. Inhibition of annual plants by Arctostaphylos. In: Biochemical interactions among plants. Nat. Acad. Sci.. pp. 33-38.

Hitchcock, C.L., and A. Cronquist. 1973. Flora of the Pacific Northwest. Univ. of Washington Press, Seattle. 730 p.

McPherson, J.K., and C.H. Muller. 1969. Allelopathic effects of Adenostoma fasciculatum, "chamise," in the California chaparral. Ecol. Monogr. 39: 177-198.

Muller, C.H. 1966. The role of chemical inhibition (allelopathy) in

vegetational composition. Bull. Torr. Bot. Club 93: 332-351.

Muller, C.H. 1970. Phytotoxins as plant habitat variables. Rec. Adv. in Phytochemistry 3: 105-121.

Muller, C.H., and R. del Moral. 1966. Soil toxicity induced by terpenes from Salvia leucophylla. Bull. Torr. Bot. Club 93: 130-137.

Muller, C.H., R.B. Hanawalt, and J.K. McPherson. 1968. Allelopathic control of herb growth in the fire cycle of California chaparral. Bull. Torr. Bot. Club 95: 225-231.

Shoemaker, D.P., and C.W. Garland. 1967. Experiments in physical chemistry. McGraw-Hill, New York.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. W.H. Freeman, San Francisco. 776 p.

U.S.D.A. Forest Service. 1974. Seeds of woody plants in the United States. Agricultural Handbook No. 450. 883 p.

Whittaker, R.H. 1970. Biochemical ecology of higher plants. In: E. Sondheimer and J.B. Simeone (eds.). Chemical ecology. Academic Press, New York. pp. 43-70.

Whittaker, R.H., and P.P. Feeny. 1971. Allelochemics: chemical interactions between species. Science 171: 757-770.

APPENDIX A

DETERMINATION OF LEAF EXTRACT MOLAR CONCENTRATION FROM FREEZING POINT DEPRESSION

Freezing point depression experiments were used to determine the molar concentration of three extract solutions which were prepared by identical methods from the same lot of dried Rhododendron macrophyllum leaves. In each experiment a weighed amount of dry solute was added to a volume of distilled water and the resultant freezing point depression was measured. Following this, the frozen solution was melted and an additional amount of dry solute was added to the solution. The freezing point depression measurements were then repeated to check for precision of molecular weight determinations.

In all cases supercooling occurred before freezing of the solution took place. To estimate the true freezing point of the solution, that part of the cooling curve that was plotted after supercooling had occurred was extrapolated back until it intersected the cooling curve of the liquid solution. The extrapolated part of the curve was assumed to be linear (Shoemaker and Garland, 1967). The exact location and slope of the extrapolated curve was determined by establishing a regression line for those points on the cooling curve which were plotted after supercooling had occurred. The freezing point of the solution was determined to be the point at which the regression line intersected the cooling curve of the liquid solution (Figure 11).

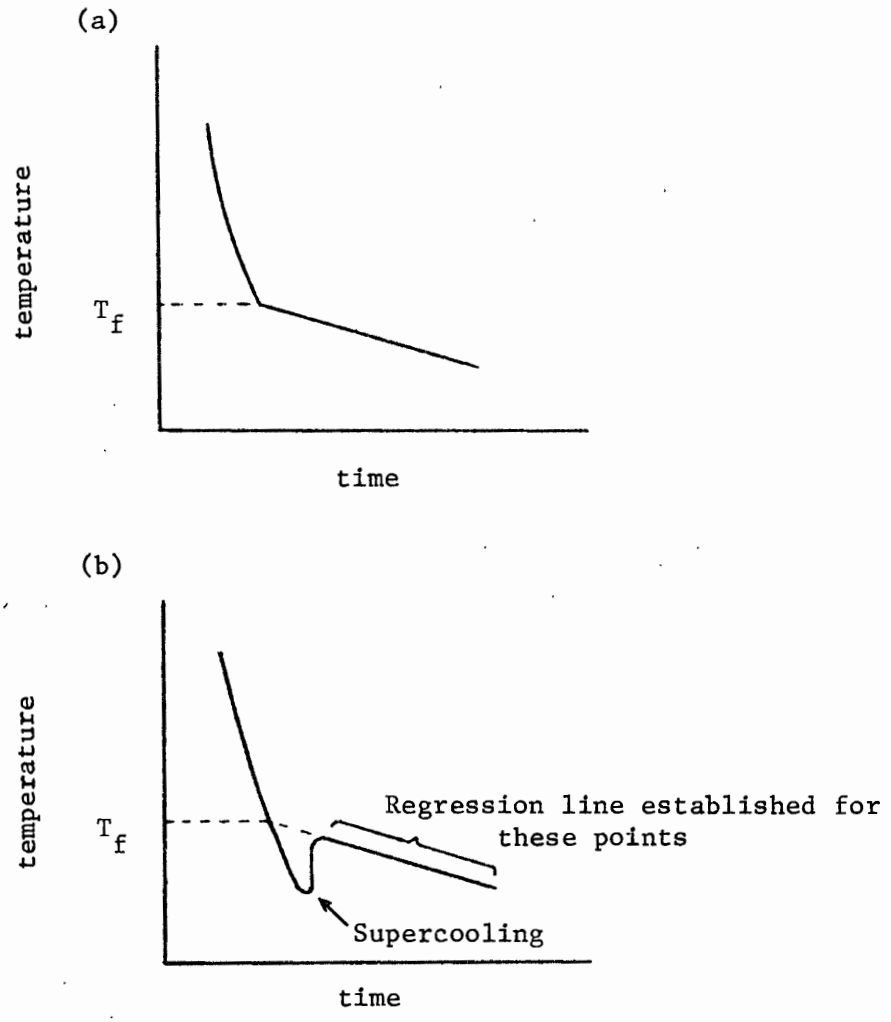

Figure 11. Cooling curves for solutions without supercooling (a) and with supercooling (b). After Shoemaker and Garland (1967).

TABLE XI

SOLUTIONS USED IN FREEZING POINT DEPRESSION EXPERIMENTS

	Solution						
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	Mannitol
Weight of solute (g)	0.6307	1.0406	0.5948	0.8180	0.6459	0.8698	0.6052
Volume of water (ml)	15.0	15.0	12.0	12.0	12.0	12.0	12.0

[a]Extract used in Bromus tectorum bioassays.

[b]Extract used in Pseudotsuga menziesii bioassays.

[c]Extract used in a preliminary bioassay.

TABLE XII

TEMPERATURE READINGS OVER TIME IN FREEZING POINT DEPRESSION EXPERIMENTS, ^{o}C

Time (min.)	Solution						
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	Mannitol
0	0.78	0.30	0.22	0.37	0.40	0.33	0.57
0.5	0.15	-0.22	-0.39	-0.33	-0.23	. . .	-0.04
1.0	-0.27	-0.72	-0.99	-1.07	-0.99	-0.88	-0.65
1.5	-0.75	-1.12	-1.50	-1.69	-1.59	-1.45	-1.13
2.0	-1.15	-1.53	-2.10	-2.38	-2.30	-2.30	-1.67
2.25	. . .	-1.63	. . .	. . .	. . .	. . .	. . .
2.5	-1.41	-0.73	-2.62	-2.91	-1.53	-1.48	-0.66
2.75	-1.48	. . .	. . .	. . .	. . .	. . .	. . .
3.0	-0.55	-0.48	-2.23	-3.40	-0.38	-0.48	-0.59
3.5	-0.33	-0.47	-0.36	-2.23	-0.385	-0.47	-0.59
4.0	-0.30	-0.50	-0.345	-0.495	-0.395	-0.48	-0.57
4.5	-0.31	-0.50	-0.335	-0.48	-0.39	-0.485	-0.58

TABLE XII--Continued

Time (min.)	Solution						
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	Mannitol
5.0	-0.315	-0.50	-0.335	-0.49	-0.39	-0.49	-0.58
5.5	-0.325	-0.50	-0.355	-0.49	-0.395	-0.49	-0.59
6.0	-0.325	-0.50	-0.36	-0.495	-0.39	-0.50	-0.59
6.5	-0.32	-0.50	-0.36	-0.50	-0.39	-0.505	-0.60
7.0	-0.32	-0.505	-0.36	-0.51	-0.39	-0.515	-0.61
7.5	-0.315	-0.505	-0.37	-0.51	-0.40	-0.52	. . .
8.0	-0.32	-0.51	-0.375	-0.52	-0.40	-0.53	. . .
8.5	. . .	-0.51	-0.38	-0.53	-0.42	-0.54	. . .
9.0	. . .	-0.51	-0.38	-0.53	-0.425	-0.545	. . .
9.5	. . .	-0.51	-0.39	-0.535	-0.425	-0.555	. . .
10.0	. . .	. . .	-0.39	-0.54	-0.425	-0.56	. . .
10.5	. . .	. . .	-0.39	. . .	-0.44	. . .	. . .
11.0	. . .	. . .	-0.39	. . .	. . .	. . .	. . .

TABLE XII--Continued

Time (min.)	Solution						
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	Mannitol
11.5	. . .	. . .	-0.	. . .	. . .	. . .	. . .
12.0	. .	. . .	-0.40	. . .	. . .	. . .	. . .

[a]Extract used in Bromus tectorum bioassays.

[b]Extract used in Pseudotsuga menziesii bioassays.

[c]Extract used in preliminary bioassays.

TABLE XIII

ANALYSIS OF FREEZING POINT DEPRESSION BY LINEAR REGRESSION

	Solution						
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	Mannitol
Time interval used for regression (min.)	4.0-8.0	3.0-9.5	3.5-12.0	4.5-10.0	3.0-10.5	3.0-10.0	4.0-7.0
r	0.5774	0.8162[d]	0.9337[d]	0.9913[d]	0.8980[d]	0.9829[d]	0.9507[d]
Slope	-0.033	-0.0045	-0.007	-0.011	-0.007	-0.0129	-0.012
y-intercept	-0.297	-0.472	-0.315	-0.431	-0.356	-0.427	-0.522
Value of y when x = 1	-0.300	-0.476	-0.322	-0.442	-0.363	-0.440	-0.534
ΔT_f (°C)	0.30	0.475	0.32	0.44	0.36	0.44	0.53

[a]Extract used in Bromus tectorum bioassays.

[b]Extract used in Pseudotsuga menziesii bioassays.

[c]Extract used in preliminary bioassays.

[d]Significant at the 0.05 confidence level.

TABLE XIV

CALCULATIONS OF LEAF EXTRACT MOLAR CONCENTRATIONS

	Solution						
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]	Mannitol
Volume freeze-dried (ml)	800	800	150	150	150	150	. . .
Weight of dry solute (g)	11.0	11.0	2.09	2.09	1.93	1.93	. . .
Weight/volume concentration ($g\text{-}liter^{-1}$)	13.8	13.8	13.9	13.9	12.9	12.9	. . .
Average molecular weight ($g\text{-}mole^{-1}$)	260	270	290	290	280	310	180[d]
Concentration ($moles\text{-}liter^{-1}$)	0.05	0.05	0.05	0.05	0.05	0.04	. . .

[a]Extract used in Bromus tectorum bioassays.

[b]Extract used in Pseudotsuga menziesii bioassays.

[c]Extract used in preliminary bioassays.

[d]This corresponds very closely with the true molecular weight of mannitol, 182 $g\text{-}mole^{-1}$.

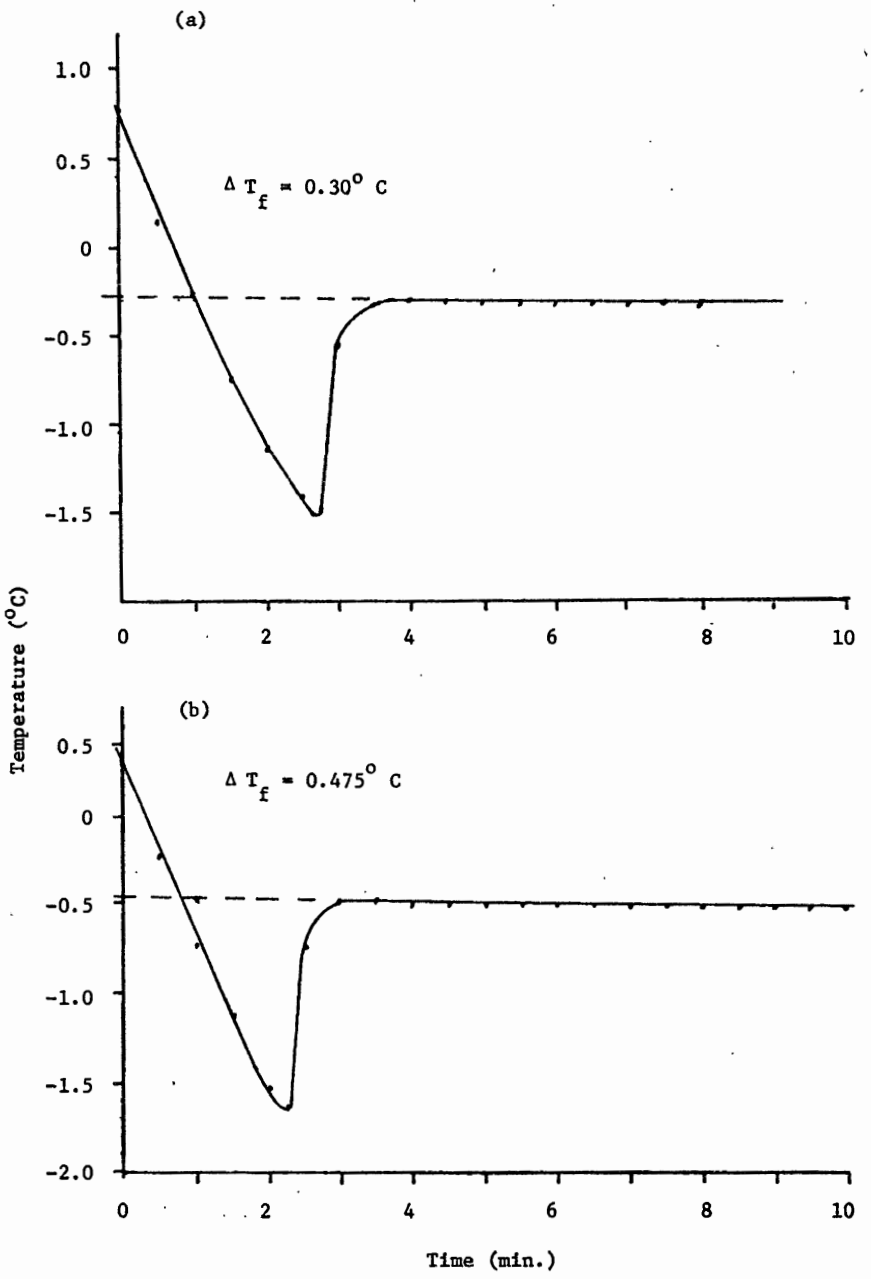

Figure 12. Cooling curves for aqueous solutions of extract used in Bromus tectorum bioassays. (a) 0.6307 g solute in 15.0 ml water. (b) 1.0406 g solute in 15.0 ml water.

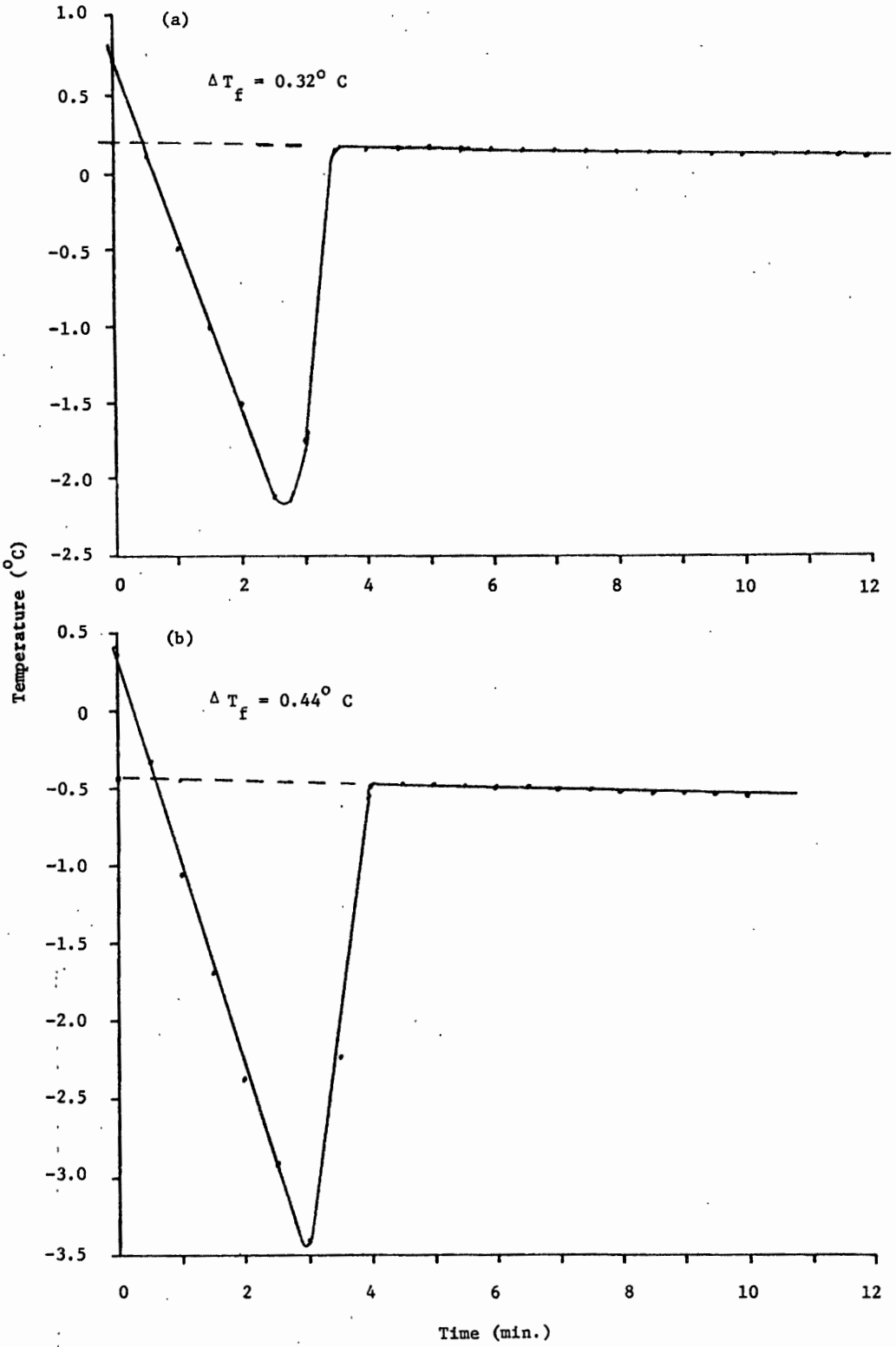

Figure 13. Cooling curves for aqueous solutions of extract used in Pseudotsuga menziesii bioassays. (a) 0.5948 g solute in 12.0 ml water. (b) 0.8180 g solute in 12.0 ml water.

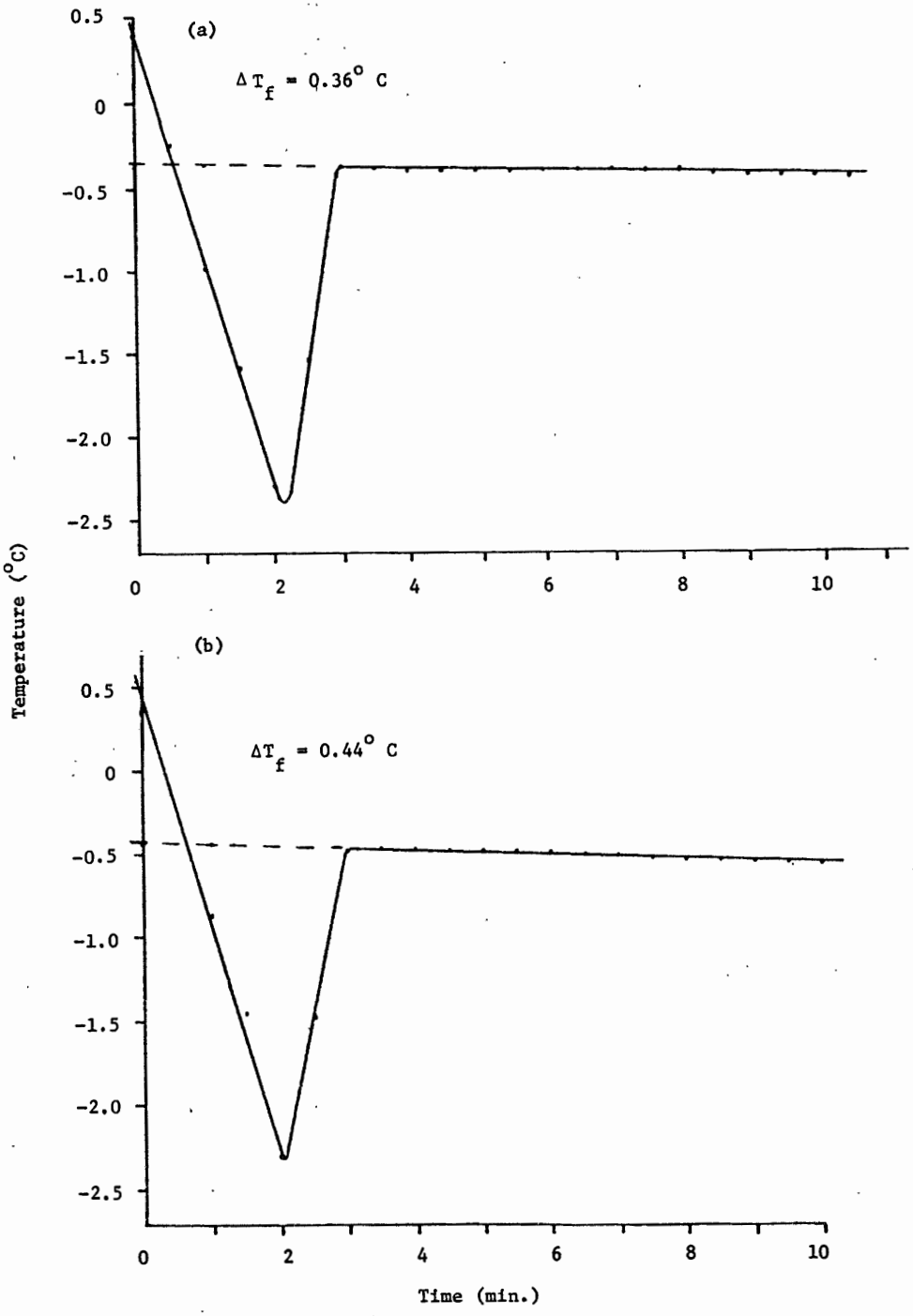

Figure 14. Cooling curves for aqueous solutions of extract used in a preliminary bioassay. (a) 0.6459 g solute in 12.0 ml water. (b) 0.8689 g solute in 12.0 ml water.

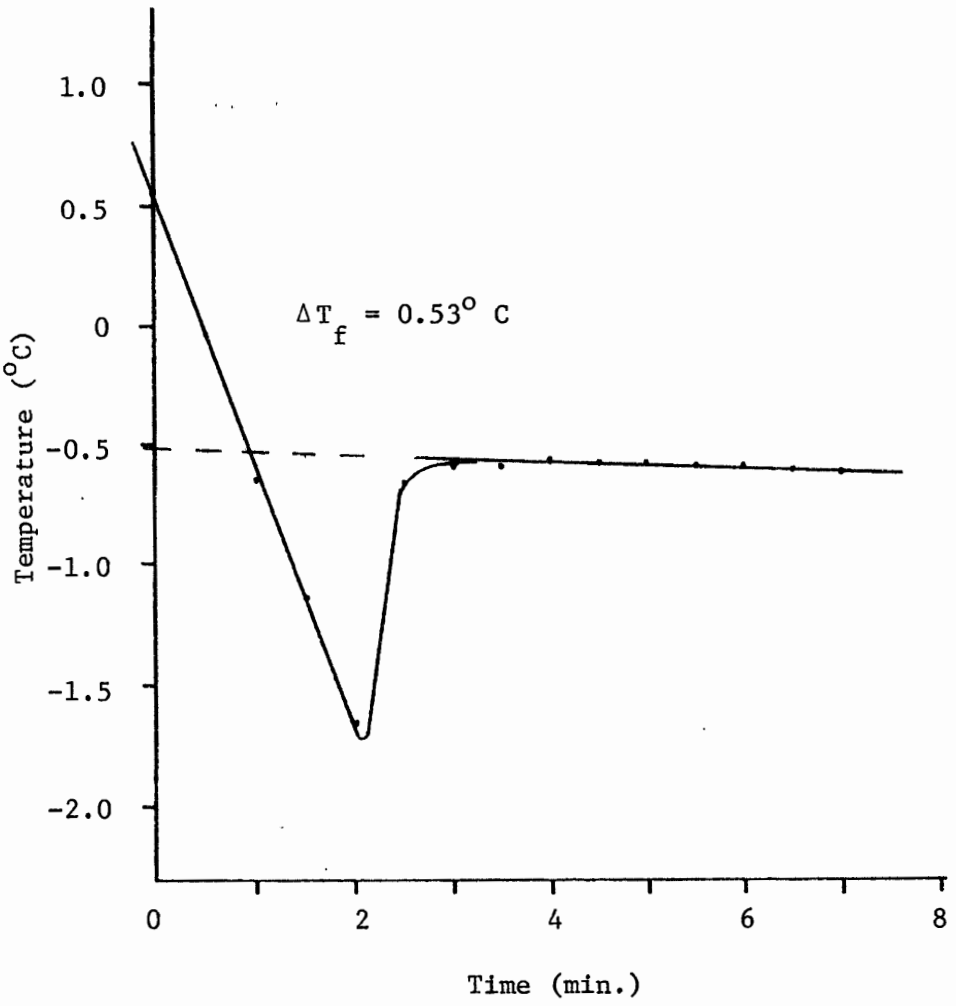

Figure 15. Cooling curve for an aqueous solution of mannitol, molecular weight 182 g-mole^{-1}. 0.6052 g mannitol dissolved in 12.0 ml water.

APPENDIX B

GERMINATION RATES AND MEAN RADICLE LENGTHS OF TEST SPECIES IN BIOASSAYS

TABLE XV

GERMINATION RATES AND MEAN RADICLE LENGTHS OF *BROMUS TECTORUM*, *EPILOBIUM ANGUSTIFOLIUM*, AND *PSEUDOTSUGA MENZIESII* IN LEAF EXTRACT BIOASSAYS

Species	Treatment	n	Germination rate (%)	Mean radicle length (mm, ± SD)
Bromus tectorum	Water	60	97	10.2 ± 4.5
	Extract	60	65	3.4 ± 2.5
Epilobium angustifolium	Water	400	81	. . .
	Extract	400	62	. . .
	½ strength extract	400	67	. . .
Pseudotsuga menziesii	Water	207	82	24.0 ± 11.1
	Extract	189	3	2.6 ± 1.8

TABLE XVI

GERMINATION RATES AND MEAN RADICLE LENGTHS OF *BROMUS TECTORUM* IN SUCROSE BIOASSAYS

Sucrose concentration (moles-liter^{-1})	n	Germination rate (%)	Mean radicle length (mm ± SD)
0.0 (water)	30	97	10.1 ± 4.3
0.01	40	100	8.7 ± 3.8
0.02	40	98	9.4 ± 3.8
0.05	40	100	7.3 ± 3.6
0.10	40	100	6.6 ± 3.7
0.15	40	93	5.2 ± 2.9
0.20	40	95	2.2 ± 1.8

TABLE XVII

GERMINATION RATES AND MEAN RADICLE LENGTHS OF *EPILOBIUM ANGUSTIFOLIUM* AND *PSEUDOTSUGA MENZIESII* IN MANNITOL BIOASSAYS

Species	Mannitol concentration (moles-liter^{-1})	n	Germination rate (%)	Mean radicle length (mm ±SD)
Epilobium angustifolium	0.0 (water)	160	79	. . .
	0.01	160	87	. . .
	0.02	160	83	. . .
	0.05	160	71	. . .
	0.10	160	74	. . .
	0.15	160	61	. . .
	0.20	160	49	. . .
	0.25	160	21	. . .
Pseudotsuga menziesii	0.0 (water)	60	85	26.2 ± 11.8
	0.01	60	87	30.5 ± 13.5
	0.02	60	85	26.3 ± 13.1
	0.05	60	85	25.1 ± 12.2
	0.10	60	83	21.1 ± 10.2
	0.15	60	83	19.6 ± 9.9
	0.20	60	70	14.4 ± 8.5

TABLE XVIII

GERMINATION RATES AND MEAN RADICLE LENGTHS OF *PICEA SITCHENSIS* AND *TSUGA HETEROPHYLLA* IN BIOASSAYS WITH LEAF EXTRACT AND MANNITOL

Species	Treatment	n	Germination rate (%)	Mean radicle length (mm ±SD)
Picea sitchensis	Water	250	81	30.1 ± 15.4
	Extract	250	49	9.3 ± 6.9
	½ strength extract	250	64	14.3 ± 9.1
	0.01 M mannitol	100	75	28.8 ± 14.6
	0.02 M mannitol	100	73	28.1 ± 14.1
	0.05 M mannitol	100	65	18.2 ± 11.7
	0.10 M mannitol	25	56	17.6 ± 9.0
	0.15 M mannitol	75	63	12.8 ± 7.7
	0.20 M mannitol	100	35	8.4 ± 6.6
Tsuga heterophylla	Water	250	76	9.3 ± 5.5
	Extract	250	42	5.2 ± 3.2
	½ strength extract	250	68	7.9 ± 5.0
	0.01 M mannitol	100	72	11.3 ± 5.5
	0.02 M mannitol	100	80	9.1 ± 5.4
	0.05 M mannitol	100	66	6.8 ± 4.0
	0.10 M mannitol	100	66	7.0 ± 3.9
	0.15 M mannitol	100	43	3.9 ± 2.8
	0.20 M mannitol	100	61	4.0 ± 2.6

APPENDIX C

LITERATURE REVIEW

Early Research on Allelopathy

The term for allelopathy, the inhibition of one plant by another through the release of chemicals into the environment, was not coined until 1937 by Molisch, but the theory began to develop over a century previous to this. De Candolle (1832) was one of the first to formalize the theory of allelopathy by suggesting that soil exhaustion by certain plants might in fact be due to phytotoxic excretions from roots. Stickney and Hoy (1881), noting the paucity of vegetation under black walnut trees (Juglans nigra), suggested that this lack of vegetation was caused by the poisonous drip from the leaves. Schreiner and his co-workers (Schreiner and Reed, 1907, 1908; Schreiner and Shorey, 1909; Schreiner and Sullivan, 1909) showed that the soil exhaustion resulting from continuous one-crop agriculture was due to the addition of growth inhibitors to the soil by the roots of crop plants. An unidentified inhibitor involved in soil exhaustion by cowpeas (Vigna catjang) was extracted from the soil by Schreiner and Sullivan (1909). They found not only that this substance inhibited the growth of cowpeas but that the soil from which it was extracted was no longer inhibitory (Rice, 1974).

In addition to soil fatigue, the fact that many plants do not grow well together had long been noticed. That inhibition of one plant

was due to more than competition for light, moisture, or nutrients was shown early by Pickering (1917, 1919). Pickering (1917) demonstrated that water that was passed through soil in which certain species of grass were growing inhibited the growth of apple-tree seedlings, while water that was passed through soil alone did not. This suggested that the inhibitor was produced by the grass and carried in the water (Krebs, 1972).

During the 1920's several workers again observed that black walnut trees had an inhibitory effect on certain plants growing under and around them. Cook (1921) noted that potato and tomato plants grown near black walnut suffered from wilting and that apple trees grew poorly. Massey (1925), in a study of the inhibition of alfalfa and tomato plants planted under and around black walnut trees, noted that the zone of dead alfalfa plants covered an area two to three times the area covered by the tree canopy. He concluded that this zone must correspond to the area occupied by the walnut roots. Massey went further to show that when pieces of bark from walnut roots were added to water cultures of tomato plants, wilting resulted, and that when pieces of root bark were added to soil in which tomato plants were growing, reduced growth resulted.

Similarly, Schneiderhan (1927) observed that black walnut trees could injure or kill apple trees up to a distance of 80 feet.

In an attempt to find the toxic substance involved, Davis (1928) extracted from the hulls and roots of black walnut the substance juglone (5-hydroxy-α-napthaquinone). This compound was shown to be quite toxic to tomato and alfalfa plants when injected into their

stems.

Elmer (1932) found that volatile substances from four varieties of ripe apples inhibited normal sprout development of germinating potatoes when the potatoes were placed in closed containers or in closed rooms with ripe apple fruits. This work with ripe apple fruits was continued by Molisch (1937) who formalized the theory of allelopathy and gave it its name, although his definition covered both harmful and beneficial chemical interactions between plants. At the same time Loehwing (1937) published a review on the subject of plant-produced toxins. He concluded, however, that the theory of differential plant growth inhibitors had yet to be conclusively proven and that it was of little consequence (Rice, 1974).

Bode (1940) and Funke (1943) found that leaves of Artemesia absinthium excreted a substance, absinthiin, which was washed off by rainfall into the soil, where it inhibited the surrounding vegetation up to one meter away.

Bonner and Galston (1944) noted that in agriculturally planted guayule (Parthenium argentatum), seedlings never grew under larger plants. Additionally, those plants in the edge rows of the plantings were much larger that those in the center of the plots. Bonner and Galston identified the toxin involved as trans-cinnamic acid, which was exuded from the roots of guayule. Guayule seedlings were extremely sensitive to this compound - 100 times as sensitive as tomato plants - in their experiments.

Went (1942) noted that annual plants were seldom associated with

Encelia farinosa, a desert shrub in California. He suggested that they were excluded by phytotoxic root exudates. Experiments by Gray and Bonner (1948) showed that this lack of annual species was due to a compound produced in the leaves of Encelia farinosa and released from decomposing leaf litter. The inhibitory substance in the leaves was isolated and identified as 3-acetyl-6-methoxy- benzaldehyde. It was additionally found that this toxin was selective - while tomato, pepper, and corn were greatly inhibited, Encelia seedlings, barley, oats, and sunflower were resistant to inhibition. It was similarly shown by Massey (1925) and Brooks (1951) that the black walnut toxin was selective in its effects.

It had also been observed that fruit trees often do not grow well when replanted in soil that has previously grown the same species - referred to as the "replant problem" (Börner, 1960). This had been particularly noted for peaches (Proebsting and Gilmore, 1941; Proebsting, 1950), and apples (Börner, 1959). It was determined that a toxic component involved in the peach replant problem might be amygdalin, exuded by peach roots. The breakdown products of amygdalin were shown to be toxic and Patrick (1955) suggested that microorganisms in the soil utilize the amygdalin and produce substances toxic to peach roots.

Beginning with Evenari's (1949) review on seed germination inhibitors, several major reviews on allelopathy or aspects of it have been published. Bonner (1950) reviewed the literature on the role of toxic substances in the interactions of higher plants and concluded that many plant interactions may be chemically influenced.

Grümmer (1955) proposed a system of nomemclature for types of plant-produced toxins, based on the type of plant producing the toxin and on the type of plant being affected. He applied the name "koline" to those inhibitors produced by higher plants and inhibitory to higher higher plants.

Garb (1961) reviewed the literature on differential growth inhibitors produced by plants. Börner's (1960) review focused on the role of plant-produced toxins in the soil sickness problem. Tukey (1969) also discussed the implications of allelopathy in agriculture.

Woods (1960) reviewed the topic of phytotoxic root exudates. Rovira (1969) covered the topic of root exudates in general.

Rice (1974), in an entire book devoted to the subject, covers almost all aspects of allelopathy.

The Role of Allelopathy in Vegetation Patterning and Plant Succession

Rice and co-workers have found several species of plants to produce phytotoxic root exudates which may have significance in old-field succession (Abdul-Wahab and Rice, 1967; Neill and Rice, 1971; Parenti and Rice, 1969; Rasmussen and Rice, 1971; Rice, 1968, 1971, 1972; Wilson and Rice, 1968). Rice (1968) showed six pioneer species of grasses and forbs to produce root exudates which significantly reduced nodulation in innoculated legumes. He postulated that this would slow the rate of succession by slowing the rate of addition of nitrogen to the fields. Parenti and Rice (1969) found root exudates of crabgrass (Digitaria sanguinalis) to be toxic to other early pioneer species and to itself. For this reason it forms relatively pure stands

which then rapidly disappear.

Jackson and Willemsen (1976) similarly found root exudates of ragweed (Ambrosia artemisiifolia), a first year dominant, to inhibit the germination and growth of early invaders of abandoned fields. Ragweed failed to become established, however, in plots cleared of second stage vegetation (dominated by Aster pilosus) despite the large number of ragweed seeds present.

Newman and Rovira (1975) tested eight species commonly found together in British grasslands for allelopathic influences on each other. Leachates from the soil from pots of all eight test species were inhibitory. Four species grew more slowly when receiving their own leachates that when receiving leachates from other species. Three species showed the opposite response.

Groner (1974, 1975) showed that established plants of Kalanchoe daigremontiana (mother-of-millions) inhibit the growth of daughter plantlets that fall within the area covered by their root systems. Kalanchoe root exudates were also shown to be allelopathic to various other test species. Davidonis and Ruddat (1974) showed that inhibitors released from the roots of the sporophyte of the fern Thelypteris normalis inhibited the growth of T. normalis gametophytes and young sporophytes.

McPherson and Thompson (1972) found that seedling growth of understory plants in oak forests was inhibited by root exudates of Quercus stellata and Q. marilandica.

Leaching from above-ground plant parts is probably the greatest source of allelopathic compounds (Rice, 1974). Plants are known to be

leaky systems. That large amounts of inorganic and organic substances are leached from above-ground plant parts has been well documented (Tukey, 1966, 1971). Aqueous extracts of foliage and litter of a number of plant species have been shown to be allelopathic. Rice and co-workers have shown that in addition to phytotoxic root exudates, inhibitory leachates of leaves and litter of pioneer and seral species can have a significant effect on rates of succession in abandoned fields in the American midwest (Abdul-Wahab and Rice, 1967; Neill and Rice, 1971; Parks and Rice, 1969; Rasmussen and Rice, 1971; Rice, 1968, 1971, 1972; Wilson and Rice, 1968). Rice (1974) has suggested that pioneer species in old-field succession produce substances inhibitory to other species and to themselves. Due to autoinhibition, the pioneer species rapidly disappear and are replaced by the dominant of the second stage, Aristida oligantha, which is resistant to the inhibitors produced by plants of the first stage (Wilson and Rice, 1968). Old-fields are low in nitrogen and there is a correlation between sequence of successional species and their increasing requirements for nitrogen (Blum and Rice, 1969). The pioneer species, along with the dominant of the second stage, Aristida oligantha, and Andropogon virginicus, a later successional dominant which often persists for years in dense stands, have all been shown to slow the rate of addition of nitrogen to the fields, through the inhibition of nitrogen-fixing bacteria (Rice, 1972; Blum and Rice, 1969), nitrogen-fixing blue-green algae (Parks and Rice, 1969), and by inhibition of nodulation of legumes (Rice, 1968, 1971).

Jackson and Willemsen (1976) found shoot extracts of Ambrosia

artemisiifolia and Aster pilosus to inhibit germination and growth of early invaders of abandoned fields. Bazzaz (1975) attributed low species diversity during the fourth to tenth year of succession in southern Illinois fields to their allelopathic suppression by Andropogon virginicus. Gant and Clebsch (1975) postulated that Sassafras albidum is able to maintain itself through many stages of old-field succession by allelopathic suppression of the understory. Aqueous leachates of leaves and litter and canopy washings of Sassafras reduced radicle growth of test species.

Aqueous leaf extracts of Rumex crispus inhibited seedling growth of Amararthus retroflexus, grain sorghum, and field corn, and inhibited germination of grain sorghum and radish (Einhellig and Rasmussen, 1973). Field sampling showed reduced plant biomass in quadrats near R. crispus. Werner (1975) found that germination of Dipsacus sylvestris (teasel) in field and greenhouse experiments was inhibited by litter from various plant species, most notably quackgrass, Agropyron repens. Removal of litter just prior to sowing greatly increased germination. Petalostemon gattingeri (prairie-clover) has been found to limit the distribution of Arenaria patula in cedar glades in Tennessee (Turner and Quarterman, 1975). The inhibitors are leached from the living plants and from litter and are retained in the soil. Bell and Muller (1973) showed Brassica nigra to be allelopathic to weedy grass species in California annual grasslands, through the leaching of water-soluble toxins from standing dead stalks and from litter. Tinnin and Muller (1971, 1972) found leachates of the straw of Avena fatua to be differentially inhibitory to surrounding vegetation. The toxin was most

effective following the first rains of the growing season.

Carley and Watson (1968) found that aqueous extracts of 23 plant species inhibited the germination of clover, lettuce, radish, and wheat seeds. Del Moral and Cates (1971) tested 40 species of western Washington for allelopathic effects both in the field and in the laboratory. Nine species were shown to be inhibitory both in the laboratory and in the field. These included six conifers and three woody angiosperms. Sixteen species were inhibitory in the laboratory but not in the field, five species showed interference in the field but no inhibition in the laboratory, and ten species were neither inhibitory in the laboratory nor in the field.

Bare areas occur not only under walnut trees but under various other trees as well, including hackberry (Celtis laevigata), sycamore (Platanus occidentalis), and oak (Quercus spp.). Seed germination and seedling growth of their respective associated herbaceous species have been significantly reduced by decaying leaves, leaf leachates, and soil from under hackberry (Lodhi and Rice, 1971; Lodhi, 1976), sycamore (Al-naib and Rice, 1971), and red oak and white oak (Lodhi, 1976). McPherson and Thompson (1972) also found leachates of green leaves of oak (Quercus stellata and Q. marilandica) to restrict growth of understory plants. Frei and Dodson (1972) suggested that the distribution of orchids which are epiphytic on certain species of Mexican oaks while not on others was correlated with the absence of inhibitors in the bark of those species which supported the orchids.

Del Moral and Muller (1970) found that the bare zone around Eucalyptus camaldulensis trees was caused by an accumulation of both water-

soluble inhibitors (phenolic acids) and volatile inhibitors (terpenes). Litter accumulates through the summer and reaches a peak just before the onset of the winter rains. Volatile inhibitors are released by the leaves and are adsorbed to the soil particles. They also accumulate during the dry season, so that inhibitors are at a maximum at the onset of the rainy season.

Shrub stands in the California chaparral are characterized by sparse herbaceous vegetation and as such have been the object of several studies of allelopathy. Shrub species in these areas have been shown to exclude other vegetation through water-soluble toxins leached from crowns and litter, thorugh volatile inhibitors released from leaves, or both.

Leaf leachates of intact crowns of Adenostoma fasciculatum reduce growth of associated herbaceous species (Muller, 1966; Muller, Hanawalt, and McPherson, 1968; McPherson and Muller, 1969; Christiansen and Muller, 1975).

Muller (1966, 1970) found that Salvia leucophylla, Artemesia californica, and other aromatic shrubs release volatile terpenes which can inhibit vegetation to a considerable distance past the canopy edge. He noted that old Salvia stands were deteriorating in the centers, presumably due to auto-inhibition.

Halligan (1976) also found leaf volatiles, leachates, and soil from under Artemesia californica to be toxic to associated herb species. It was noted that this toxicity existed only at the beginning of the wet season. Muller (1970) found that the effectiveness of the volatile

toxins produced by Salvia also varied with the weather. The effectiveness of the water-soluble toxins did not.

Water-soluble toxins are released from the crowns and litter of Arctostaphylos glandulosa (Muller, Hanawalt, and McPherson, 1968; Chou and Muller, 1972) and A. glauca (Muller, Hanawalt, and McPherson, 1968). Salvia mellifera and Lepichinia calycina release volatile terpenes (Muller, Hanawalt, and McPherson, 1968). All are effective in inhibiting seed germination and herb growth.

The California chaparral is characterized by recurrent fires which destroy the shrub cover. Following fire or artificial removal of the shrubs there is a luxuriant regrowth of annual herbs and bulb-forming perennials which were not evident prior to the fire. It is suggested that fire serves to remove the source of the toxins (the shrubs) and to destroy some of the toxins present in the soil. The herbs appear as a result of germination of seeds which have been held dormant in the soil, inhibited by the toxins. As the shrubs regenerate from surviving roots and from seeds, the herbs are again eliminated (Muller, Hanawalt, and McPherson, 1968; McPherson and Muller, 1969; Chou and Muller, 1972; Rice, 1974).

Appendix C
References Cited

Abdul-Wahab, A.S., and E.L. Rice. 1967. Plant inhibition by Johnson grass and its possible significance in old-field succession. Bull. Torr. Bot. Club 94: 486-497.

Al-Naib, F.A., and E.L. Rice. 1971. Allelopathic effects of Platanus occidentalis. Bull. Torr. Bot. Club 98: 75-82.

Bazzaz, F.A. 1975. Plant species diversity in old-field successional ecosystems in Southern Illinois. Ecology 56: 485-488.

Bell, D.T., and C.H. Muller. 1973. Dominance of California annual grassalnds by Brassica nigra. Am. Midl. Nat. 90: 277-299.

Blum, U., and E.L. Rice. 1969. Inhibition of symbiotic nitrogen-fixation by gallic and tannic acid, and possible roles in old-field succession. Bull. Torr. Bot. Club 96: 531-544.

Bode, H.R. 1940. Uber die Blattausscheidung des Wermuts und ihre Werkung auf andere Pflanzen. Planta 30: 567-589.

Bonner, J. 1950. The role of toxic substances in the interaction of higher plants. Bot. Rev. 16: 51-65.

Bonner, J., and A.W. Galston. 1944. Toxic substances from the culture media of guayule which may inhibit growth. Bot. Gaz. 106: 185-198.

Börner, H. 1959. The apple replant problem. I. The excretion of phlorizon from apple root residues. Contrib. Boyce Thompson Inst. 29: 39-56.

Börner, H. 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. Bot. Rev. 26: 393-424.

Brooks, M.G. 1951. Effects of black walnut trees and their products on other vegetation. West. Virg. Exp. Sta. Bull. 347: 1-37.

Carley, H.E., and R.D. Watson. 1968. Effect of various aqueous plant extracts upon seed germination. Bot. Gaz. 129: 57-62.

Cook, M.T. 1921. Wilting caused by walnut trees. Phytopathology 11: 346.

Chou, C., and C.H. Muller. 1972. Allelopathic mechanism of Arctostaphylos glandulosa var. zacaensis. Am. Midl. Nat. 88: 324-347.

Christensen, N.L., and C.H. Muller. 1975. Effects of fire on factors controlling plant growth in Adenostoma chaparral. Am. Midl. Nat. 93: 71-78.

Davidonis, G.H., and M. Ruddat. 1974. Growth inhibition in gametophytes and oat coleoptiles by thelypterin A and B released from roots of the fern Thelypteris normalis. Am. J. Bot. 61: 925-930.

Davis, E.F. 1928. Toxic principle of Juglans nigra as identified with synthetic juglone, and its toxic effect on tomato and alfalfa plants. Am. J. Bot. 15: 620.

De Candolle, M. A-P. 1832. Physiologie vegetale. Vol. III. Bechet Jeune, Lib. Fac. Med., Paris.

del Moral, R., and R.G. Cates. 1971. Allelopathic potential of the dominant vegetation of Western Washington. Ecology 52: 1030-1037.

del Moral, R., and C.H. Muller. 1970. The allelopathic effects of Eucalyptus camaldulensis. Am. Midl. Nat. 83: 254-282.

Einhellig, F.A., and J.A. Rasmussen. 1973. Allelopathic effects of Rumex crispus on Amaranthus retroflexus, grain sorghum and field corn. Am. Midl. Nat. 90: 79-86.

Elmer, O.H. 1932. Growth inhibition of potato sprouts by the volatile products of apples. Science 75: 193.

Evenari, M. 1949. Germination inhibitors. Bot. Rev. 15: 153-194.

Frei, Sister John Karen, O.P., and C.H. Dodson. 1972. The chemical effect of certain bark substrates on the germination and early growth of epiphytic orchids. Bull. Torr. Bot. Club 99: 301-307.

Funke, G.L. 1943. The influence of Artemesia absinthium on neighboring plants. Blumea 5: 281-293.

Gant, R.E., and E.E.C. Clebsch. 1975. The allelopathic influences of Sassafras albidum in old-field succession in Tennessee. Ecology 56: 604-615.

Garb, S. 1961. Differential growth inhibitors produced by plants. Bot. Rev. 27: 422-443.

Gray, R., and J. Bonner. 1948. An inhibitor of plant growth from the leaves of Encelia farinosa. Am. J. Bot. 35: 52-57.

Groner, M.G. 1974. Intraspecific allelopathy in Kalanchoe daigremontiana. Bot. Gaz. 135: 73-79.

Groner, M.G. 1975. Allelopathic influence of Kalanchoe daigremontiana on other species of plants. Bot. Gaz. 136: 207-211.

Grümmer, G. 1955. Die gegenseitige Beeinflussung hoherer Pflanzen, Allelopathie. Gustav Verlag, Jena. 162 p.

Halligan, J.P. 1976. Toxicity of Artemesia californica to four associated herb species. Am. Midl. Nat. 95: 406-421.

Jackson, J.R., and R.W. Willemsen. 1976. Allelopathy in the first stages of secondary succession on the piedmont of New Jersey. Am. J. Bot. 63: 1015-1023.

Krebs, C.J. 1972. Ecology: the experimental analysis of distribution and abundance. Harper and Row, New York. 694 p.

Loehwing, W.F. 1937. Root interactions of plants. Bot. Rev. 3: 195-239.

Lodhi, M.A.K. 1976. Role of allelopathy as expressed by dominating trees in a lowland forest in controlling productivity and pattern of herbaceous growth. Am. J. Bot. 63: 1-8.

Lodhi, M.A.K., and E.L. Rice. 1971. Allelopathic effects of Celtis laevigata. Bull. Torr. Bot. Club 98: 83-89.

Massey, A.B. 1925. Antagonism of the walnut in certain plant associations. Phytopathology 15: 773-784.

McPherson, J.K., and C.H. Muller. 1969. Allelopathic effects of Adenostoma fasciculatum, "chamise," in the California chaparral. Ecol. Monogr. 39: 177-198.

McPherson, J.K., and G.L. Thompson. 1972. Competitive and allelopathic suppression of understory by Oklahoma oak forest. Bull. Torr. Bot. Club 99: 293-300.

Molisch, H. 1937. Der Einfluss einer Pflanze auf die andere - Allelopathie. Gustav Fischer Verlag, Jena. 106 p.

Muller, C.H. 1966. The role of chemical inhibition (allelopathy) in vegetational compostition. Bull. Torr. Bot. Club 93: 332-351.

Muller, C.H. 1970. Phytotoxins as plant habitat variables. Rec. Adv. in Phytochemistry 3: 105-121.

Muller, C.H., R.B. Hanawalt, and J.K. McPherson. 1968. Allelopathic control of herb growth in the fire cycle of the California chaparral. Bull. Torr. Bot. Club 95: 225-231.

Neill, R.L., and E.L. Rice. 1971. Possible roles of Ambrosia psilostachya on pattern and succession in old-fields. Am. Midl. Nat. 86: 344-357.

Newman, E.I., and A.D. Rovira. 1975. Allelopathy among some British grassland species. J. Ecol. 63: 727-737.

Parenti, R.L., and E.L. Rice. 1969. Inhibitional effects of Digitaria sanguinalis and possible role in old-field succession. Bull. Torr. Bot. Club 96: 70-78.

Parks, J.M., and E.L. Rice. 1969. Effects of certain plants of old-field succession on the growth of blue-green algae. Bull. Torr. Bot. Club 96: 345-360.

Patrick, Z.A. 1955. The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. Can. J. Bot. 33: 461-486.

Pickering, S.V. 1917. The effect of one plant on another. Ann. Bot. 31: 181-187.

Pickering, S.V. 1919. The action of one crop on another. J. Roy. Hort. Soc. 43: 372-380.

Proebsting, E.L. 1950. A case history of a "peach replant" situation. Proc. Amer. Soc. Hort. Sci. 56: 46-48.

Proebsting, E.L., and A.E. Gilmore. 1941. The relation of peach root toxicity to the re-establishing of peach orchards. Proc. Amer. Soc. Hort. Sci. 38: 21-26.

Rasmussen, J.A., and E.L. Rice. 1971. Allelopathic effects of Sporobolus pyramidatus on vegetational patterning. Am. Midl. Nat. 86: 309-326.

Rice, E.L. 1968. Inhibition of nodulation of innoculated legumes by pioneer plant species from abandoned fields. Bull. Torr. Bot. Club 95: 346-358.

Rice, E.L. 1971. Inhibition of nodulation of innoculated legumes by leaf leachates from pioneer plant species from abandoned fields. Am. J. Bot. 58: 368-371.

Rice, E.L. 1972. Allelopathic effects of Andropogon virginicus and its persistence in old fields. Am. J. Bot. 59: 752-755.

Rice, E.L. 1974. Allelopathy. Academic Press, New York. 353 p.

Rovira, A.D. 1969. Plant root exudates. Bot. Rev. 35: 35-57.

Schneiderhan, F.J. 1927. The black walnut (Juglans nigra) as a cause of the death of apple trees. Phytopath. 17: 529-540.

Schreiner, O., and H.S. Reed. 1907. The production of deleterious excretions by roots. Bull. Torr. Bot. Club 34: 279-303.

Schreiner, O., and H.S. Reed. 1908. The toxic action of certain organic plant constituents. Bot. Gaz. 45: 73-102.

Schreiner, O., and E.D. Shorey. 1909. The isolation of harmful organic substances from soils. U.S., Bur. Soils, Bull. 53.

Schreiner, O., and M.X. Sullivan. 1909. Soil fatigue caused by organic compounds. Jour. Biol. Chem. 6: 39-50.

Stickney, J.S., and P.R. Hoy. 1881. Toxic action of black walnut. Trans. Wis. State Hort. Soc. 11: 166-167.

Tinnin, R.O., and C.H. Muller. 1971. The allelopathic potential of Avena fatua: influence on herb distribution. Bull. Torr. Bot. Club 98: 243-250.

Tinnin, R.O., and C.H. Muller. 1972. The allelopathic influence of Avena fatua: the allelopathic mechanism. Bull. Torr. Bot. Club 99: 287-292.

Tukey, H.B., Jr. 1966. Leaching of metabolites from above-ground plant parts and its implications. Bull. Torr. Bot. Club 93: 385-401.

Tukey, H.B., Jr. 1969. Implications of allelopathy in agricultural plant science. Bot. Rev. 35: 1-16.

Tukey, H.B., Jr. 1971. Leaching of substances from plants. In Biochemical interactions among plants. Nat. Acad. Sci., Washington, D.C. pp. 25-32.

Turner, B.H., and E. Quarterman. 1975. Allelopathic effects of Petalostemon gattingeri on the distribution of Arenaria patula in cedar glades. Ecology 56: 924-932.

Went, F.W. 1942. The dependence of certain annual plants on shrubs in Southern California deserts. Bull. Torr. Bot. Club 69: 100-114.

Werner, P.A. 1975. The effects of plant litter on germination in teasel, Dipsacus sylvestris Hud.. Am. Midl. Nat. 94: 470-476.

Wilson, R.E., and E.L. Rice. 1968. Allelopathy as expressed by Helianthus annuus and its role in old field succession. Bull. Torr. Bot. Club 95: 432-448.

Woods, F.W. 1960. Biological antagonisms due to phytotoxic root exudates. Bot. Rev. 26: 549-569.