Investigation of Possible Autotoxicity in Daucus Carota L.

Carole Greer Smith

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INVESTIGATION OF POSSIBLE AUTOTOXICITY IN DAUCUS CAROTA L.

by

Carole Greer Smith

A thesis presented to the Faculty of the School of Graduate Studies in partial fulfillment of the Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
June, 1964
ACKNOWLEDGEMENTS

The investigator wishes to express her sincere appreciation to Drs. Leo C. VanderBeek and Beth Schultz for their help in the guidance and evaluation of this study.

Special thanks are due also to Drs. Richard W. Pippen and Richard Brewer, and to Mr. Jack Meagher of the Computer Center at Western Michigan University.

Carole Greer Smith
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Section I

INTRODUCTION

Results of recent research indicate the probable existence of plant-produced chemical substances which affect the behavior of other plants. Germination-inhibiting, and growth-inhibiting substances have been found to be widely distributed throughout the Plant Kingdom (Garb, 1960). Although the presence of inhibitors has been demonstrated in laboratory experiments, their ecological significance has not been clearly defined.

A preliminary study by the writer indicated the probability of autotoxicity in *Daucus carota* L., a biennial common to fields and roadsides in the northeastern United States. This study suggested the following investigation which was undertaken to determine more specifically the effects of extracts from various parts of *Daucus* plants on the growth of *Daucus* itself as well as on that of other species.
Section II

CHEMICAL ASPECTS OF PLANT INTERACTION

A Review of the Research

Competition among plants for such factors as water, soil nutrients, and sunlight, has long been widely accepted as the most important aspect governing natural plant relationships (Clements, Weaver, and Hanson, 1929). Within the last forty years, methods of plant interaction by means of chemical substances have come under investigation. These chemical substances are believed to be produced and exuded by living plants or released with the decomposition of the dead plant. These substances may affect the germination and/or growth of the same, or other species.

Germination inhibitors

Germination inhibitors have been found in practically all of the structures that act as coverings of the seed, or as coverings of the embryo within the seed (Toole, Hendricks, and Botworth, 1956). In nature, these inhibitors prevent, by self-inhibition, premature germination; extend the germination over a long period of time; and/or suppress germination of other species in the area surrounding the plants producing the inhibitors. Many investigators have found germination inhibitors in other plant parts such as roots, bulbs, leaf sap, and fruit, which are not always structurally associated with
seeds, but which come in contact with them in the soil after the seeds are shed (Evenari, 1949; Konis, 1947).

Natural germination inhibitors have been identified by Evenari (1949), as ammonia, hydrogen cyanide, ethylene, essential oils including both aldehydes and mustard oils, alkaloids, unsaturated lactones, and unsaturated acids.

The specificity or non-specificity of these inhibitors is unknown. In some cases, the toxic effects are specific, affecting one kind of plant and exerting no effect on another kind of plant. For example, flax, which is suspected of exuding toxic substances, should not be planted after flax crops, but grains or legumes may be planted after it (Becquerel and Rousseau, 1941, in Woods, 1960). Other studies have indicated the non-specificity of germination inhibitors. The inhibitor in seeds of Trifolium pratense and fruits of Beta vulgaris inhibited the germination of seeds in 28 species belonging to 14 families (Froeschel, 1939, in Evenari, 1949). Tomato juice is an effective inhibitor on tomato seeds, wheat, barley, oat, and maize grains (Konis, 1940).

Growth inhibitors

Growth inhibitors, by definition, may be any chemical substances which affect the vegetative or reproductive growth of plants. Growth inhibitors have been demonstrated abundantly under laboratory conditions (Bonner, 1950). Attempts
to relate these noted inhibitors to actual field occurrences have been difficult. The many aspects of the biological and physical environment make it almost impossible to identify a single explanation for plant associations. For this reason, laboratory findings have been limited in their ecological application.

Some work, however, seems to have been successful in explaining phytosociological phenomena in nature.

In analyzing the "soil sickness" problem, Schriener (1907) found that soil solutions from fields repeatedly planted to one crop inhibited the growth of wheat seedlings. Keever, (1950) reported that succession of abandoned fields in North Carolina was influenced by the decay of old plant parts. Horseweed, *Leptilon canadense* L., lost its dominance after the first year because its seedlings were stunted by decay products from horseweed roots.

Living rhizomes of *Agropyron repens* appear to inhibit the growth of some weedy species (Hamilton and Buchholtz, 1955). Decomposing rhizomes of *Helianthus occidentalis* have been reported to inhibit growth and flowering of the same species, thus effecting the "fairy ring" of sunflowers (Cottam and Curtis, 1950).

Toxic substances are believed to be washed out of leaves during a rain, as theorized by Bode (1940, in Grummer, 1961) to account for the action of *Artemisia leucophylla*. Volatile substances produced in the leaves of *Salvia leucophylla* are
thought to be deposited when dew condenses on affected seedlings in the field. (Muller, Muller and Haines, 1961).

Went (1942) noted a pattern of shrub and annual association in a southern California desert, in which herbs seldom grew around the base of the shrub Encelia farinosa, while other shrubs harbored several species of annuals growing in their shade. Gray and Bonner (1948) found that the leaves of Encelia inhibited the growth of tomato and other plants. Muller (1953) reported evidence that threw some doubt on the ecological significance of this phenomenon. Leaf extracts of several desert shrubs inhibited the growth of the very plants that were associated with them in the desert. Grummer (1961) has postulated that the amount of detritus collected beneath the shrubs is an important factor in determining the effectiveness of the toxic substances, and that Muller's work does not eliminate toxic principles as methods of plant interaction.

Although methods of plant interaction by means of underground routes have been questioned, preliminary work has indicated possible mechanisms of interaction.

Preston, Mitchell, and Reeve (1954) demonstrated that plants can take up foreign metabolites through their roots. Alpha-methoxyphenylacetic acid, introduced into the stems and leaves of bean plants, was found within nine hours in untreated plants that were growing in the same pots as the treated ones.

Adsorption of antibiotics on clay colloids occurs in soil, so that concentration of toxic substances in soil water is greatly reduced, thereby limiting their effectiveness (Brian,
1957). However, adsorbed antibiotic is not necessarily biologically inert, since streptomycin, strongly adsorbed in soil and unextractable by solvent techniques, was still inhibitory to bacterial cells sufficiently near to the clay-streptomycin complex. (Pramer and Starkey, 1951). The type of soil plays an important part in the effectiveness of a plant excretion. The effect of Artemisia absinthium on neighboring plants was much more marked in sand than in normal, rich soil (Grummer, 1961).

Microorganisms are believed to affect plant relationships. In many cases the result is probably the inactivation of toxic substances, however, the possibility exists that nontoxic compounds may be decomposed or transferred to phytotoxic ones. Amygdalin, a natural constituent of peach root bark, is nontoxic to peach seedlings, while the breakdown product, benzaldehyde, shows considerable toxic effect (Borner, 1960).

Factors affecting germination

Although many factors affect seed germination, certain conditions are universally essential. One of the most important of these is temperature. The temperature must be high enough to activate enzymes and thereby initiate seed respiration, but not so high that the enzymes would be denatured. Fluctuating temperatures are believed to be important in bringing about a physiological balance within the seed that initiates germination. Moisture needed for rehydration and oxygen for respiration are also essential factors. The amounts needed vary with each species, since water sufficient for imbibition, but not an
amount that would inhibit gaseous exchange, necessarily differs with the size of the seed.

Before germination can occur, seeds must be in a non-dormant state. Dormancy may be caused by a variety of conditions (Amen, 1963); (1) rudimentary embryos, (2) physiologically immature embryos, (3) mechanically resistant seed coats, (4) impermeable seed coats, and (5) presence of germination inhibitors. Seeds of annual plants are usually non-dormant after a period of exposure to harsh physical conditions.

Factors affecting growth

The rate of plant growth, after germination, depends upon the availability of appropriate soil nutrients, moisture, sunlight, and carbon dioxide. The universal need for these factors results in plant competition, when one or more factors is in inadequate supply.

A study of the effects of toxic substances is best carried out when competition for these factors is minimal, such as during the first few days following germination. At that time, the seedlings need no light, and are able to utilize the nutrients in the endosperm of the seed.

Discussion of research methods

The prevalent method for testing germination inhibitors is to germinate various seeds in Petri dishes on filter paper moistened by the solutions to be tested (Konis, 1947). Sometimes the plant parts containing inhibitors are put into the same dishes with the seeds.
Some authors have attributed inhibition solely to the osmotic pressure of the extracts used. Oppenheimer (1922, in Evenari, 1949) concluded that the osmotic pressure of tomato juice is not responsible for its inhibitory action. Work by others (Evenari, 1949) indicates that in many cases osmotic pressure is one of the inhibiting factors, sometimes the most important, but that there are other factors also.

The question as to whether pH values in inhibitory aspects are important, has been raised by some workers. In an extreme case, that of lemon juice, the extremely acid pH (about 2.5) makes it probable that this factor may be responsible for the germination inhibition. However, experiments by Eny and Sinclair (1945) have shown that even in this extreme case, pH alone was not enough to explain the inhibition. Hydrogen ion concentration contributes to inhibition but is not its only cause. Konis (1939, in Evenari, 1949) has shown that there is not a relation between the inhibitory action of leaf sap and its acidity. The sap with the lowest pH inhibited germination least. Saps with neutral or slightly basic pH are no less inhibitory than the acid ones.

Substances which inhibit the germination of wheat have been demonstrated in the leaf sap and root sap of Daucus carota var. sativa, by Konis (1947) and Evenari (1949). The following investigation continues the study of Daucus carota extracts with emphasis on autotoxic effects.
Section III

DESCRIPTION OF THE EXPERIMENT

Statement of Problem

The purpose of this study was to determine whether or not *Daucus carota* L. contains chemical substances which inhibit germination and/or growth of its own seedlings. That is, does *Daucus carota* L. have autotoxic effects that might be of significance in explaining growth patterns of said species in the field. Fogg (1945) has reported that *Daucus* is seldom a dominant plant but occurs sporadically with little tendency toward mass effect.

A second line of study was conducted to learn whether observed effects were unique to *Daucus*.

Methods

To determine any autotoxic principles in *Daucus carota* L., seeds were germinated in extracts of the mature plant under controlled conditions. Germination rates and growth of seedlings were recorded.

In order to determine whether or not noted effects were species specific, similar tests were conducted on seeds of *Avena sativa* (Clinton variety), *Triticum vulgare* (Monon variety), and *Cucumis sativus*.

Test procedure for determining autotoxicity

Petri dishes, two inches in diameter, were sectioned into eight squares by cross-hatched lines made with wax pencil on
the outside of the lower halves of the dishes. The dishes were lined with Armstrong's filter paper which was then moistened with 1.5 ml. of the test solution. One D. carota seed was placed in each section and the lid was sealed with vaseline. One hundred and sixty seeds were used in each test.

The Petri dishes were placed in a controlled environment laboratory in which light was entirely eliminated except for brief periods during checking. Temperature was regulated at 75°F and 65°F for 16-hour and 8-hour periods, respectively. Relative humidity remained at 75% to minimize the possibility of evaporation of solutions. The date of germination was noted for each seed and growth was measured one week from germination. Length of hypocotyls and epicotyls was recorded.

**Preparation of test solutions**

Extracts of Daucus carota were prepared from both live and dead plants. Leaves or roots were chopped and macerated using a mortar and pestle. A stock solution was prepared by adding distilled water to the plant parts in the ratio of 2 ml. water/1 gm. substance. The resulting solution was strained through cheesecloth and filtered through Armstrong's filter paper. Varying concentrations were prepared by adding distilled water to the stock solution.

Exudates from Daucus carota seedlings and leaves were also tested on Daucus seeds. Water in which week-old seedlings had been growing was used as a test solution. A leaf exudate was prepared by immersing intact Daucus leaves in distilled
water for a 24-hour period prior to testing.

Distilled water provided the control solution for each experiment.

Solutions tested:

Stock solution, dead leaves extract
Stock solution, dead roots extract
Stock solution, fresh leaves extract
50% stock solution, fresh leaves extract
25% stock solution, fresh leaves extract
12.5% stock solution, fresh leaves extract
6.25% stock solution, fresh leaves extract
3% stock solution, fresh leaves extract
Stock solution, fresh roots extract
50% stock solution, fresh roots extract
25% stock solution, fresh roots extract
12.5% stock solution, fresh roots extract
6.25% stock solution, fresh roots extract
3% stock solution, fresh roots extract
Live leaf exudate
Live seedling exudate

Test procedure for determining antibiotic effects

Seeds of Clinton oats, Monon wheat, and cucumber were used to demonstrate the effects of wild carrot extracts on germination and growth.

Plastic growth chambers, 6" in diameter and 2½" tall, were lined with Armstrong's #6 filter paper and moistened with 13 ml. of test solution. Twenty seeds, in rows of five,
were placed in each container and the lid was tightly secured. One hundred seeds were used in each test.

The containers were placed in the controlled environment laboratory where relative humidity was maintained at 75% and temperature regulated at 75°F and 65°F for 16-hour and 8-hour periods, respectively.

Date of germination was recorded for each seed and length of hypocotyls and epicotyls was measured after four days. The stock solution of fresh leaves, 50% stock solution of fresh leaves, 25% stock solution of fresh leaves, 12.5% stock solution of fresh leaves, stock solution of fresh roots, 50% stock solution of fresh roots, 25% stock solution of fresh roots, and 12.5% stock solution of fresh roots, were tested for their effects on wheat, oats, and cucumber.

All results were statistically analyzed to determine differences of significance. Germination data were treated with the chi square test and chi square correction factor for two samples. Data on seedling growth were treated with the "t" test of significance for two sample means, with unpaired variates.
Section IV

RESULTS

Effects on Germination

Extracts of fresh *Daucus carota* roots and leaves did not affect the germination rate of *Daucus* seeds except at highest concentrations. It is possible that the inhibition of germination by both root and leaf extracts at this concentration can be attributed to the osmotic pressure of the test solutions. However, when the same concentrations were tested on seeds of Clinton oats, Monon wheat, and cucumber, no inhibition of germination was noted.

Neither germination nor growth of *Daucus carota* seedlings was affected by extracts prepared from dead plants of *Daucus*. No effect was noticed on *Daucus* seedlings grown in exudates from live leaves and live seedlings of the same species.

Effects on Growth

*Daucus carota*

Extracts prepared from live *Daucus carota* plants inhibited the growth of *Daucus* seedlings in the most concentrated solutions (Fig. 1). Leaf extract inhibited seedling growth more severely at high concentrations than did root extract. All dilutions of root extract caused inhibition of root growth, whereas leaf extract lost its effectiveness on roots at the 3% level.

The roots of *Daucus carota* seedlings were more sensitive
to growth inhibiting substances present in both root and leaf extracts of mature Daucus plants, than the seedling hypocotyls. Only the two highest concentrations exerted any effect on the hypocotyls.

In all of the seedlings tested, inhibition of growth by Daucus extracts was most pronounced in Daucus. Stimulation of Daucus growth by Daucus was not observed in any instance, whereas marked stimulation occurred in each of the other three species tested.

*Avena sativa*

The roots of Clinton oat seedlings were inhibited in growth by all concentrations of *Daucus carota* root extract (Fig. 3). The pattern of inhibitory action differs from that of the other three tests and may be the result of experimental error. It would be necessary to repeat this one experiment to verify the results. The strongest solution of *Daucus carota* root extract inhibited the growth of oat shoots.

Both root and shoot growth of oat seedlings was inhibited by concentrated solutions of *Daucus* leaf extract and stimulated by dilute solutions (Fig. 2).

*Cucumis sativus*

The growth of cucumber shoots was stimulated by all concentrations of *Daucus carota* root and leaf extracts (Figs. 2 & 3). Stimulation appeared to be inversely proportional to the concentration of the test solutions.
Root growth of cucumber seedlings was retarded by all dilutions of root and leaf extracts of Daucus, with the exception of the weakest solution of root extract which stimulated growth (Figs. 2 & 3).

_Triticum vulgare_

All four dilutions of Daucus root extract inhibited the growth of roots and shoots of wheat seedlings (Fig. 3). The roots of these wheat seedlings appeared to be more sensitive to the inhibitory substances than did the shoots.

_Daucus carota_ leaf extract inhibited wheat root and shoot growth in the solution of highest concentration (Fig. 2). The three lowest concentrations of leaf extract stimulated growth of wheat shoots but had no apparent effect on wheat roots.
Fig. 1. The effects of *Daucus carota* L. root and leaf extracts on the growth of *Daucus carota* L. seedlings (based on data in Tables 1, 2, 3, and 4).

<table>
<thead>
<tr>
<th>Stock solution</th>
<th>Hypocotyls</th>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>6.25%</td>
<td></td>
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</tr>
<tr>
<td>12.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td></td>
<td></td>
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<tr>
<td>50%</td>
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<td></td>
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<tr>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- : root extract
- : leaf extract
Fig. 2. The effects of *Daucus carota* L. leaf extract on the growth of oat, wheat, and cucumber seedlings (based on data in Tables 1, 2, 3, and 4).
Fig. 3. The effects of Daucus carota L. root extract on the growth of oat, wheat, and cucumber seedlings (based on data in Tables 1, 2, 3, and 4).

<table>
<thead>
<tr>
<th>Stock solution</th>
<th>Shoots</th>
<th>Roots</th>
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<tr>
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<td>![Shoots Bar Chart]</td>
<td>![Roots Bar Chart]</td>
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<tr>
<td>25%</td>
<td>![Shoots Bar Chart]</td>
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<tr>
<td>50%</td>
<td>![Shoots Bar Chart]</td>
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<tr>
<td>water</td>
<td>![Shoots Bar Chart]</td>
<td>![Roots Bar Chart]</td>
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Legend:
- ■■■■ wheat
- •••• cucumber
- // oats
Table 1. Mean growth in millimeters of shoots grown in carrot leaf extract.

<table>
<thead>
<tr>
<th></th>
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<th>25% stock</th>
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<td>32</td>
<td>33</td>
<td>36</td>
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<td></td>
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<tr>
<td>Oats</td>
<td>53</td>
<td>38</td>
<td>50</td>
<td>59</td>
<td>62</td>
<td></td>
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</tr>
<tr>
<td>Wheat</td>
<td>44</td>
<td>37</td>
<td>47</td>
<td>50</td>
<td>54</td>
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Table 2. Mean growth in millimeters of shoots grown in carrot root extract.

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<td>33</td>
<td>33</td>
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Table 3. Mean growth in millimeters of roots grown in carrot leaf extract.

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<td></td>
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<td>Oats</td>
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<td>98</td>
<td>107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>69</td>
<td>49</td>
<td>68</td>
<td>70</td>
<td>79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Mean growth in millimeters of roots grown in carrot root extract.

<table>
<thead>
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<th>50% stock</th>
<th>25% stock</th>
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<th>6.25% stock</th>
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<td>9</td>
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<td>Cucumber</td>
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<td>49</td>
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</table>
Section V

REMARKS

Discussion

Konis (1947) and Evenari (1949) in their work on germination and growth inhibitors, found that substances in root and leaf extracts of *Daucus carota* var. *sativa* inhibited the germination and growth of Nursi wheat grains. Evenari's work is unavailable, so a comparison of procedure and results can only be made between my work and the work of Konis.

Konis found that the leaf extract of carrot, which was boiled in preparation, inhibited the germination of wheat in dilutions up to 1:25. In a 1:1 solution he observed complete inhibition of germination. My experiments involved the use of extracts from *Daucus carota* L. which were tested on Monon wheat. I found no significant inhibition of germination in a 1:2 (stock) solution of leaf extract, or in any lower concentrations.

Growth of wheat radicles in Konis' experiment was inhibited to a greater extent than in my study. He observed 100% inhibition of growth in a 1:1 solution, and 53% inhibition in a 1:5 solution. In my study, leaf extracts of *Daucus carota* L. caused 29% inhibition of wheat radicles grown in a 1:2 (stock) solution, and no
inhibition of growth in a 1:5 (50% stock) solution.

Differences in results may perhaps be attributed to the difference in varieties tested, however, boiling of the extract (by Konis and Evenari) may have altered its composition and thus changed its inhibitory action. If ecological application is the ultimate aim of these studies, then it is of utmost importance to approximate natural conditions in so far as possible. Heating to boiling of plant-produced substances does not usually occur in nature.

Konis measured growth by average weight of radicles and therefore his results did not show the effects of the extract on shoot growth. To determine any differential growth pattern caused by the extracts, I measured growth by length of roots and shoots. This provided a more accurate picture of the effects of the extracts on seedling growth. For example, growth of cucumber roots was inhibited by both extracts tested, but shoot growth was stimulated. This phenomenon would not have been noted if measured by the procedure used by Konis.

Evenari (1949) states that "Germination - and growth-inhibition are nearly always associated with one another". Evidence from my experiments indicates that this is not always the case since the substances that inhibited the growth of oat, wheat, and cucumber seedlings were inactive upon germination.

My study indicates that root and leaf extracts of Daucus carota L. contain substances which affect the growth of not only wheat, but also Daucus itself as well as other
seedlings. The effects vary with the origin of the extract, the concentration of the solution, and the species tested. There is also variation in effect on different parts of the plant.

Conclusions

Origin of extract

Daucus root extract was particularly inhibitory to the root growth of the four species tested. Leaf extract inhibited root growth but not to the same extent as root extract. Leaf extract stimulated the growth of oat and wheat shoots to a greater extent than root extract, while the reverse was true of cucumbers.

Concentration of solution

The inhibiting effect is a function of the concentration of the solution, being less marked at low concentrations. In several tests, inhibition was replaced by stimulation of growth at low concentrations.

Species tested

The effect of Daucus extracts upon seedlings of the same species differed notably from the effects exerted on the other three species tested. Inhibition of growth was more severe and there was little significant stimulation of growth even at extremely low concentrations. It is doubtful that osmotic pressure of the test solutions is the only factor retarding growth, since even the most concentrated solutions stimulated growth of cucumber shoots.
Part of plant tested

In general, roots appeared to be more sensitive to the inhibitory action of the extracts than did the shoots, whereas the shoots were more sensitive to stimulatory action. The difference in inhibitory action may be caused, in part, by the fact that any substance entering the plant does so through the roots and concentration there would necessarily be greater.

Ecological and Agricultural Application

The ecological values of these findings is difficult to assess. Few cases of autotoxicity have been reported but the mechanism would presumably aid in the perpetuation of the species by preventing overgrowth of plants on inadequate soil, with subsequent death of the entire group (Garb, 1960). If autotoxicity plays a role in the distribution of Daucus carota L. in the field then its method of interaction needs to be studied.

The effects of Daucus on cultivated plant forms may have significant value in agriculture. It has been known for centuries that weeds are injurious to crops. The explanation that weeds utilize moisture and nutrients that are needed for the cultivated plant has been questioned, since most weeds are smaller than the cultivated plants with which they compete. It appears that some additional mechanism is involved in the competition between some weeds and crops. In a few cases it has been suggested that this mechanism involves secretion of differential growth inhibitors (DeCandolle, 1832, and Thatcher, 1923; in Garb, 1960).
SECTION VI
SUMMARY

Extracts from the roots and leaves of *Daucus carota* L. were tested for their effects on the germination and growth of *Avena sativa* (Clinton variety), *Triticum vulgare* (Monon variety), *Cucumis sativus*, and *Daucus carota* L., itself. It was observed that substances present in both root and leaf extracts affected the growth of the four species tested. The effects varied with the origin of the extract, the species tested, the concentration of extract, and the part of the plant affected. *Daucus carota* L. was most severely affected by the extracts.

Results were compared to those of a previous study. A conclusion was reached that germination-inhibition is not necessarily coupled with growth-inhibition.

The value of possible phytochemical plant interactions to ecology and agriculture was discussed.


