Reciprocal Allelopathy Between the Gametophytes of Osmunda cinnamomea and Dryopteris intermedia

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Allelopathy is the chemical inhibition of growth and/or development of one organism by another. The literature on allelopathy is very extensive and has been summarized in several reviews (Muller, 1970; Pickett & Baskin, 1973; Rice, 1974). See also Swain (1977) for a synoptic review of secondary compounds as allelopathic agents.

In the life cycle of any species, one portion, designated by Petersen & Fairbrothers (1973) as the weakest link, is likely to be the most vulnerable to allelopathic interactions. As an evolutionary strategem, allelopathy would be developed most effectively against the weakest link in an organism's life cycle, such as germinating spores and developing prothalli of ferns or germinating seeds and seedlings of higher plants. Furthermore, it is at these critical points that one ought to be able to best detect allelopathy.

Intra- and interspecific allelopathic interactions occur in four ways: (1) sporophytes acting on sporophytes; (2) sporophytes acting on gametophytes; (3) gametophytes acting on sporophytes; and (4) gametophytes acting on gametophytes. Seed plants reduce gametophytic vulnerability structurally by enclosing the gametophyte and ovule and functionally by pollination and fertilization. Therefore, the weakest link in seed plants is shifted from their gametophytes to their germinating seeds and seedlings. In pteridophytes, presumably the gametophyte generation—spore, prothallus and gametes—is the most vulnerable portion of the fern life cycle with regard to interspecific interactions for habitat maintenance (allelopathy and competition), for a smaller amount of chemicals would be required to eliminate a gametophyte from effective competition than a sporophyte.

Of the four interactions, gametophytes acting on gametophytes of different species permits study of the weakest link hypothesis in an experimental design where interspecific competition is minimal. Fern gametophytes were selected as experimental organisms because they are easily cultured and amenable to the experiments' requirements of control, replication, manipulation, and minimization of competition. Six fern species usually found growing in similar natural habitats were selected for the initial survey experiment which was conducted to determine if allelopathic effects were detectable between any of the species. By similar natural habitats we mean that, among the species selected, there is some overlap between their respective ecological amplitudes so that it is possible that they would be in competition for the same space.

The literature on fern allelopathy is limited but increasing. Froeschel (1953) reported that water extracts of *Polypodium aureum* and *Lycopodium clavatum*

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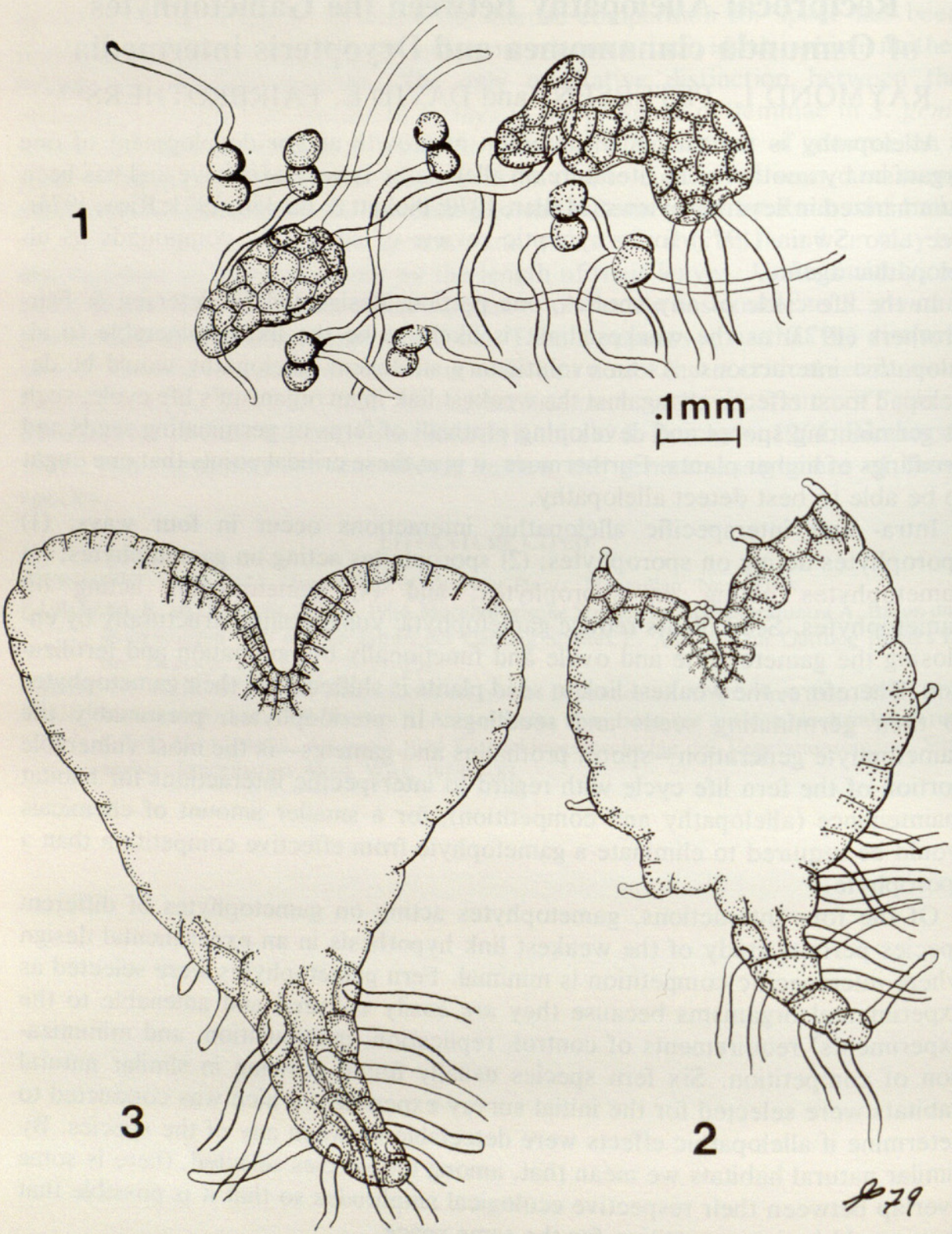


FIGS. 1–3. Gametophytes of *Dryopteris intermedia* and *Osmunda cinnamomea* after 30 days of growth. FIG. 1. Gametophytes of *D. intermedia* (2 prothallial cell stage) and *O. cinnamomea* (multicellular prothalli). FIG. 2. Typical *D. intermedia* gametophyte from a control monoculture. FIG. 3. Typical *O. cinnamomea* gametophyte from a control monoculture.

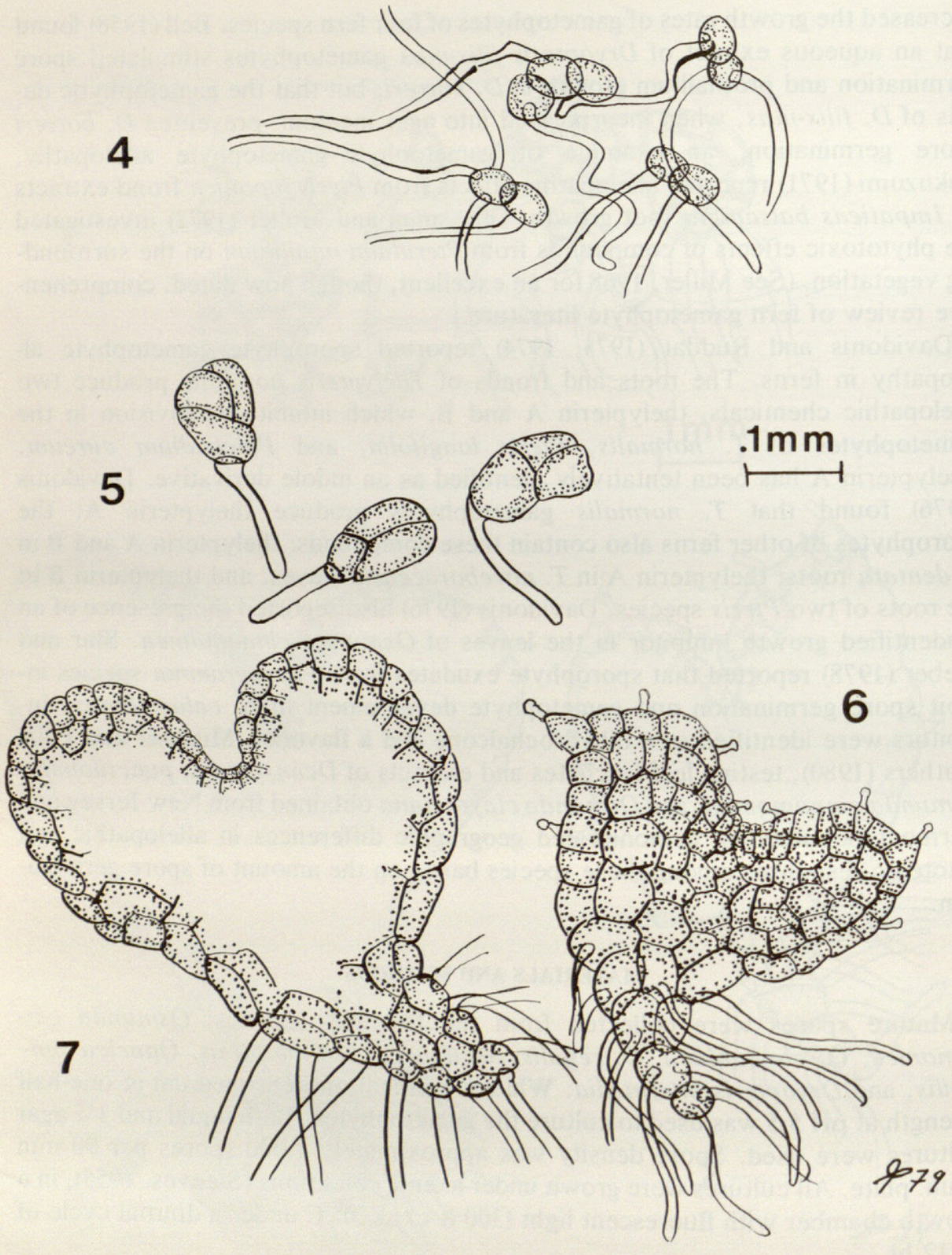
decreased the growth rates of gametophytes of four fern species. Bell (1958) found that an aqueous extract of *Dryopteris filix-mas* gametophytes stimulated spore germination and prothallium growth in *D. borreri*, but that the gametophytic debris of *D. filix-mas*, when incorporated into agar medium, prevented *D. borreri* spore germination, an example of gametophyte–gametophyte allelopathy. Fukuzumi (1971) reported allelopathic effects from *Pteris japonica* frond extracts on *Impatiens balsamina* root growth. Gliessman and Muller (1972) investigated the phytotoxic effects of compounds from *Pteridium aquilinum* on the surrounding vegetation. (See Miller, 1968 for an excellent, though now dated, comprehensive review of fern gametophyte literature.)

Davidonis and Ruddat (1973, 1974) reported sporophyte–gametophyte allelopathy in ferns. The roots and fronds of *Thelypteris normalis* produce two allelopathic chemicals, thelypterin A and B, which inhibit cell division in the gametophytes of *T. normalis*, *Pteris longifolia,* and *Phlebodium aureum*. Thelypterin A has been tentatively identified as an indole derivative. Davidonis (1976) found that *T. normalis* gametophytes produce thelypterin A; the sporophytes of other ferns also contain these compounds: thelypterin A and B in *T. dentata* roots, thelypterin A in *T. noveboracensis* leaves, and thelypterin B in the roots of two *Pteris* species. Davidonis (1976) also reported the presence of an unidentified growth inhibitor in the leaves of *Osmunda cinnamomea*. Star and Weber (1978) reported that sporophyte exudates from *Pityrogramma* species inhibit spore germination and gametophyte development in *P. calomelanos*. Inhibitors were identified as a dihydrochalcone and a flavonol. Munther and Fairbrothers (1980), testing leaf leachates and extracts of *Dennstaedtia punctilobula, Osmunda cinnamomea*, and *Osmunda claytoniana* obtained from New Jersey and Vermont populations, demonstrated geographic differences in allelopathic and autotoxic responses among these species based on the amount of spore germination.

MATERIALS AND METHODS

Mature spores were collected from the following species: *Osmunda cinnamomea, O. claytoniana, O. regalis, Matteuccia struthiopteris, Onoclea sensibilis*, and *Dryopteris intermedia*. White's minimal nutrient medium at one-half strength at pH 5.5 was used to culture the gametophytes. Both liquid and 1% agar cultures were used. Spore density was approximately 1,000 spores per 90 mm diam. plate. All cultures were grown under axenic conditions (Steeves, 1955), in a growth chamber with fluorescent light (300 ft-c) at 20° C under a diurnal cycle of 12/12 hr.

In the initial survey experiment, spores of the six species were sown on agar plates in paired strips adjacent to one another in all possible combinations. Control plates containing spores from one species also were sown. Plates were examined daily at the interfaces of adjacent species for symptoms of allelopathy or competition such as decreases from the control plates in percent germination or growth.



FIGS. 4–7. Gametophytes of *Dryopteris intermedia* and *Osmunda cinnamomea* after 20 days of growth. FIG. 4. *D. intermedia* gametophytes from a plate sprayed with *O. cinnamomea* supernatant. FIG. 5. *O. cinnamomea* gametophytes from a plate sprayed with *D. intermedia* supernatant. FIG. 6. Typical *D. intermedia* gametophyte from a plate sprayed with *D. intermedia* supernatant. FIG. 7. Typical *O. cinnamomea* gametophyte from a plate sprayed with *O. cinnamomea* supernatant.

On the basis of this experiment, *D. intermedia* and *O. cinnamomea* were selected as the most promising taxa for further experimentation because the gametophytes of these species appeared to inhibit each other's growth. A minimum of 10 replicates was run for each experiment. Initially, control plates and experimental plates containing a mixture of *Dryopteris* and *Osmunda* spores were prepared. The next phase was designed to eliminate the possibility of competition. Separate liquid cultures of *D. intermedia* and *O. cinnamomea* gametophytes (0.5g spores/liter of half-strength White's Medium at pH 5.5) were initiated and grown for two weeks. The gametophytes were then filtered off and the supernatants were conserved. Agar plates were prepared as above. Control plates consisted of spores of one species sprayed with one ml of the supernatant of the same species; experimental plates contained spores of one species sprayed with one ml of the supernatant of the other. This experimental design eliminates the possibility of interspecific competition (e.g., differential nutrient assimilation by one species over the other).

RESULTS AND DISCUSSION

In the survey of six species for allelopathic symptoms, a clear area was detected on the plates at the interface between *O. cinnamomea* and *D. intermedia* gametophytes. This was the result of progressive inhibition of *D. intermedia* gametophyte growth, which was proportional to the proximity of *Osmunda* gametophytes.

In the first phase of the *Dryopteris–Osmunda* growth rate analyses, *Dryopteris* spore germination was initially lower on the experimental plates (60% germination after 7 days) compared to the control plates (90% germination after 7 days). But after 10 days, 90% germination was reached on the experimental plates. Post-germination rate data was discontinued because it soon became apparent that the gametophytes of both species on the experimental plates were growing very slowly (*Fig. 1*) and no longer had significantly different growth rates. In contrast, the control plate gametophytes developed normally (*Figs. 2* and *3*).

The gametophytes of *D. intermedia* and *O. cinnamomea* inhibit the growth and development of one another, but one cannot distinguish from these results whether the inhibition is the result of allelopathy or competition, and so a set of supernatant experiments was designed to do so. These experiments essentially reproduced the results of the preceeding set of experiments. Control plates sprayed with supernatant from the same species produced normally developed gametophytes (*Figs. 4* and *5*). But experimental plates sprayed with supernatant from the other species yielded gametophytes having a severely retarded growth rate; after germination and a few cell divisions, development essentially ceased (*Figs. 6* and *7*).

This reciprocal supernatant experiment proves that the gametophytes of each species were suppressing cell division of the gametophytes of the other species and that this was done through the release of inhibitory compounds, rather than by competition.

This is the first recorded example in ferns of reciprocal allelopathy, in which two antagonistic species act on one another, that has been demonstrated *in vitro* between gametophytes.

Previous investigators working with ferns have demonstrated unidirectional allelopathy in the following systems: sporophyte acting on gametophyte (Froeschel, 1953; Davidonis & Ruddat, 1973, 1974; Star & Weber, 1978; Munther & Fairbrothers, 1980) and gametophyte acting on gametophyte (Bell, 1958).

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