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Investigations of Inhibitors, Dormancy, and Photoperiod in Common Ragweed

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INVESTIGATIONS OF INHIBITORS, DORMANCY, AND PHOTOPERIOD IN COMMON RAGWEED

BY

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

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INTRODUCTION

Investigation of common ragweed, Ambrosia artemisiifolia, variety elatior L., revealed several interesting aspects. Those reported in the literature include requirement of a short photoperiod for flowering and changes in the sex ratio in relation to photoperiod (6). Seed dormancy was reported in Ambrosia trifida L., a related species (3). Other possible aspects which deserve investigation include an extractable plant growth inhibitor, modification of flower parts in relation to photoperiod, and modification of leaf shapes in relation to photoperiod.

The primary objectives of this research were to determine the location within the plant, solubility characteristics, a method of purification, and the effects of the inhibitor on other biological organisms. Secondary objectives dealt with the dormancy of ragweed seeds and the effects of photoperiod upon ragweed plants.

The method selected to carry out the objectives involved: (a) location and extraction of the inhibitor at various stages of development of ragweed, (b) purification of the inhibitor, and (c) determining the effects of the inhibitor on other biological organisms. It was suspected that in this way the actual role of the inhibitor in ragweed could be elucidated. That the inhibitor has a definite morphogenetic role in controlling growth is possible. It may be a metabolic product having no effect on the ragweed, but having an effect on other biological organisms. It was hoped that this research would provide information on these questions.

REVIEW OF THE LITERATURE

Inhibitors are chemical compounds which repress the germination of plant seeds and the growth of plants. The naturally occurring growth inhibitors are numerous and show great variance in their chemical structure (1, 11). Evidence indicates that by far the majority are organic compounds, but in some cases inorganic products can be effective (1). Some of the groups of chemical compounds known to have significance as inhibitors are the unsaturated lactones, phthalides, mustard oils, essential oils, organic acids, alkaloids, cyanides, aldehydes, and flavonoids (1, 2, 4). Inhibitors can be isolated from practically all plant parts. Many actively growing plant parts are excellent sources of inhibitors and these same inhibitors may be found in the plant seeds (10).

There is some controversy as to the changes of inhibitor levels in contrast with the degree of dormancy of seeds. By far the greatest number of investigations indicated that there are no significant differences in inhibitor levels and degree of seed dormancy. It has been shown that when dormant seeds, which contain inhibitors, were given a cold treatment for periods up to six months, and despite the fact that they germinated readily when transferred to warm conditions, little change in the inhibitor level resulted (13). This same investigation showed that extracts from seeds having their dormancy broken did have certain substances not present in unchilled embryos.

The causes of dormancy are either physical, chemical, or a combination of physical and chemical (9). The physical causes of dormancy are the structure of the seed coat, the layer of tissue surrounding the embryo, and the embryo itself. The seed coat may be impermeable to water, oxygen, acid, or some specific mineral nutrient. The seed coat may be mechanically resistant to the expansion of the embryo, and only when this seed coat is broken or decayed will the seed germinate. The embryo may be completely immature and thus require a period of time for maturation. Only when such an embryo matures will the seed germinate. A high salt concentration may be present in the seed, and only when this salt is removed will the seed germinate (14).

The chemical causes of dormancy are the inhibiting substances within the seed. An inhibiting substance may be located within the tissues surrounding the embryo and/or within the embryo itself (9).

The combination of physical and chemical causes of dormancy functions in those seeds which require specific light and temperature as a conditioning for germination. Many seeds require a period of low temperature in order to condition them for germination. It has been shown that the embryos of Ambrosia trifida L. are dormant at maturity and that the time required for the after-ripening of freshly harvested fruits, at the optimum low temperature of 5 degrees centigrade, is about 3 months (3).

An experiment has been carried out, using Fraxinus excelsior, to determine whether the application of the inhibitor to the non-dormant embryos of Fraxinus prevented their growth (13). Embryos were

first leached for 48 hours. One-half of the leached embryos were planted on moist filter paper and one-half were planted on filter paper containing the inhibitor extracted from seeds. The leached embryos readily germinated while those in the inhibitor solution failed to germinate. It seemed that dormancy was controlled by the inhibitor.

The mechanism and site by which the inhibitor acts are quite obscure due to the large variety of chemical inhibitors. Krebs (7) proposed three possible sites; namely, the specific ribosome, the structural gene, and the enzyme itself.

Robinson (10) listed the functions of alkaloids in plants as waste products, storage reservoirs, protection of the plant against parasites and herbivores, growth regulation, and the replacement of mineral bases in the soil. These same functions can be applied to all inhibitors with only slight limitations.

Audus (1) left little doubt as to the significance of plant inhibitors. He stated that many plants excrete substances which inhibit the growth of other plants and thus partially eliminate competition with natural and cultivated plants. This is how some of our noxious weeds, even though they are small, may be able to compete with the large cultivated crops.

Concerning the effects of photoperiod treatments of ragweed, an investigation by Jones (6) showed that the development of pistillate ragweed flowers was accelerated and that of staminate flowers was retarded by continuous short days. When the plants were returned to natural light conditions, the pistillate flowers were replaced by staminate flowers, even to the extent that some of the plants became entirely staminate.

MATERIALS AND METHODS

Locating the Inhibitor

Two hundred ragweed plants were planted in the botany garden and from these plants the seeds were collected. From plants growing in waste areas, both vegetative and flowering plant parts were collected. The vegetative plants were divided into roots, old stems, young stems, young leaves, and terminal buds. The flowering plants were divided into roots, old stems, old leaves, terminal flowering buds, and male flowers. The fresh weight of each portion was then determined and sufficient deionized water added to obtain the proportion of one gram of ragweed to five grams of deionized water. The ragweed material with the liquid was ground in a Waring blender, and filtered through cheesecloth. Six milliliters of the filtrate were placed in each of three Petri dishes containing a germination blotter and 50 radish seeds (Raphanus sativus L.). The seeds germinated, in an incubator at 27 degrees centigrade, for three days. The germination percentage of all the seeds plus the average root length of ten radish seedlings, picked at random, were recorded. Radish seeds were used because they show the effects of the inhibitor, have a high percentage of germination, germinate and grow rapidly, mature rapidly, and can be easily obtained.

Purifying the Inhibitor

Several methods of purification of the inhibitor were used. An inhibitor was extracted from ragweed male flowers, using a Soxhlet extractor, with the period of extraction being approximately three

days. The solvents used in the extraction process were deionized water, ethyl alcohol, methyl alcohol, and petroleum ether with a boiling point of 30 degrees to 60 degrees centigrade.

Both column chromatography and paper chromatography were used in the preliminary purification procedures. In column chromatography the column packings used were inulin, alumina, and magnesium oxide. The dimensions of the columns used were 7/8 by 12 inches. The method used in packing the column consisted of: (a) closing the bottom of the column, (b) filling the column with the solvent, as used in the extraction, to a depth of 8 to 10 inches, (c) adding the solid column packing to the liquid within the column to a depth slightly greater than 12 inches, and (d) opening the closed end of the column, letting the liquid flow through (12). When the liquid was within one-fourth inch from the top of the solid, the plant extract was carefully added to the top of the column. The extract was concentrated and 15 milliliters were added. A circular piece of filter paper, or a portion of glass wool, was placed on top of the packing to prevent the disruption of the top of the column when the extract was poured into the column.

Both descending and ascending paper chromatography were used. The descending chromatographic solvents used contained various combinations of butanol, acetic acid, and water. The ascending chromatographic solvents used were: combinations of butanol, acetic acid, and water; 1 per cent hydrochloric acid; 1 per cent ammonium hydroxide; butanol saturated with water; and various concentrations of

acetic acid. The length of the paper used in the descending paper chromatography was approximately 15 inches, while the ascending paper length was only 8 inches. The paper used for each was Whatman number 1 and Whatman number 3 MM, respectively. The extract was spotted on the paper according to normal chromatographic procedures.

The final method of purification involved a fractionation process in which ragweed male flowers were extracted, using absolute ethyl alcohol in the Soxhlet extractor. This extract was centrifuged, the solution decanted, and evaporated to dryness. Petroleum ether was added to the solid portion remaining after the evaporation of the decanted liquid. The solution was brought to boiling and thoroughly mixed with the solid remains. The solution and remains were centrifuged and the solution decanted. The petroleum ether was poured on top of a large container of water and the ether evaporated. The water solution was filtered and evaporated to dryness. The remaining solid material was dissolved in absolute methyl alcohol for further use. The methyl alcohol material was further separated into its components by using ascending paper chromatography. The solvents used were 1, 10, 20, 30, 40, 50, and 60 per cent glacial acetic acid, 1 per cent ammonium hydroxide, and butanol saturated with water.

Testing the Inhibitor Effects

In each of the column and paper chromatography procedures, radish seeds were used for determining the presence of an inhibitor. For column chromatography, each portion of the liquid collected was tested by evaporating the liquid in a Petri dish. Six milliliters of water, a germination blotter, and 25 radish seeds were added to the dish. The seeds germinated for three days at 27 degrees centigrade, after which the germination percentage was recorded.

The method used for paper chromatography was quite similar to the column chromatography method, except that in this case, the paper was cut in strips with radish and cucumber seeds planted on these strips. The seeds germinated for three days at 27 degrees centigrade. The germination percentage was then recorded.

The method used to test the effect of the inhibitor on plants growing in soil was carried out in the greenhouse. Fifteen radish seeds were planted within one-fourth inch from the surface of the soil in each of ten pots. Dried and ground ragweed male flowers were sprinkled on top of the soil in amounts of 1, 5, 10, and 20 grams. The control consisted of no ragweed material on the soil surface. The experiment was repeated using only the control and 20 gram level, with the seeds planted one inch below the soil surface. The plants were then allowed to grow for one month.

A dilution of the extract of ragweed terminal buds was made, using the water extract in the normal 1 to 5 ratio. The dilutions were 1 to 10, 1 to 100, and 1 to 1000. Three replications were made

of each, and the solutions were used as the moisture source for germinating seeds.

Determining Seed Dormancy

In conjunction with the inhibitor experiment, ragweed seeds were wrapped in a moist cloth and kept in a refrigerator at approximately 5 degrees centigrade for 48 days. At the end of 0, 3, 6, 10, 13, 16, 21, 27, 33, 39, and 48 days, 25 seeds were removed and their germination percentage recorded.

Five concentrations of thiourea, ranging from 0.1 to 0.7 per cent solution, were also used in order to break the dormancy of the ragweed seeds. The seeds were placed in Petri dishes with germination blotters. Six milliliters of thiourea solution served as a moisture source for the germinating seeds. After one week the germination percentages were recorded.

Photoperiod Treatments

The photoperiod treatments involved exposing the ragweed plants to three 8-hour light periods. The method involved covering the plants at night and part of the succeeding day to keep them in continuous darkness for 18 hours. The plants were covered when they were approximately 6 inches tall and one and one-half months old. They were treated during the latter part of May and the month of June, 1962. It was thought that the photoperiod treatments might influence the amount of inhibitor produced by the plants.

RESULTS

Locating the Inhibitor

An analysis of the amount of inhibitor was made from both vegetative and flowering ragweed plants. One gram of ragweed material (fresh weight) was added to five grams of water. The germination percentage of 50 radish seeds was recorded and the average root length of 10 seedlings, picked at random, was recorded in millimeters. The effects of extracts from vegetative ragweed plant parts are recorded in Table 1 and are the means of three replications.

Table 1. The Effects of Water Extract from Vegetative Ragweed Plant Parts on Germinating Radish Seeds

Materials extracted	Per cent germination	Average root length in millimeters
Roots	100	33.6 ± 2.1
Old stems	99	29.9 ± 2.2
Young stems	97	29.1 ± 3.9
Old leaves	75	18.8 ± 0.7
Young leaves	19	2.4 ± 0.3
Terminal buds	4	2.0 ± 1.0
Control	99	37.4 ± 1.1

The effects of the water extract from flowering ragweed plant parts are shown in Table 2. The procedure used was identical to that used for the extraction of vegetative ragweed plant parts.

Table 2. The Effects of Water Extract from Flowering Ragweed Plant Parts on Germinating Radish Seeds

Materials extracted	Per cent germination	Average root length in millimeters
Roots	100	32.6 ± 2.0
Old stems	98	31.0 ± 1.4
Young stems	95	16.8 ± 1.5
Old leaves	75	15.2 ± 1.3
Young leaves	29	2.3 ± 0.4
Flowering buds	7	1.3 ± 0.4
Male flowers	10	2.7 ± 0.4
Control	99	51.2 ± 1.1

Purifying the Inhibitor

It was determined that the inhibitor can be extracted in solvents ranging in molecular polarity from slightly polar petroleum ether to polar water. The inhibitor extracted in benzene was chromatographed through column packings ranging from inulin, with a low adsorption capacity, to magnesium oxide, with a high adsorption capacity. As colored bands passed through the column, they were collected and tested for inhibitor activity. The inhibitor could be eluted with benzene from alumina, which has an above average adsorption capacity, and inulin, with a low adsorption capacity, but could not be eluted from magnesium oxide. Other eluting solvents were used without success.

Purifications of the various extracts were attempted by using ascending and descending paper chromatography. The solvents used contained combinations of butanol, acetic acid, water, hydrochloric acid, and ammonium hydroxide. None of the solvents used fulfilled

the necessary requirements for purification.

The final method of purification involved an absolute ethyl alcohol extraction, and subsequent purification with petroleum ether and water. The liquid remaining after each step of the procedure was evaporated to dryness in a Petri dish. A germination blotter was placed in the Petri dish and 50 radish seeds were planted. A diagram of the purification procedure and the resultant germination percentage is represented in Figure I. An inhibitor was present in the pellet of the petroleum ether insoluble portion of the ethyl alcohol extract (C), the residue remaining from the water insoluble portion of the petroleum ether solution (E), and the final water soluble fraction (F).

The methyl alcohol solution containing the partially purified inhibitor can be further purified, using ascending paper chromatography. In a 1 per cent acetic acid solvent, the inhibitor remains stationary. In butanol saturated with water (a neutral solvent) and in 1 per cent ammonium hydroxide (a basic solvent) the inhibitor flows with the solvent front. By using different percentages of acetic acid, the materials within the methyl alcohol solution can be separated. In 10, 20, and 30 per cent acetic acid solutions, only one compound flowed with the front. A 40 per cent acetic acid solution caused a second compound to flow with the front. A 50 per cent acetic acid solution caused three compounds to flow with the front, leaving one compound remaining stationary. The 60 per cent acetic acid solution caused all the compounds to flow with the front.

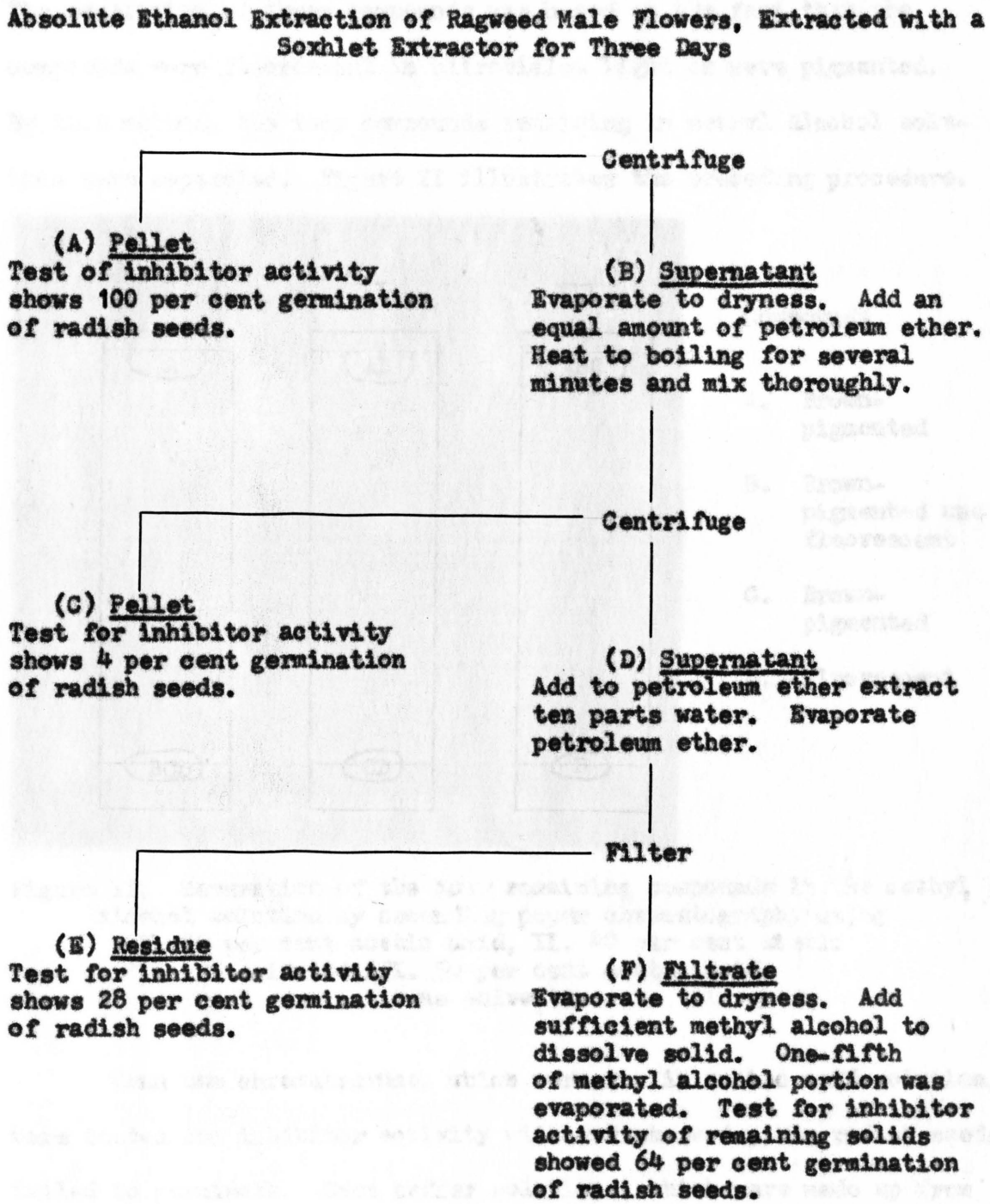

Figure I. Purification and testing an inhibitor extracted from common ragweed

The separation of these compounds was based on the fact that the compounds were fluorescent in ultraviolet light or were pigmented. By this method, the four compounds remaining in methyl alcohol solution were separated. Figure II illustrates the preceding procedure.

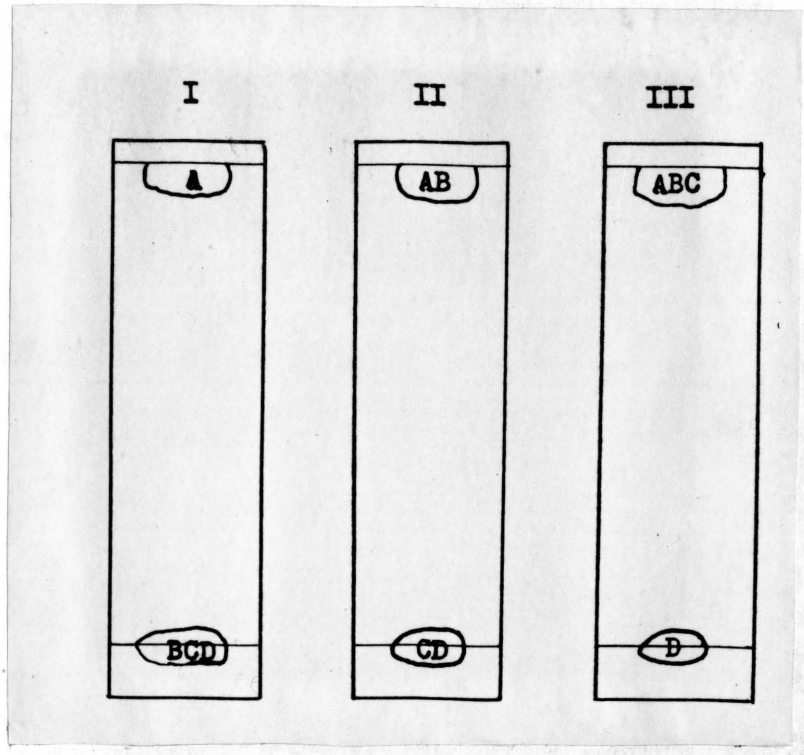

Figure II. Separation of the four remaining compounds in the methyl alcohol solution by ascending paper chromatography using I. 10 per cent acetic acid, II. 40 per cent acetic acid and III. 50 per cent acetic acid as solvents

When the chromatograms, which were run in acetic acid solution, were tested for inhibitor activity with radish seeds, the radish seeds failed to germinate. Even buffer solutions, which were made up from Na_2HPO_4 and KH_2PO_4, at pH of 5.6, 6.0, 7.0, and 7.4, produced no germination. Cucumber seeds were also planted on the chromatogram paper. Here the cucumber seeds germinated but the variability in

germination and growth was greater than the effects of the inhibitor. Under normal growing conditions the germination percentage of these cucumber seeds range from 95 to 100 per cent.

Testing the Inhibitor

The results of the application of the dried and ground ragweed male flowers on top of shallow planted radish seeds are illustrated in Figure III.

Figure III. The effects of sprinkling 0, 1, 5, 10, and 20 grams of ragweed male flowers on top of soil in pots A, B, C, D, and E, respectively

The approximate area of the soil within a pot was 1050 square centimeters. Two replications of this experiment were run with both yielding approximately the same results. Pots A and B had 100 per cent germination, C had 80 per cent germination, D had 40 per cent germination, and E had 13 per cent germination.

Using the proportion of 1 gram ragweed terminal vegetative buds to 5 grams of water, a bio-assay was made of the effects of diluting the ragweed terminal bud material. The results of the dilution are shown in Table 3. The procedure follows the method used in extracting vegetative ragweed plant portions. Only 4 per cent of the radish seeds germinated in the undiluted extract, while all of the control group germinated.

Table 3. The Effects of a Water Extract of Ragweed Terminal Buds Upon Germinating Radish Seeds

Dilution	Per cent germination	Average root length in millimeters
Normal extract	4	2.0 ± 1.0
1 : 10	99	29.7 ± 3.3
1 : 100	98	43.3 ± 1.3
1 : 1000	100	45.7 ± 2.1
Control	100	44.4 ± 4.5

Determining Seed Dormancy

The results of the cold treatment of ragweed seeds are shown in Table 4. None of the ragweed seeds germinated when they were exposed to 33 days of cold treatment at 5 degrees centigrade. At the end of 48 days, 24 per cent of the ragweed seeds germinated.

Table 4. The Effects of Cold Treatment at 5 Degrees Centigrade on Ragweed Seed Germination

Days	Germination percentage
0	0
3	0
6	0
10	0
13	0
16	0
21	0
27	0
33	0
39	8
48	24

The application of thiourea to dormant ragweed seeds gave the results shown in Table 5. The ragweed seeds were allowed to germinate for one week at 25 degrees centigrade. The thiourea concentration in which the maximum number of ragweed seeds germinated was 0.2 per cent.

Table 5. The Effects of Thiourea Solutions on Ragweed Seed Germination

Per cent thiourea solution	Per cent germination
0	0
0.1	12
0.2	18
0.3	10
0.5	4
0.7	0

Photoperiod Treatments

The final set of experiments deals with the effects of photoperiod treatment upon ragweed plants. Ragweed plants were exposed to three 8-hour light periods during the time that the daylight was 15 hours. The characteristic aftereffects of the photoperiod treatment are the production of flowers after two weeks, the production of staminate flowers, and the production of large numbers of slightly lobed leaves. Fasciation of the flowers also occurs but not as often as the entire leaves.

The following figures represent the effects of the photoperiod treatment upon ragweed plants. Figure IV shows the various leaf shapes obtained from the photoperiod treatments. Figure V shows the fasciation of flowers which occurs from the effects of photoperiod treatments. The information dealing with the effects of photoperiod upon inhibitor concentration within the plant was not obtained.

Figure IV. Various leaf shapes obtained from short light period treatment of ragweed plants (A) normal (B) result of short light period

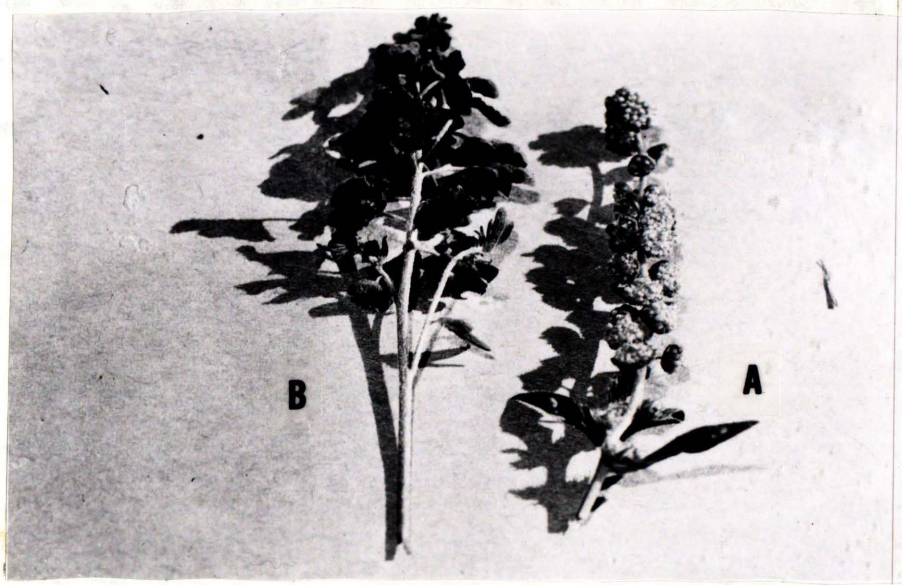

Figure V. Flower fasciation obtained from short light period treatment of ragweed plants (A) normal (B) fasciated

DISCUSSION

The results of the plant analysis, as to inhibitor content, indicated that the inhibitor was found, in the highest concentration, in the terminal buds. The actively growing regions adjacent to the terminal buds contain the next highest inhibitor concentration.

The extracts of the flowering buds were taken from the buds of the side branches as well as the primary terminal buds. This indicated that the inhibitor was found in these regions as well.

The inhibitor showed properties resembling auxins, due to its location and its action. It tends to be concentrated in the upper portions of the plant, possibly diffusing downward and acting as a growth regulator. The serial dilution of the inhibitor, as well as the experiment where the ragweed male flowers were placed on the surface of the soil, indicated that, at a very low concentration of the inhibitor, it may actually stimulate plant growth rather than inhibit plant growth. These qualities, along with the fact that the inhibitor seems to be synthesized in the terminal buds, go along with the implication that it is an auxin-like compound. More investigation should be completed in order to substantiate the auxin-like characteristics.

Observing the diagram of the final purification procedure, it would appear that there may be more than one inhibitor present in the ethyl alcohol extract and that the other inhibitors were lost during the purification process. From personal observations of the purification procedures, it was determined that the inhibitor was

not very soluble in petroleum ether or water. From these observations, it would seem likely that a large portion of the inhibitor was lost during the purification process rather than the presence of more than one inhibitor.

The possibility that the inhibitor may be a product of the extraction process can be rejected because the inhibitor can be extracted with a large variety of solvents, and it can be extracted from both dried and fresh ragweed material. Also, each solvent was tested for inhibitor activity by evaporating a portion of the solvent in a Petri dish and testing the remains with germinating radish seeds. The solutions tested did not inhibit the germination of radish seeds.

The inhibitor was extracted with hot solvents and the extract was evaporated to dryness. This extraction procedure indicated that the inhibitor was reasonably heat stable and nonvolatile.

The purification procedure shows that the inhibitor was soluble in solvents ranging in polarity from petroleum ether to water. It was much more soluble in low molecular weight alcohols than in petroleum ether or water, because it can be dissolved much more easily in the alcohol solvents. The solubility characteristics indicate a low molecular weight compound with possibly a polar and a nonpolar region. It would not have polar and nonpolar ends because if this condition existed, foaming would occur.

The compounds which remained were separated by using various concentrations of acetic acid as the solvents (See Figure II). It was proposed that if the inhibitor happened to travel with the

40 per cent acetic acid solution, then a chromatogram using the 10 per cent acetic acid solution would be run first. This paper would be dried and the top cut off to remove the front and the first compound. Using the 40 per cent acetic acid solution as the solvent, the inhibitor would then be isolated on the same paper.

The apparent reason that the direct column and paper chromatographic methods, using water, benzene or alcohol extract, did not separate the inhibitor, was that the number of compounds present in the extract was too numerous to be purified in one chromatographic process. In the column, many compounds come through at the same time as the inhibitor. On paper, the separation does not occur because many compounds have approximately the same rate of flow as the inhibitor.

It was found that when water was added to the chromatograph paper, run in acetic acid, the pH was approximately three, and this was too acid for the germination and growth of the test seeds. Therefore, a buffer solution containing N/15 Na_2HPO_4 and N/15 KH_2PO_4 was used as a solution for the germinating seeds. The buffer solutions were prepared at pH 5.6, 6.0, 7.0, and 7.4. The pH of the test solution was now raised from 3 to that of the respective buffer. The radish seeds still did not germinate readily in this solution. Even when the buffer solution was diluted 1 to 10, no increase in the radish seed germination was observed. The cucumber seeds did germinate in the solution buffered at pH of 6.0, but when recording the average root length and the per cent germination, greater

variability resulted than the effects of the inhibitor. A possible explanation of the variable germination is that either the salt concentration was too high, an inhibitor was present as a contaminate, or an inhibitor was generated by a chemical action. The high salt concentration obtained directly from the buffer seems unlikely, because this is a standard buffer for seed germination (8) in which cucumber seeds are planted at a pH of 5.6. Further tests showed that sodium acetate inhibited radish and cucumber seeds at 500 parts per million so the presence of another inhibitor seemed probable.

It is suggested that barley or asparagus seeds be planted on the chromatograph paper with the buffer solution at a pH of 6.0 as the moisture source. Both barley and asparagus are quite salt tolerant and may do satisfactorily, the location of the inhibitor and the possibility of the presence of more than one inhibitor could be determined.

Another suggestion is a solvent combination containing butanol, acetone, and water which, when prepared in the proper combination, could prove to be satisfactory for the separation of the remaining compounds. In any case, more investigation should be undertaken in this area.

When ragweed male flowers were sprinkled on top of soil in pots, and the radish seeds were planted approximately 1 inch below the ground, no effect of the inhibitor was observed. When the seeds were planted very near or at the surface, a definite inhibition of growth and germination of the radish plants resulted. If this

situation were compared to that occurring in nature, it would be expected that competing plants would drop their seeds at the soil surface. These seeds would then be in contact with ragweed plant materials. The inhibition resulting could give the ragweed plants growth advantage over the other competing plants.

The cold treatment of ragweed seeds indicated that over a month of 5 degrees centigrade cold treatment was needed for minimum percentage of germination. At the end of 48 days, one-fourth of the seeds germinated. It has been shown (3) that three months at 5 degrees centigrade is the optimum length of time for breaking the dormancy of Ambrosia trifida L. If sufficient seeds would have been given the cold treatment, the experiment should have shown results comparable to the three-month figure.

Thiourea is effective in breaking dormancy of some species of seeds. In the case of ragweed, the optimum concentration for breaking dormancy is approximately 0.2 per cent. With the application of a thiourea solution stronger than 0.7 per cent, none of the ragweed seeds germinated.

One of the effects of thiourea is the breaking of sulfur bonds. Possibly the mechanism of breaking dormancy involves the release of some protein which had been tied up by an inhibitor to a sulfur bond of an amino acid. Another possibility is that thiourea released a compound which acted as a stimulator in overcoming the effect of the inhibitor.

During the course of investigation several other aspects of ragweed growth were noticed. Jones (11) indicated that, on short days, ragweed plants developed pistillate flowers and on long days, staminate flowers. During intermediate days an equal amount of both staminate and pistillate flowers developed. The same results were found during this research.

The application of three 8-hour light periods stimulated the production of flowers. The reason why staminate flowers developed after the short day treatment was that the actual development took place during long days, and short days were only the stimulus for flowering. This investigation confirmed the stimulation of flowering by short light periods and the development of staminate flowers during long light periods.

Abnormal leaf development and the fasciation of the flowers apparently were the result of the short light period. The plant was growing under long day conditions and the immediate reversion to short days may have caused a sudden change in a growth regulating compound. A logical question would be whether the growth regulator was the same compound as the inhibitor studied in this paper, or whether it was some other compound. Due to insufficient time, a correlation with the inhibitor content and the effects of photoperiod was not completed. It is hoped that future research may be able to determine whether the ragweed inhibitor has any morphogenetic effect on the growth of flowers and leaves.

SUMMARY

Investigation of the common ragweed, Ambrosia artemisiifolia L., revealed the following:

1. The maximum inhibitor concentration was located within the terminal regions of the ragweed plant. These regions included the lateral buds, terminal buds, young leaves, flowers, and fruits. Other plant parts contained the inhibitor but in a lesser concentration.
2. The inhibitor was partially purified by a fractionation procedure involving an absolute ethyl alcohol extraction, a transfer to petroleum ether, and a final transfer to deionized water. The remaining compounds were separated by ascending paper chromatography using various concentrations of acetic acid as the solvent.
3. The ragweed inhibitor material inhibited the germination and growth of radish seeds when these were planted near the surface of the soil.
4. At the end of 48 days at 5 degrees centigrade, 25 per cent of the ragweed seeds germinated. No seeds germinated at the end of 33 days of cold treatment.
5. The optimum concentration of thiourea used for breaking the dormancy of ragweed seed was 0.2 per cent.
6. The flowering of a ragweed plant was stimulated by short light periods. Abnormal leaf and flower development occurred as the aftereffects of the treatment involving three 8-hour light periods when the plant was growing normally in long day conditions.

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