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Beyond antheridiogens: chemical competition between gametophytes of *Polypodium appalachianum* and *Polypodium virginianum*

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Testo, W. L., M. S. Grasso, and D. S. Barrington (Pringle Herbarium, Department of Plant Biology, University of Vermont, 27 Colchester Avenue, Burlington, Vermont, USA 05405) Beyond antheridiogens: chemical competition between gametophytes of *Polypodium appalachianum* and *Polypodium virginianum*. J. Torrey Bot. Soc. 141: 302–312. 2014.—To understand the mechanisms by which fern gametophytes compete and the consequences of their interactions, we studied the effects of intraspecific and interspecific chemical competition on spore germination, sexual development, and gametophyte size in two closely related and co-occurring species of *Polypodium* from eastern North America. We cultured gametophytes of diploid *Polypodium appalachianum* Haufler & Windham and tetraploid *Polypodium virginianum* L. and recorded spore germination, sexual development, and gametophyte size and growth rates in a series of treatments representing different levels of competition within and between the two species. We found that mature gametophytes of both species reciprocally inhibit spore germination, sexual development, and growth of neighboring plants; we show that these effects are due to chemical interactions and not competition for resources. Competitive effects on spore germination and sexual development are similar for within- and between-species interactions, but repression of growth and gametophyte size is greater when competing gametophytes are heterospecific. An additional mechanism of intergametophytic competition via proliferous vegetative growth is also reported. We conclude that gametophytes of these species interact through a novel context-dependent chemical system that is independent from sex expression-regulating pheromones (antheridiogens). Our findings indicate that inhibition of growth and development among fern gametophytes is not solely a side effect of antheridiogens, as is generally thought.

Key words: allelopathy, competition, fern, mating system.

Ferns play important roles as ecosystem engineers in many habitat types and their persistent ecological prominence (George and Bazzaz 1999a, b, Watkins et al. 2006, Cardelús et al. 2009) has drawn attention to the importance of competition in fern community establishment and assembly. An extensive body of literature suggests that many fern taxa are exceptional competitors, notably via the use of chemical inhibition between sporophytes. Most studies of chemicals in ferns have focused on the cosmopolitan weed *Pteridium aquilinum* (L.) Kuhn, which has been demonstrated to possess a strong allelopathic system inhibiting growth of co-occurring ferns and angiosperms throughout its range and contributing to its prominence as a major agricultural pest (Glass 1976, Gliessman 1978, Dolling 1996, Silva Matos and Belinato 2010). Allelopathic agents produced by the sporophyte phase of the life cycle have also been shown to contribute to the success of weedy species in the Gleicheniaceae (Kato-Noguchi et al. 2012a, b, Voltarelli et al. 2012), and chemical competition has been described in several other families, notably the Osmundaceae (Munther and Fairbrothers 1980, Wagner and Long 1991), Pteridaceae (Star 1980, Melos et al. 2007) and Thelypteridaceae (Davidonis and Ruddat 1973, 1974).

Our knowledge of competitive interactions among fern gametophytes centers on antheridiogens, gibberellin-like pheromones known to mediate breeding systems in many fern species by inducing maleness in small, presexual gametophytes (Döpp 1950, Näf et al. 1975). In addition to promoting outcrossing and the reproductive success of both the producing and responding gametophytes (Haufler and Gastony 1978, Schneller 1979, Schneller et al. 1990), antheridiogens may act as competitive agents by generating a favorable gender ratio among nearby conspecifics and repressing archegonium formation in nearby gametophytes. Support for these hypotheses from both laboratory- and field-based studies is derived from the demonstration that antheridiogen-related competition occurs both within and between species via impacts on

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gametophyte growth, survivorship, and reproductive success (Willson 1981, Schneller 1988, Hamilton and Lloyd 1991, Quintanilla et al. 2007, Jiménez et al. 2008).

A few studies report chemical interactions between fern gametophytes that include suppression of spore germination and slowed gametophyte growth, but taxonomic coverage is weak (Petersen and Fairbrothers 1980, Testo and Watkins 2013). Though these inhibitory interactions appear to be related to antheridiogen effects in some species (Petersen and Fairbrothers 1980, Wagner and Long 1991, Quintanilla et al. 2007, Jiménez et al. 2008), gametophyte competition has also been reported for species that lack antheridiogen systems. For instance, Chiou and Farrar (1997a) reported repression of gametophyte growth independent of antheridiogen effects in tropical members of the Polypodiaceae, leading them to infer the presence of a separate growth-inhibiting chemical cue. Similarly, Testo and Watkins (2013) demonstrated that gametophytes of several North American fern species inhibited germination of the rare endemic *Asplenium scolopendrium* L. var. *americanum* (Fernald) Kartesz & Gandhi, a taxon which lacks a response to antheridiogens. Together, these findings suggest that non-antheridiogen cues may play a role in fern gametophyte competition.

Given our limited knowledge of competition in fern gametophytes, many pertinent questions remain unanswered. To what extent does competition occur between mature gametophytes? Do immature gametophytes compete with mature gametophytes, and with what outcome? What is the distribution of different mechanisms of competition across taxonomic groups?

Here we begin to address these questions through an investigation of chemical interactions between gametophytes of *Polypodium appalachianum* Haufler & Windham and *Polypodium virginianum* L. These species present an excellent system in which to study gametophyte competition, as they are closely related (Haufler and Windham 1991, Haufler and Zhongren 1991, Haufler et al. 1995), occur together throughout much of their range, and do not respond to gibberellin-based antheridiogens (Voeller 1964). *Polypodium virginianum* is an allotetraploid derived from a cross between diploid *P. appalachianum* and *Polypodium sibiricum* Sipliv., the latter a diploid species that occurs to the north and west of these species' current ranges (Haufler and Windham 1991). Gametophytes of these two species appear to interact frequently in nature, as their triploid hybrid, *Polypodium* × *incognitum* Cusick is common and widespread (Evans 1971, Haufler and Zhongren 1991, Haufler et al. 1995, Montgomery 1996, Cusick 2002). Our goal in this work was to investigate the ecological significance of gametophyte competition in order to develop a more comprehensive understanding of chemical interactions in ferns. Our approach was to study the effects of intraspecific (between conspecific gametophytes) and interspecific (between heterospecific gametophytes) competition on spore germination, sexual development, and gametophyte growth rates, three traits that strongly influence gametophyte fitness in natural communities (Willson 1981, Haig and Westoby 1988, Hamilton and Lloyd 1991).

Materials and Methods. Plant Material and Culture Conditions. Three sporophytes of *Polypodium virginianum* and *Polypodium appalachianum* were collected in Chittenden County, Vermont, in October 2012. Species were identified by sporophyte morphology and spore size; vouchers (*P. appalachianum*: *Grasso LT-1, Grasso LT-2, Grasso LT-4*; *P. virginianum*: *Grasso OL-1*, *Grasso OL-2*, *Grasso OL-5*) are deposited at VT. Fertile fronds from each individual were placed in glassine envelopes and dried for 3–5 days in an air-conditioned lab to allow for the release of spores. Gametophytes were cultured on Bold's nutrient media (Bold 1957) supplemented with Nitsch's micronutrients (Nitsch 1951); spore sowing methodology and growth conditions follow Testo and Watkins (2011). Stock cultures were maintained in 100 × 20 mm Petri dishes sealed with parafilm, and gametophytes were transferred to 35 × 10 mm dishes for experimental treatments.

Spore Germination. To determine the possible effects of various competition types on spore germination, the proportion of spores that had germinated was determined every three days from 0–30 d post-sowing by viewing 50 spores per parental sporophyte per treatment. Spores were scored as germinated if exerted rhizoid initials and/or prothallial cells were observed. In rare cases, 50 spores could

not be counted for all time intervals; however, at least 47 spores were recorded for all counts.

A total of five treatments were established for each species: (1) spores sown in normal conditions (control), (2) spores sown in the presence of a mature female conspecific gametophyte, (3) spores sown in the presence of a mature female heterospecific gametophyte, (4) spores sown in the presence of an aqueous extract obtained from a mature female conspecific gametophyte, and (5) spores sown in the presence of an aqueous extract obtained from a mature female heterospecific gametophyte.

In all treatments, spores were sown at an approximate density of 50 spores per plate, and mature female gametophytes were placed in the center of the Petri dishes for treatments in which they were used. Treatments using an aqueous solution were used to control for possible resource competition (versus chemical interactions) observed in treatments with mature gametophytes; solutions were prepared following Petersen and Fairbrothers (1980): mature female gametophytes were rinsed with 1 mL of distilled water; the supernatant was then collected and sprayed on spores immediately following sowing. For each treatment, mean spore germination percentage was determined by averaging counts of fifty spores each from three parental sporophytes per species.

GAMETANGIUM FORMATION. To investigate possible repression of sex expression between gametophytes of *Polypodium appalachianum* and *P. virginianum*, we observed the time required for gametophytes of both species to develop archegonia and antheridia in various competitive environments. To assess archegonium formation, 45 gametophytes of each species (90 for paired treatments) were transferred to experimental plates at 60 d post-sowing and the percent of gametophytes with archegonia was scored weekly from 60–109 d. Observation of antheridia required temporary removal of gametophytes from culture and inspection with a compound microscope performed biweekly on a subset (15 per treatment) of gametophytes to minimize disturbance. The percentage of antheridiate gametophytes was scored at the same time intervals as for archegoniate gametophytes. Treatment design paralleled the spore germination experiment: (1) an isolated gametophyte, (2) a gametophyte paired with a conspecific gametophyte, (3) a gametophyte paired with a heterospecific gametophyte, (4) an isolated gametophyte in the presence of an aqueous extract obtained from a conspecific mature female gametophyte, and (5) an isolated gametophyte in the presence of an aqueous extract obtained from a heterospecific mature female gametophyte. In all paired gametophyte treatments, gametophytes were of the same age and placed 1 cm apart.

GAMETOPHYTE SIZE. To determine the effects of competition on gametophyte size, 45 gametophytes from each treatment were measured at 90, 120, and 150 d. Measurements were obtained by imaging the gametophyte with a SPOT Insight Firewire 2.0 (Diagnostic Instruments, Inc., Sterling Heights, MI, USA) camera mounted on a Leica MZ8 stereoscope (Leica Microsystems, Weztlar, Germany) and measuring the gametophytes across their widest axis using ImageJ image analysis software (Abramoff et al. 2004). For each species, seven treatments were established: (1) isolated gametophytes (isolate), (2) a gametophyte paired with a conspecific gametophyte, (3) a gametophyte paired with a heterospecific gametophyte, (4) an isolated gametophyte in the presence of an aqueous extract obtained from a conspecific mature female gametophyte, (5) an isolated gametophyte in the presence of an aqueous extract obtained from a heterospecific mature female gametophyte, (6) an isolated gametophyte in the presence of conspecific spores and young gametophytes, and (7) an isolated gametophyte in the presence of heterospecific spores and young gametophytes sown at 60 d post-germination.

STATISTICAL ANALYSES. All statistical analyses were performed using JMP 10.0 statistical analysis software (SAS Institute Inc., Cary, NC, USA). Spore germination rates and archegonium formation were expressed as a percentage of the total number of plants examined; both were partitioned within species by parental sporophyte for analyses. Maximal germination percentage, archegonium formation at 109 d, and gametophyte size at 150 d were tested for normality, log-transformed when necessary, and then compared across species by ANOVA and post-hoc Tukey tests.

Results. SPORE GERMINATION. *Polypodium appalachianum.* Germination was first observed

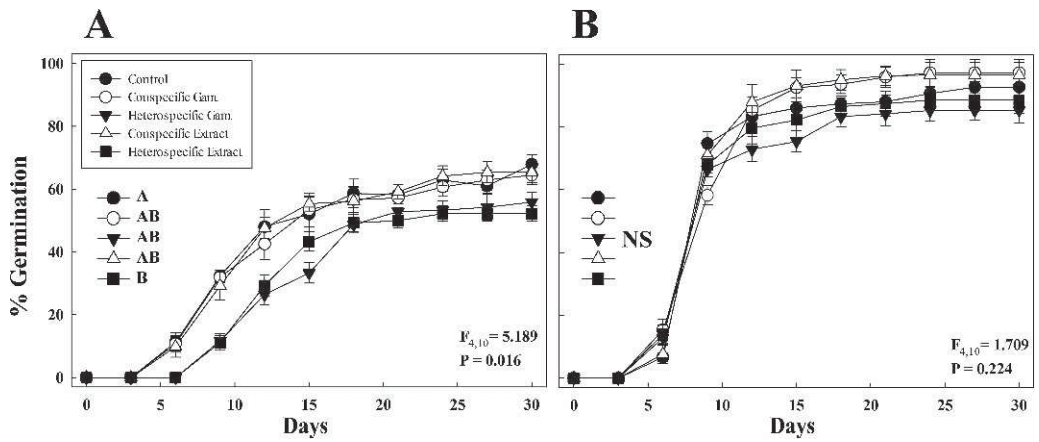

FIG. 1. Spore germination of *Polypodium appalachianum* (A) and *P. virginianum* (B) under different levels of competition from 0–30 d. Letters following symbols represent homogeneous subsets recovered by post-hoc Tukey test with $\alpha = 0.05$; ANOVA summary statistics are included. Error bars represent ± 1 SEM. For all treatments, $N = 3$, $n = 50$.

at 6 d in the control treatment and spores grown in the presence of a mature conspecific gametophyte or aqueous extract derived from a conspecific gametophyte. For spores sown in the presence of a mature gametophyte of *P. virginianum* or its extract, germination was first observed at 9 d post-sowing (Fig. 1A). Spores sown in the presence of a *P. virginianum* gametophyte or its extract germinated more slowly than those sown in the presence of a conspecific gametophyte or its extract or the control; this delay was greatest from days 6–12 and decreased thereafter. Maximal germination percentage (at 30 d) was highest in the control treatment (mean 68% ± SEM 3.0%—format parallel in the data reported throughout) but was not significantly different across treatments, except for the control treatment and spores sown in the presence of aqueous extract derived from *P. virginianum* ($F_{4,10} = 5.189$; $P = 0.016$).

Polypodium virginianum. Germination was first observed at 6 d in all treatments; by 12 d post-sowing, all treatments had exceeded 70% (Fig. 1B). No evidence of consistent delay or repression of germination was observed. At 30 d, more than 97% (± 4.2) of spores grown in the presence of a conspecific gametophyte had germinated; values were lower for other treatments through this time point, but maximal germination percentage did not differ significantly across treatments ($F_{4,10} = 1.709$; $P = 0.224$).

GAMETANGIUM FORMATION. *Polypodium appalachianum.* Archegonia were first observed at 67 d in gametophytes grown in the presence of a *P. virginianum* gametophyte (35 ± 6.3%) and its extract (27 ± 4.8%); a small proportion of isolated gametophytes had also developed archegonia by 67 d (Fig. 2A). Archegonia were observed in gametophytes from all treatments by 74 d post-sowing. All gametophytes grown in isolation were archegoniate after 81 d; those in all other treatments exhibited delayed archegonium formation—they attained stable maximal values of 75–84% by 102 d. The percentage of gametophytes that had become archegoniate at the final time point (109 d) differed between isolated gametophytes and those from all other treatments was statistically significant (Figure 2A; $F_{4,10} = 5.514$; $P = 0.013$). Following the development of archegonia, most gametophytes became bisexual by 120 d; however, no antheridia were observed prior to 102 d and no solely antheridiate gametophytes were observed in any treatment at any time point.

Polypodium virginianum. Archegonia were observed on gametophytes from all treatments by 67 d; fewer than 20% of those in each treatment (except isolated gametophytes; 42 ± 5.5%) were archegoniate by this time (Fig. 2B). By 74 d, all isolated gametophytes were archegoniate, whereas in the remaining treatments only 26.4% of gametophytes bore archegonia. By 102d, gametophytes in all treatments (except those grown in the presence

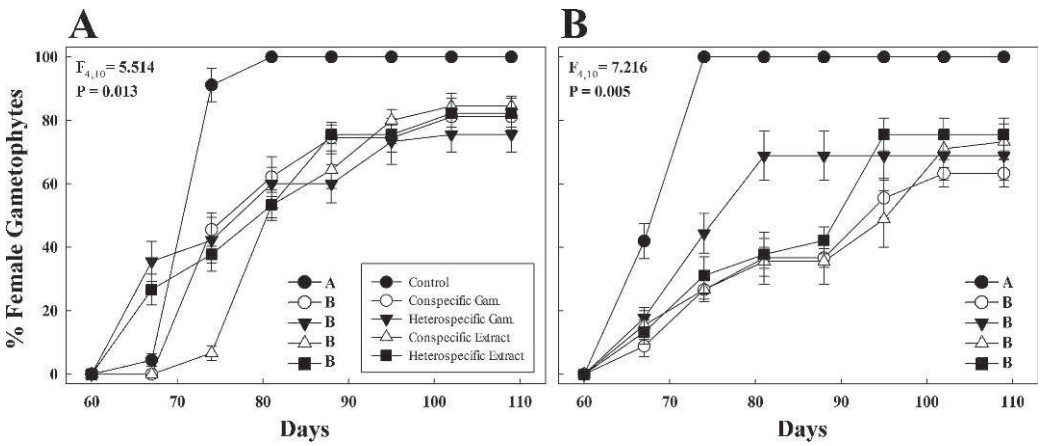

FIG. 2. Archegonium formation in *Polypodium appalachianum* (A) and *P. virginianum* (B) gametophytes under different levels of competition from 60–109 d. Letters following symbols represent homogeneous subsets recovered by post-hoc Tukey test with $\alpha = 0.05$; ANOVA summary statistics are included. Error bars represent ± 1 SEM. For all treatments, $N = 3$, $n = 45$.

of *P. virginianum*-derived extract) reached stable levels of archegonium formation, with 63.8% of gametophytes in each treatment bearing archegonia. The exception was the isolate treatment, with 100% archegoniate gametophytes. The percentage of archegoniate gametophytes at 109 d differed between those grown in isolation and those from all other treatments ($F_{4,10} = 7.216$; $P = 0.005$). As with *P. appalachianum*, most archegoniate gametophytes later became bisexual, with antheridia first observed at 81 d. No solely antheridiate gametophytes were observed at any time point in any of the treatments.

GAMETOPHYTE SIZE AND GROWTH RATE. *Polypodium appalachianum.* At 90 d, mean thallus width ranged from 4.6 ± 0.2 mm for gametophytes grown in the presence of a *P. virginianum* gametophyte to 8.5 ± 0.5 mm for isolated gametophytes (Fig. 3A, Fig. 4A–E). At 150 d, isolated gametophytes were on average the largest (12.9 ± 0.8 mm), and those grown in the presence of a *P. virginianum* gametophyte or an aqueous extract derived from that species were the smallest (5.9 ± 0.8 mm and 6.0 ± 0.9mm, respectively). ANOVA and post-hoc Tukey tests recovered significant differences across treatments in

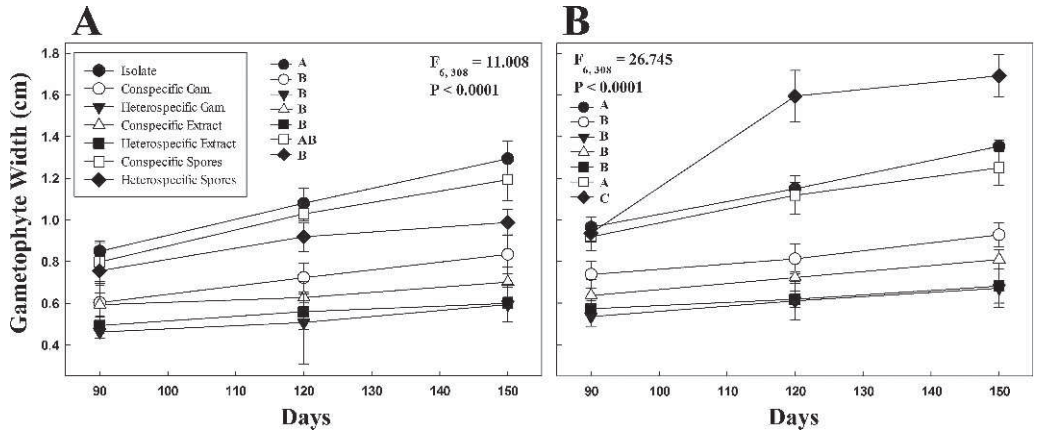

FIG. 3. Width of *Polypodium appalachianum* (A) and *P. virginianum* (B) gametophytes under different levels of competition measured at 90, 120, and 150 d. Letters following symbols represent homogeneous subsets recovered by post-hoc Tukey test with $\alpha = 0.05$; ANOVA summary statistics are included. Error bars represent ± 1 SEM. For all treatments, $N = 3$, $n = 45$.

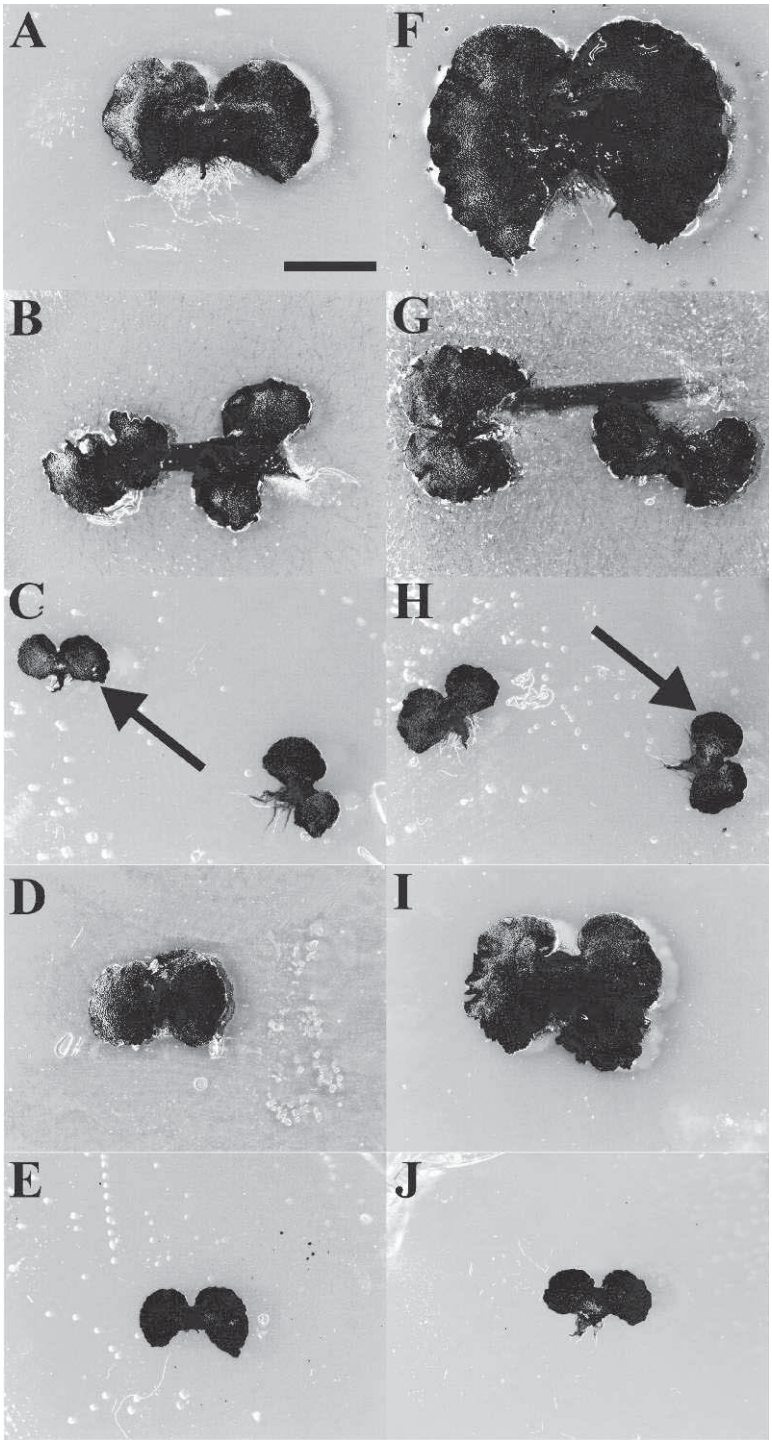

FIG. 4. Gametophytes of *Polypodium appalachianum* (A–E) and *P. virginianum* (F–J) grown under different levels of competition at 150 d (after 90 d in designated treatment). Top row, isolated gametophyte; second row, conspecific pairs; third row, heterospecific pairs (arrows indicated relevant individual); fourth row, isolated gametophytes treated with aqueous solution from conspecific gametophyte; and bottom row, isolated gametophyte treated with aqueous solution from heterospecific gametophyte. Scale bar (top left) = 5 mm.

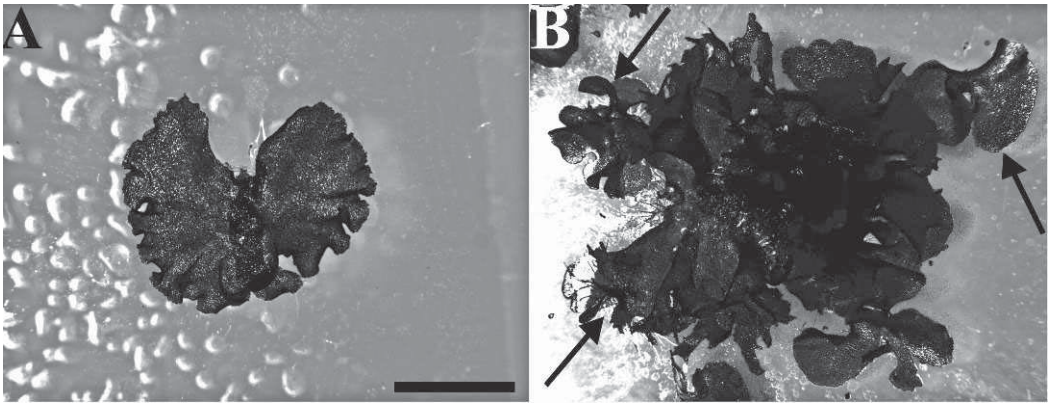

FIG. 5. Two hundred day-old *Polypodium virginianum* gametophyte grown in isolation and exhibiting normal cordiform morphology (A) and same-aged *P. virginianum* gametophyte, grown in the presence of spores and young gametophytes of *P. appalachianum*, with spatulate outgrowths (B). Arrows indicate young *P. appalachianum* gametophytes that have been partly overshadowed by the proliferation of the older *P. virginianum* gametophyte. Scale bar = 1 cm.

gametophyte width at 150 d; isolated gametophytes and those grown in the presence of conspecific spores and young gametophytes differed from all other treatments. Mean growth rate was also substantially different across treatments— gametophytes grown in isolation grew at an average rate of 0.52 mm · week^{-1} whereas those grown in the presence of *P. virginianum* extract grew at one-quarter that rate (0.13 mm · week^{-1}). Intermediate growth rates were observed in the other treatments (Fig. 3A).

Polypodium virginianum. At 90 d, mean thallus width ranged from 5.4 ± 0.5 mm for gametophyte grown in the presence of a *P. appalachianum* gametophyte to 9.7 ± 0.5 mm for isolated gametophytes (Fig. 3B, Fig. 4F–J). At 150 d, the relationships between treatments had changed, with gametophytes grown in the presence of heterospecific spores and young gametophytes being wider than those grown in isolation (16.9 ± 1.0 mm vs. 13.6 ± 2.8 mm); those grown in the presence of a single mature *P. appalachianum* gametophyte or its aqueous extract were still the smallest (6.7 ± 0.9 mm and 6.8 ± 0.8 mm, respectively; Fig. 3B). Mean growth rate ranged from 0.88 mm · week^{-1} for gametophytes grown in the presence of spores and young gametophytes of *P. appalachianum* to 0.13 mm · week^{-1} for gametophytes grown in the presence of *P. appalachianum* extract.

The large size of gametophytes grown in the presence of heterospecific spores and young gametophytes was due to an unusual growth response exhibited by all *P. virginianum* gametophytes in this treatment (Fig. 5). Shortly after the germination of nearby heterospecific spores (80–110 d), small spatulate outgrowths were observed on the margins and thallus body of the mature *P. virginianum* gametophyte. These outgrowths grew to 10–15 cells in length, developed notch meristems, and further proliferated (Fig. 5). In many cases, the proliferating gametophyte grew over small nearby *P. appalachianum* gametophytes. No such outgrowths were observed in any other treatment.

Discussion. SPORE GERMINATION. Inhibition of spore germination, as with seed germination, is a commonly reported consequence of allelopathy; several examples of this phenomenon are known among ferns (Bell 1958, Davidonis and Ruddat 1973, 1974; Petersen and Fairbrothers 1980). Our results suggest that germination inhibition does occur in eastern North American *Polypodium*, but that such effects are limited. The only clear evidence of germination inhibition in our study was that of *P. appalachianum* spores grown in the presence of a mature female *P. virginianum* gametophyte or its extract; the similar responses in these two treatments demonstrate that the inhibition is chemical in nature and not the result of resource competition. The inhibition of *P. appalachianum* spore germination by mature *P. virginianum* gametophytes and their extract demonstrates

that the latter species produces a chemical compound responsible for the effects observed here. It is unclear whether secretion of these chemicals is suppressed in the presence of conspecific spores and young gametophytes or if spore germination of *P. virginianum* is simply unaffected by their presence.

Antheridium Formation. The pattern of antheridium formation observed in both species is consistent with that described for fern species that lack an antheridiogen response: gametophytes form antheridia only after developing a notch meristem and archegonia (Haufler and Ranker 1985, Watkins and Farrar 2005). If an antheridiogen system were present in either species, we would have expected presexual gametophytes grown in the presence of mature female gametophytes to become antheridiate, resulting in populations of large female gametophytes and small male gametophytes as has been described for taxa with active antheridiogen systems (Tryon and Vitale 1977, Haufler and Gastony 1978, Haufler and Soltis 1984, Chiou and Farrar 1997a, Quintanilla et al. 2007, Prada et al. 2008). We interpret the lack of any solely antheridiate gametophytes or a population structure as described above as compelling evidence for the lack of an antheridiogen system in these species; the same conclusion as in other studies encountering this pattern of sex expression (Haufler and Ranker 1985, Chiou and Farrar 1997a, Watkins and Farrar 2005) and suggests that the competitive effects observed in this study are the result of another mechanism.

Archegonium Formation. In nature, many fern gametophyte communities (particularly among terrestrial taxa) develop in habitats that are frequently disturbed and inherently transient (Cousens et al. 1985, Peck et al. 1990, Dyer and Lindsay 1992, Barrington 1993, but see Watkins et al. 2007a). In these scenarios, there is strong selection for early archegonium formation and antheridiogen-producing gametophytes, which mediate sexual development of neighboring gametophytes, are thought to convey a considerable advantage. Several variations of this general model of community dominance by antheridiogen-secreting female gametophytes have been proposed (Willson 1981, Haig and Westoby 1988, Hamilton and Lloyd 1991), and it is likely that many natural gametophyte communities conform to this pattern (Tryon and Vitale 1977, Schneller et al. 1990, Korpelainen 1994, Quintanilla et al. 2007). However, this model is inapplicable to taxa that lack antheridiogen responses.

Our findings provide evidence of chemically mediated suppression of archegonium formation in non-antheridiogen systems. In both *Polypodium* species, archegonium formation was delayed significantly in all competition treatments relative to those grown in isolation, and a significant proportion of gametophytes never formed sex organs (Fig. 2). The similar levels of inhibition of archegonium formation exerted by both gametophytes and their aqueous extracts provides evidence that the repression of archegonium formation is not due to crowding or resource limitation but is instead chemical in nature. To our knowledge, this is the first experiment to report non-antheridiogen inhibition of sexual development in ferns, providing novel insight into mechanisms by which fern gametophytes lacking antheridiogen systems can influence sex expression in competitors. Repression of archegonium formation may be an important competitive mechanism in these species; by delaying reproduction in neighbors, mature female gametophytes enhance their own chances of sporophyte production.

The similar responses to intraspecific and interspecific competition in both species suggest that this mode of competition solely favors individual success rather than the mutual benefit promoted by antheridiogen systems (Willson 1981, Haig and Westoby 1988, Hamilton and Lloyd 1991). In populations influenced by antheridiogen, both signaling and responding gametophytes benefit because young, presexual gametophytes are provided with an increased opportunity for successful mating. In contrast, the system observed here for *Polypodium appalachianum* and *P. virginianum* delays the formation of archegonia on neighboring gametophytes without regard to relatedness but has no effect on antheridium formation, thus conferring an advantage only on the signaling gametophyte. In natural populations comprised of gametophytes of mixed ages and species, this system may promote establishment of these species.

Gametophyte Size. Both laboratory and field experiments indicate that large gametophytes exhibit greater survivorship and fecundity than smaller ones (Willson 1981, Schneller

et al. 1990, Hamilton and Lloyd 1991, Quintanilla et al. 2007). In both species of *Polypodium* studied here, gametophytes grown in isolation grew faster and were larger than those paired with another gametophyte or treated with aqueous extract; and repression was greater in heterospecific treatments than in conspecific ones. This pattern, also reported by Chiou and Farrar (1997a) for tropical Polypodiaceae, provides additional evidence that repression of gametophyte growth in this family is mediated by a chemical cue distinct from antheridiogen. At the same time, our observations provide a compelling argument for rejecting the view that antheridiogens act primarily to mediate gametophyte size rather than gender.

The spatulate outgrowths observed in *P. virginianum* gametophytes grown in the presence of spores and young gametophytes of *P. appalachianum* appear to allow the mature *P. virginianum* gametophyte to outcompete nearby heterospecific gametophytes by dominating the local environment—in many cases overshadowing them. Additionally, indeterminate growth may promote gametophyte longevity as has been demonstrated for epiphytic fern gametophytes with similar morphology (Chiou and Farrar 1997b, Dassler and Farrar 1997, 2001; Watkins et al. 2007b, Farrar et al. 2008). As has been demonstrated for epiphytic fern gametophytes, extensive vegetative reproduction and indefinite persistence of gametophyte clones may enhance opportunity for outcrossing by increasing the probability of interaction of genetically distinct individuals both spatially and over time.

With independent, free-living sporophyte and gametophyte phases, ferns present unique challenges to researchers—the autecology of both phases must be studied in order to attain a comprehensive understanding of the organism's biology as a whole. Nevertheless, a disproportionately small number of studies address basic features of gametophyte ecology, including how, when, and to what extent fern gametophytes compete with each other for resources and reproductive opportunities. Since its conception and development in the latter half of the last century, ''antheridiogen theory'' has been used to frame most inquiries into fern gametophyte interactions. Although antheridiogen systems play an important role in the structuring of natural fern gametophyte communities, other mechanisms of interactions should not be ignored. In this study, we demonstrate the presence of complex, chemically mediated competition in the absence of an antheridiogen system and show that these competitive interactions significantly affect gametophyte growth and sexual development. Our findings strongly support the earlier claim by Chiou and Farrar (1997a) that growth inhibition can be caused by a different chemical from that responsible for antheridium formation. In light of these findings, it is best to conceive of gametophyte competition as mediated by multiple chemical systems acting either sequentially or concomitantly. Understanding these interactions will provide important insight into the developmental and reproductive biology of fern gametophytes as well as the dynamics of natural gametophyte communities.

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