

ALLELOPATHIC EFFECTS OF *LYCOPODIUM FLABELLIFORME* SPOROPHYTES

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ABSTRACT

Experiments were conducted to test the effects of *Lycopodium flabelliforme* (Ferns.) Blanch sporophyte material on the germination and growth of seedlings of *Lycopersicum esculentum* Mill. and *Raphanus sativa* L. Three different treatments were undertaken using grindate, fresh, and extracts of sporophyte tissue. The grindate treatment produced the greatest inhibitory effects. Further tests indicated that the inhibitory substances were heat stable and concentratable. Column chromatography of extracts at 37.7°C, yielded a crude alkaloid extract with a molecular weight and size near that of glucose. *Lycopersicum esculentum* germination and seedling growth was inhibited by this crude alkaloid extraction.

INTRODUCTION

Allelopathy is defined as any direct or indirect harmful effect by one plant on another through the production of chemical compounds that escape into the environment (Rice, 1974). Many different types of organic compounds have been identified as inhibitors. Evenari (1949) stated that all seeds and fruits known for their high alkaloid content are strong germination inhibitors, and it can be shown that the alkaloids are the main, if not the only cause of inhibition in these cases.

The two genera *Lycopodium* and *Equisetum* are notable exceptions to the fact that alkaloids are rarely found in the lower vascular plants. Manske (1955) reviews much of the early literature concerned with the presence of alkaloids in the genus *Lycopodium*, and found the alkaloids lycopodine, complanatine, bases L2 to L5, obscureine and nicotine in *Lycopodium flabelliforme* (Fern.) Blanch. Later, Curcumelli-Rodostamo and MacLean (1962) identified flabelliformine, and Young and MacLean (1963) identified flabelline.

The present work is concerned with the problem of whether or not inhibitors arise from, and affect the composition of the plants associated with *Lycopodium flabelliforme* colonies. Experiments were conducted to test the effect of sporophyte material on the germination and growth of seedlings of *Lycopersicum esculentum* Mill. (tomato) and *Raphanus sativa* L. (radish). Also determined is the heat stability and molecular size of the inhibitory substance or substances.

MATERIALS AND METHODS

Preparation of Sporophyte Material: Sporophyte material of *Lycopodium flabelliforme* was collected from a colony 2 miles NW of Flat Rock, Crawford County, Illinois (Sect. 36 T6N R12W). Approximately one-third of the collected material was air-dried for 3 days, ground to a coarse grade using a Wiley Mill and placed in an air-tight container (grindate). Another one-third-portion yielded an extract by placing 25 g of sporophytes plus 250 ml of distilled water into a Waring blender and homogenizing for 10 minutes. The solution was then centrifuged at 1500xg for 10 minutes. The supernatant was centrifuged again at 1500xg for 10 minutes. The final supernatant was suction-filtered and placed in a polyethylene bottle (extract). The last one-third portion was sealed fresh in a plastic bag (fresh). All forms were stored in a refrigerator.

Allelopathic Investigations: In three separate studies, using 10 replicates per treatment, 0.25 g of grindate, 0.25 g of fresh material, and 0.10 ml of extract were placed in separate Petri dishes, with 10 ml of distilled water and 4 seeds of *Raphanus sativa* L. (radish) on a disk of Whatman No. 1 filter

paper. A control was prepared in the same way with sporophyte material excluded. The plates were incubated for three days at approximately 27°C. After the incubation period, number of seeds germinated and radicle lengths (mm) were recorded. A "germinated" seed possessed a radicle 4 times its own length.

Heat Stability of Allelopathic Substance: *Lycopodium* extract was subjected to the following test to determine its heat stability, and its ability to be concentrated. The extract material used was collected in September since it was thought that this material would possess the highest inhibitor concentrations.

Test tubes containing one ml of *Lycopodium* extract was placed in boiling water (100°C.) for the following lengths of time; 30 seconds 1, 3, 10, and 30 minutes. After this heat treatment, the test tubes were placed in an ice bath and cooled to room temperature. Extract volume was brought up to 10 ml with distilled water and poured into Petri dishes along with 10 tomato seeds (*Lycopersicon esculentum* Mill.) on a disk of Whatman No. 1 filter paper. Eight control dishes were prepared containing only 10 seeds and 10 ml of distilled water. Percent inhibition and germination was calculated after 5 and 10 days.

Molecular Size of Allelopathic Substance: Column chromatography was used to separate the different molecular sized compounds present in *Lycopodium* extract. Again, the extract was prepared from sporophytes collected in September.

Twenty ml of extract was dehydrated to dryness using a Rotary Evapo-Mix. The dried substance was rehydrated to 1 ml with distilled water, shaken using a Vortex Junior, and then loaded into a column filled with Bio-Gel P-2 (100-200 mesh), as two 0.5 ml portion followed by two 0.5 ml portions of distilled water. Fifteen two ml samples were collected and brought up to a 20 ml volume with distilled water. The solutions were then divided into two 10 ml portions and placed into separate Petri dishes along with 5 radish seeds on a disk of Whatman No. 1 filter paper. Percent germination and radicle length (mm) were recorded after 9 days. An Inhibition Value (I-Value) was calculated after del Moral and Cates (1971), where the magnitude of the I-Value is directly proportional to the inhibitory capacity of the fraction examined.

To determine the molecular size of the substance present in each faction, 0.1 ml of blue dextran/glucose solution was loaded into a column filled with Bio-Gel P-2 and thirty 1 ml fractions were collected. Each fraction was then analyzed for sugar content by adding 3 ml of anthrone, boiling for 10 minutes, cooling to room temperature, and its absorbtion read on a Bausch and Lomb spectronic 20 colorimeter.

Alkaloid Extraction: Alkaloid extraction procedures were adapted from Manske and Marion (1942). After extraction, 1 ml of distilled water was added to 10 ml of the dehydrated "crude alkaloid" and shaken using a Voltex Junior. One ml of this extract was placed with 10 ml of distilled water and 5 tomato seeds on a disk of Whatman No. 1 filter paper in a Petri dish. Plates were allowed to incubate for 9 days at room temperature (27°C.). The number of seeds germinated and radicle lengths were recorded.

Field Observations: The physiognomy of the colonies suggested more abundant growth of herbaceous plants outside of the *Lycopodium* colonies. Four randomly tossed 0.25 m² quadrat samples were taken inside, and not more than 5 m outside of the colony. The number of individuals of each species encountered per sample unit were recorded.

RESULTS AND DISCUSSION

Allelopathic Investigations: Results of the allelopathic investigations show that *Lycopodium* sporophyte material does inhibit the germination and growth of radish seedlings under laboratory conditions. Of the forms of sporophyte material used (grindate, extract, fresh), the grindate form had the greatest inhibitory effect. With this treatment the mean radicle length was 4.8 to 5.1 mm while the percent germination varied from 14 to 18%. These results are significant from all other

means at the .05% level (Table 1). Also, mean radicle length of seedlings subjected to extract and fresh sporophyte material was significantly different from the control means in two of the three replicates. The extract results for these experiments are difficult to explain since the extract was prepared from grindate material. Possible explanations are problems with the extraction procedure, and/or low concentrations of inhibitors in these spring collected sporophytes. According to del Moral and Cates (1971), if inhibitors are present in high concentrations in mature tissue, they could inhibit the growth of developing sporophytes. The low inhibition with fresh material is not surprising, since in living material it is quite possible that the inhibitor will not diffuse out of the cells.

Heat Stability of Allelopathic Substance: Results of the heat stability experiment indicates that the inhibitory substance is heat stable and can be concentrated by heating. Five-day readings (Table 2) of the number of seeds germinated revealed about 95-100% inhibition of germination for all lengths of heat treatment, taking control germination as 100%. A ten-day reading was made, but values were not significantly different from the five-day readings (t-test).

Molecular Size of Allelopathic Substance: Molecular size experiments were conducted at two temperatures. When *Lycopodium* extract was analyzed by column chromatography at room temperature (27°C.), no inhibitory effect was observed on seed germination or seedling growth. But when the temperature of the study area accidentally reached 37.7°C., an inhibitory effect was observed in fractions 11, 12, and 13 (Table 3). Utilizing a blue dextran/glucose standard curve prepared previous to the experiment and plotted simultaneously, glucose was eluted in fraction 12. This suggests the actual inhibitory substance possesses a molecular size and a molecular weight somewhere close to that of glucose (Mw = 180). Replicates of this experiment at both temperatures were performed, and the results for 37.7°C. are shown in Table 3.

When the temperature of the experimentation area reached 37.7°C. an artificial high temperature stress condition was probably created. Perhaps the inhibitory substance present in *Lycopodium flabelliforme* is effective only under high temperature conditions. Although temperature readings of the colony area were not made, soil surface temperatures in Illinois have been known to reach 37.7°C. Although the test plants were exposed to only one night of high temperature, perhaps this was sufficient to activate the inhibitors present.

Table 1. Mean radicle length (mm) and germination percentages (%G) of radish seeds subjected to 3 treatments in 3 replicates.

Control		Grindate		Extract		Fresh	
Length	%G	Length	%G	Length	%G	Length	%G
49.8	87	5.1 *	18	27.5 +	87	30.0 +	78
45.9	92	4.8	14	34.0 +	87	34.2 +	82
33.3	85	5.1 *	16	30.7	95	30.8	90

* Significantly different at the .05% level from all other means.

+. Significantly different at the .05% level from the controls.

Table 2. Percent germination (%G) and percent inhibition (%I) of tomato seeds growing in *Lycopodium* sporophyte extract subjected to various lengths of heat treatment.

Length of heat treatment	Five days		Ten days	
	%G	%I	%G	%I
30 sec	5.1	94.9	4.5	95.5
1 min	0.0	100.0	9.1	90.9
3 min	0.0	100.0	0.0	100.0
10 min	0.0	100.0	4.5	95.5
30 min	5.1	94.9	9.1	90.9
Control	49.0	0.0	55.0	0.0

Table 3. Inhibition values (I-values) for fractions of *Lycopodium flabelliforme* extracts from column chromatography (del Moral and Cates, 1971). The I-value listed is directly proportional to the degree of inhibition the collected fraction had on radish seed germination and seedling growth. Fractions 1, 2, and 3 served as controls, therefore possess no I-values. This experiment was conducted at 37.7°C. The optical density of each fraction is also given.

Fraction Number	Optical Density	I-values			
		Seed Germination		Seedling Growth	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	.55	0	0	0	0
5	.01	1	0	15	8
6	.04	10	1	19	10
7	.02	0	0	0	0
8	.03	0	0	0	0
9	.01	0	0	0	0
10	.05	0	0	0	0
11	.16	16	17	24	10
12	.49	33	47	13	12
13	.22	55	48	34	33
14	0	22	0	19	8
15	0	10	16	0	16

Alkaloid Extraction: There are many compounds possessing a molecular weight around that of glucose, ie. alkaloids and their perchlorates. Results of the alkaloid extraction experiment infer that a "crude alkaloid" fraction does exist in *Lycopodium flabelliforme*, and that this fraction does exhibit some inhibitory effect on tomato seedlings. The data obtained were analyzed using a Student t-test with a 0.1 protection level. Mean lengths of tomato radicles subjected to the "crude alkaloid" treatment were significantly shorter than the control means in two replicates. Taking control germination as 100%, a 32% decrease in germination was observed for those seeds subjected to the "crude alkaloid" treatment.

Field Observations: Because a slight change in the microenvironment can have a great effect on the inhibitory power of a compound, it cannot be assumed that because an extracted fraction of a plant inhibits another plant under laboratory conditions that this would be the case in the field. Even though a plant might release inhibitory compounds into the soil, several things could occur to render these compounds inactive. According to Muller and Muller (1956) the inhibitory material could be destroyed or altered by soil microflora to yield a harmless compound, or it may never reach a concentration high enough to inhibit surrounding flora, or it may be absorbed by the colloidal components of the soil.

From field observations and sampling (Table 4), it is postulated that *Lycopodium flabelliforme* exhibits an inhibitory effect in the field which tends to produce a large homogenous population. Six species of plants were observed growing outside the colony and were absent in the colony, while one species, *Potentilla simplex*, was found both inside and outside the colony. However, there were five times as many individuals outside the colony. *Parthenocissus quinquefolia* seemed quite tolerant of any inhibitory substance present, and perhaps even stimulated, as 32 individuals were recorded inside the colony while none were found outside. Two other species (3 individuals) were also found only inside the colony. *Pycnanthemum flexuosum* and *Solidago altissima* seemed the least tolerant as 24 and 14 individuals were recorded outside the colony respectively, and none inside. A total of 4 species and 38 individuals were found inside the colony, while 7 species and 76 individuals, twice that of inside the colony, were recorded outside the colony.

Table 4. The species and the number of plants per square meter inside and outside a *Lycopodium flabelliforme* colony.

Species	Outside Colony	Inside Colony
<i>Achillea millefolium</i> L.	5	0
<i>Aster pilosus</i> Willd.	8	0
<i>Elymus virginicus</i> L.	0	1
<i>Parthenocissus quinquefolia</i> (L) Planch.	0	32
<i>Poa compressa</i> L.	2	0
<i>Potentilla simplex</i> Michx.	15	3
<i>Pycnanthemum flexuosum</i> (Walt.) BSP.	24	0
<i>Rudbeckia hirta</i> L.	8	0
<i>Solidago altissima</i> L.	14	0
<i>Solidago</i> spp. (seedlings)	0	2
Totals	76	38

The allelopathy resulting from alkaloids produced by *Lycopodium flabelliforme* appears to be at least a partial explanation of the expanding homogenous colonies of *L. flabelliforme* in Illinois. This study supports the suggestion by Ebinger (1979) that the allelopathic conditions produced by *L. flabelliforme* were responsible for the homogenous nature of the ground pine colonies.

The introduction and spread of *L. flabelliforme* colonies in Illinois appears to be related to conifer plantings where the ground pine was incorporated in root ball packing materials (Ebinger 1979). Zimmerman and Kucera (1977) found that disturbance of natural ecosystem control mechanisms apparently caused the occurrence of large homogeneous goldenrod populations in Missouri. The relationship between allelopathy and ecosystem disturbance, for *L. flabelliforme* colonies in Illinois and adjacent sites, will be investigated in future studies.

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LITERATURE CITED

- Curcumelli-Rodostamo, M. and D.B. MacLean. 1962. *Lycopodium* alkaloids. XII. Flabelliformine. Can. Jour. Chem. 40:1068-1070.
- del Moral, R. and R.G. Cates. 1971. Allelopathic potential of the dominant vegetation of western Washington. Ecology 52:1030-1037.
- Ebinger, J.E. 1979. *Lycopodium flabelliforme* in central Indiana and Illinois. Proc. Ind. Acad. Sci. 88:357-363.
- Evenari, M. 1949. Germination inhibitors. Bot. Rev. 15:153-194.
- Manske, R.H.F. 1955. The alkaloids: chemistry and physiology. Vol. V. Pharmacology. Academic Press, Inc., New York, pp. 295-300.
- Manske, R.H.F. and L. Marion. 1942. The alkaloids of *Lycopodium* species. I. *Lycopodium complanatum* L. Can. Jour. Res. Sect. B. Chem. Sci. 20:87-92.
- Muller, W.H. and C.H. Muller. 1956. Association patterns involving desert plants that contain toxic products. Amer. Jour. Bot. 43:354-361.
- Rice, E.L. 1974. Allelopathy. Academic Press, New York.
- Young, J.C.F. and D.B. MacLean. 1963. *Lycopodium* alkaloids. XIV. Flabelline. Can. Jour. Chem. 41:2731-2736.
- Zimmerman, U. Douglas and C.L. Kucera. 1977. Effects of composition changes on productivity and biomass relationships in tallgrass prairie. Amer. Midl. Nat. 97:465-469.