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Allelopathic Effects of *Osmunda cinnamomea* on Three Species of *Dryopteris*

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Plants can reduce competition for limited resources by chemically inhibiting the growth and development of other species (allelopathy). In moist environments, the inhibitory chemicals are leached from growing or senescent leaves by rainfall, are exuded from the roots, or result from decomposition of the plant (Rice, 1984).

Most studies of allelopathy in pteridophytes have focused on the effect of fern species on angiosperms. For example, Gliessman & Muller (1978) found in southern California meadows that toxins from *Pteridium aquilinum* severely affected some species of herbs and annual grasses while not affecting or only slightly affecting others. Of the few investigations of allelopathy among fern species, even fewer have focused on species which are found in the same general habitat. Two studies involving species found in the same general habitat investigated chemical interactions at the gametophyte stage of the life cycle. Bell (1958) found that various extracts of prothalli from *Dryopteris filix-mas* inhibited germination or reduced growth in *D. borreri* gametophytes; Petersen & Fairbrothers (1980) found that gametophytes of *Osmunda cinnamomea* reduced growth in *Dryopteris intermedia* gametophytes and that *D. intermedia* gametophytes reduced growth in *O. cinnamomea* gametophytes.

Other studies of species found in the same general habitat looked at the effect of the sporophyte on the gametophyte stage of the life cycle. Bell & Klickoff (1979) discovered that sporophytes of *Polystichum acrosticoides, Polypodium vulgare,* and *Onoclea sensibilis* reduced gametophyte growth for all species but *P. vulgare.* Munther & Fairbrothers (1980) found autotoxic as well as allelopathic inhibition of spore germination from sporophytes of *Osmunda cinnamomea, O. claytonia* (no allelopathic effects), and *Dennstaedtia punctilobula.*

All of the species used in these studies, although found in the same general habitat, do not generally occur in close proximity. This paper reports on an experiment performed to determine the allelopathic effect of sporophytes of *Osmunda cinnamomea* on the number and growth rate of gametophytes of *Dryopteris carthusiana, D. cristata,* and *D. goldiana. O. cinnamomea* grows in close proximity with *D. carthusiana* and *D. cristata* but not with *D. goldiana. O. cinnamomea* is, however, found in the same general habitat as *D. goldiana.*

Both of the experiments with *O. cinnamomea* mentioned above showed allelopathic effects on *Dryopteris* and other species (Munther & Fairbrothers, 1980; Petersen & Fairbrothers, 1980). Cinnamic acid and benzoic acid derivatives, which have been implicated as allelopathic agents in a number of studies (Rice, 1984), have been found in *O. cinnamomea* (Bohm & Tryon, 1967).

Materials and Methods

Dryopteris carthusiana and *D. cristata* grow together with *Osmunda cinnamomea* in alder swamps in the southwestern Virginia mountains. Sporophytes of the three species are found in the swamps on hummocks covered with sphagnum or grass. All three species occur together occasionally on the same hummocks where *O. cinnamomea* tends to dominate because of its relatively greater size. More often, the species are segregated within the habitat. *D. goldiana,* found in steep, loamy mountain valleys in the southwestern Virginia mountains (Wagner, 1963) is not usually found growing with *O. cinnamomea.*

Fronds with mature spores were collected from *D. carthusiana, D. cristata,* and *D. goldiana* near Mountain Lake Biological Station, Giles County, Virginia, on 22 June 1988, 8 July 1988, and 9 July 1988, respectively. Each species was collected from three sites. Fronds were rinsed with water to remove extraneous spores and placed in a plant press at room temperature for 48 hrs. Spores released from sporangia were collected and stored with dessicant at 0°C.

Fresh fronds of *O. cinnamomea* were collected near Capon Bridge, Hampshire County, West Virginia, on 17 September 1988, and stored for one week at 4°C. Leachate was then prepared by placing the fronds 2 thick on top of a fiberglass screen which covered a plastic tray, 28 × 18 cm. Fronds were misted with 300 ml of deionized water. The water collected in the tray below, the leachate, was bottled and stored at 4°C (Munther & Fairbrothers, 1980) for the duration of the experiment.

Sterile potting soil was placed in six 48-cell tissue culture plates. Fifty spores of each of the three *Dryopteris* species were sown separately in each cell, yielding 16 cells per plate of each species. The environment within each culture plate was presumably more homogeneous than between plates; therefore, each plate contained spores of all species placed in randomly selected cells.

The cultures were maintained in a growth chamber with a 12 hr photo- and thermo-period. A light intensity of 200 $\mu Em^{-2}s^{-1}$ photosynthetically active radiation, 2% of full sunlight, was produced by fluorescent tubes and incandescent bulbs to simulate the average commonly found in a temperate forest understory (Hutchinson & Matt, 1977). "Day/night" temperatures were maintained at 25/18°C, simulating temperatures commonly found during the summer at the Mt. Lake Biological Station (National Climatic Center, N.O.A.A.). Half the plates were watered weekly with deionized water and half with leachate.

After 4 weeks, the number of gametophytes was counted in each cell of the tissue culture plates. Gametophyte size was measured on a maximum of 5 gametophytes in each cell using a square grid ocular micrometer in a dissecting microscope.

Analysis of variance (SAS, version 5.16) was used to determine if the leachate from *O. cinnamomea* affected the number and size of *Dryopteris* spp. gametophytes. Attempts to normalize or equalize the variances of the data by various transformations failed, thus the data were analyzed untransformed.

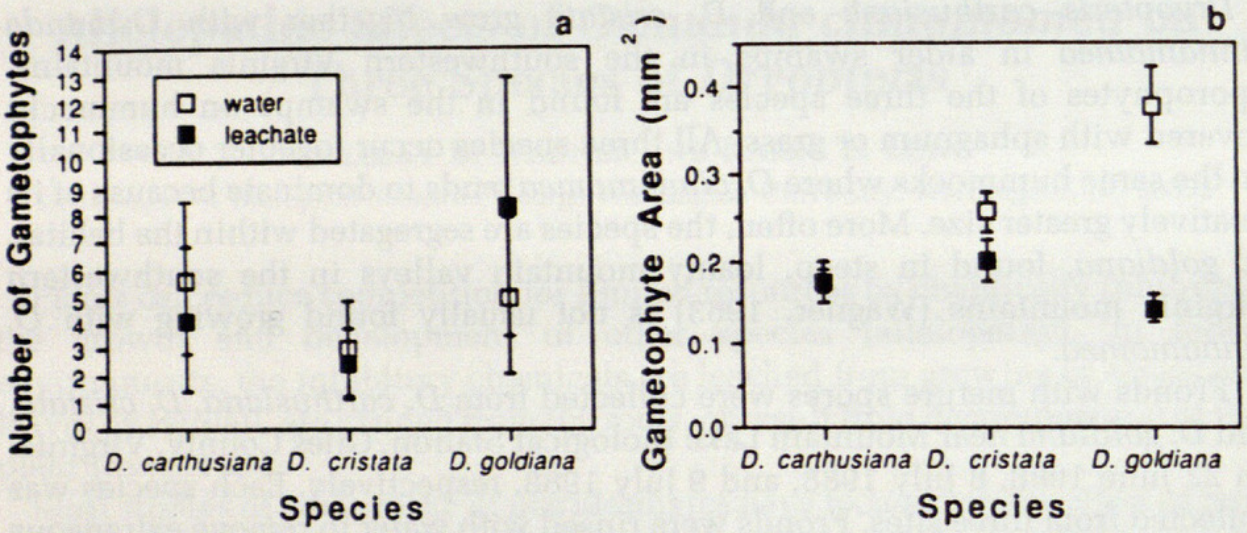

FIG. 1. Effect of leachate from *Osmunda cinnamomea* fronds on (a) survival and (b) growth of *Dryopteris* gametophytes. (Means ± S.E.).

Fortunately, ANOVA is relatively insensitive to data that are not normally distributed and have unequal variances. The placement of all species on each culture plate isolated the effect of the variation in the environment between plates from that of species and treatment, and allowed the culture plate to be handled as a block effect in the analysis. The Tukey-Kramer multiple comparison test was used to determine statistically significant differences among means (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Exposure to leachate from *O. cinnamomea* did not significantly affect the number of spores which germinated or gametophytes that survived in any of the three *Dryopteris* species (ANOVA: $F=.51$, $df=47$, $P>.767$) (Figure 1a). However, leachate significantly reduced gametophyte size in *D. goldiana* (Tukey-Kramer: $MSD=.125$, $df=86$, $P<.001$; ANOVA: $F=10.19$, $df=92$, $P<.001$) (Figure 1b). Leachate did not significantly affect gametophyte size in *D. carthusiana* (Tukey-Kramer: $MSD=.027$, $df=86$, $P>.50$) or *D. cristata* (Tukey-Kramer: $MSD=.072$, $df=86$, $P>.10$).

The results reflect a negative relationship between response to frond leachate and species that exist in the same habitat. The two *Dryopteris* species that coexist with *O. cinnamomea* are not sensitive to the leachate; the *Dryopteris* species that rarely occurs with *O. cinnamomea* is sensitive to the leachate. This suggests the development of resistance to the leachate by species that may frequently encounter it. Alternatively, the lack of resistance of *D. goldiana* to the leachate may explain the lack of close proximity to *O. cinnamomea* in the field.

Results of this study show that although the growth of gametophytes may be sensitive to leachate, the number of spores that germinate and survive is not. Germination and survival are indistinguishable in this experiment because of the manner in which the data were collected. These results agree with the conclusions of Leather & Einhellig (1985) that seedling growth in angiosperms is more sensitive than germination to allelochemicals. However, germination/survivorship rates were low and quite variable for all species (Figure 1a) with no germination/survival in 50% of the cells sown with *D. cristata* and in 36% of the cells sown with either *D. carthusiana* or *D. goldiana*. A higher germination rate of spores or a higher survival rate of gametophytes overall might have allowed statistical discrimination between treatments for numbers of gametophytes.

While a few studies have established toxicity *in situ* as well as in the laboratory (Gleissman & Muller, 1978), other studies suggest that demonstration of allelopathy in the laboratory may not reflect allelopathic interaction in the field (Stowe, 1979). The lack of correlation between lab and field may be due to lower concentrations of the allelochemical in the field than those used in the lab. Less of the toxic chemicals may be exuded or leached from the plant than predicted (Stowe, 1979) or interaction with soils, micro-organisms, and mycorrhiza may modify the chemicals or their absorption (Rice, 1984). In this experiment, concentrations of leachate in the field were unknown as were the concentrations in the lab. However, the different *Dryopteris* species responded to the concentration used in a manner consistent with their association in the field with *O. cinnamomea*.

In ferns, as in angiosperms, size is an important component of success. In competition for light, nutrients, and water, small size is a handicap at all stages of the life cycle. The smaller, slower growing gametophyte would suffer delayed development of archegonia, leading to delayed development of a less provisioned, smaller, and therefore less successful sporophyte (Näf, 1979). A sporophyte that can reduce the growth rate of potential competitors with its own gametophytes would be increasing the potential success of its progeny. The difficulty in using this argument with *O. cinnamomea* is that Munther & Fairbrothers (1980) discovered that the species exudate is autopathic as well as allelopathic. A study in which spores and gametophytes of all four species, the three *Dryopteris* and *O. cinnamomea*, are placed beneath *O. cinnamomea* in the field would help to clarify the allelopathic interactions among the species.

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