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ANT PROTECTION OF THE NECTARIED FERN *POLYPODIUM PLEBEIUM* IN CENTRAL MEXICO[1]

SUZANNE KOPTUR,[2,4] VICTOR RICO-GRAY,[3] AND MÓNICA PALACIOS-RIOS[3]

[2]Department of Biological Sciences, Florida International University, Miami, Florida 33199; and
[3]Departamento de Ecología Vegetal, Instituto de Ecología, Apdo. Postal 63, Xalapa, Veracruz 91000, México

Nectaries on fronds of *Polypodium* spp. have been studied previously only in cultivated specimens. We conducted field observations in middle-elevation forests in Mexico and found five ant species associated with nectaries of *Polypodium* plebeium and *P. lepidotrichum.* To investigate whether nectaries promote protection against herbivores, we performed ant-exclusion experiments with nectary-bearing ferns (*P. plebeium*) and other ferns without nectaries *(Polypodium plesiosorum, P. furfuraceum,* and *Phlebodium pseudoaureum*). When ants were excluded from the developing fronds of *Polypodium plebeium,* damage from foliage-feeding sawfly and lepidopteran caterpillars was significantly greater than in control fronds. Ferns without nectaries did not show a difference in damage between ant-excluded and control fronds. Our results demonstrate that fern nectaries can support ant defense of the plant body as do the extrafloral nectaries of many angiosperms.

Key words: ant–plant interactions; antiherbivore defense; ferns; nectaries; plant protection; *Polypodium plebeium*; Polypodiaceae; pteridophytes.

There are many examples of protection of plants by ants and other beneficial insects attracted to extrafloral nectaries (reviewed in Bentley, 1977; Buckley, 1982; Jolivet, 1986; Koptur, 1992), however, none of these examples involve pteridophytes. Nectaries in ferns have been observed in various genera (Koptur, Smith, and Baker, 1982), but their role in ecological interactions has been studied only in the bracken fern (*Pteridium aquilinum*) in various parts of the world (Lloyd, 1901; Page, 1982; Tempel, 1983; Lawton and Heads, 1984; Heads and Lawton, 1984, 1985; Rashbrook, Compton, and Lawton, 1991, 1992). In ant-exclusion experiments performed thus far, there has been no evidence for protection of the ferns (Tempel, 1981; Heads and Lawton, 1984; Dennis O'Dowd, personal communication), though Heads (1986) found that one ant species had weak but significant effects on populations of certain insect herbivores of bracken.

The confirmation of nectaries in species of the *squamatum* group of *Polypodium* was determined by nectar analysis (Koptur, Smith, and Baker, 1982) of exudates previously presumed to be water from hydathodes (de la Sota, 1966). Nectaries in the genus have been noted in more species than just this section of the genus (Tryon and Tryon, 1982).

Since earlier studies found little or no support for the ant-protection hypothesis, it is important to consider alternatives. Myrmecophytic ferns show various adaptations for spore dispersal, such as echinate spores in *Solanopteris* or filamentous elements on spores in *Lecanopteris* (Tryon, 1985), though this is debated (Walker, 1985). It seems reasonable that the nectaries of ferns may serve to attract ants or other insects as dispersal agents.

In this study we used field observations and experiments to determine the role of ant visitors to nectaries of ferns. We documented visitors to nectaries of *Polypodium* spp. We performed controlled ant-exclusion experiments on both epiphytic ferns with nectaries and on epiphytic ferns without nectaries. Our objectives were to test the hypotheses of ant protection and spore dispersal.

Three study sites were located in or near Xalapa, Veracruz, Mexico, in lower montane wet forest habitat at an elevation of 1500 m. The most disturbed of the sites was Jardin Francisco J. Clavijero, the botanical garden of the Instituto de Ecología, where ferns occurred on trees in forested borders along paths and around the perimeter of the garden. A site of intermediate disturbance was Rancho Guadalupe, the ''ecological reserve'' on the grounds of the institute, containing secondary forest and many cleared areas. The most pristine of the sites is a privately owned forest at Banderilla, ~8 km outside of Xalapa, where firewood is harvested by local people but the forest is largely intact. Observations were made at all three sites, but ant-exclusion experiments were conducted only at the least disturbed Banderilla site.

Beginning in January and February 1994, ten individuals of each *Polypodium* species (except for *P. lepidotrichum,* where only four individuals were encountered) growing as epiphytes on tree trunks at heights from 0.5 to 2.0 m from the ground were tagged and observed every 2 wk at the Rancho Guadalupe site for frond development, herbivores, herbivory, other insects (including ants), nectary presence, and nectary visitor activity (if nectaries were present). The presence of nectaries was suggested in some individuals by the presence of black

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[4] Author for correspondence.

sooty mold (*Ceratocystis* sp.; Ophiostomales, Ascomycotina) on the acroscopic lobes of the simple fronds. This technique was also useful in detecting the presence of nectaries on herbarium specimens. Nectar production was verified in living specimens using glucose-test strips (Clinistix, TM, Miles Inc., Elkhart, Indiana). Ants and other arthropods observed at the baits were collected for identification.

Leafing activity of the *Polypodium* spp. with nectaries accelerated in April, and in mid-April we set up ant-exclusion experiments at the Banderilla site. We chose 20 pairs of fronds (in the advanced crozier, or fiddlehead, stage) for both *Polypodium plebeium* (a species with nectaries) and several ferns without nectaries (*Polypodium furfuraceum, N* = 10; *P. plesiosorum, N* = 5; and *Phlebodium pseudoaureum, N* = 5). It was necessary to pool fern species without nectaries because new leaf production was less frequent on these species. One of each pair was designated the exclusion frond, the other the control. We removed any ants or other insects present from both exclusion and control fronds. Using Tanglefoot (TM) resin (Nixalite, East Moline, Illinois), we banded the petiole of exclusion fronds for an area of 3 cm at least 1 cm from the base. We monitored the fronds during their development (once or twice weekly), removing any debris from the sticky bands and performing corrective maintenance where necessary. One month (27 d) later the fronds had all expanded, and we assessed damage and ended the experiment. We used a clear plastic grid (0.09 cm^2 squares) to count the number of squares with at least 50% of the square damaged and multiplied to obtain a conservative estimate of square centimeters damaged for each frond. We compared the amount of damage experienced by control and ant-excluded fronds within each group of ferns using paired *T* tests. We constructed leaf area regressions for each of the fern species used by measuring the length, width, and area (using a leaf area meter) of ten perfect fronds. We then measured the length and width of fronds involved in the experiment and calculated their area using the regressions. The percentage damaged was figured by dividing the area damaged by the total area of the frond. Sample sizes <20 were due to frond death or disappearance during the course of the experiment. Final sample sizes were 19 pairs of *P. plebeium* fronds, nine pairs of *P. furfuraceum* fronds, three pairs of *P. plesiosaurum* fronds, and four pairs of *Phlebodium pseudoaureum* fronds (combined nonnectaried *N* = 16).

Fern nectary activity—We encountered two species of *Polypodium* with nectaries at our study sites: *P. plebeium* Schldl. & Cham. and *P. lepidotrichum* (Fée) Maxon. Nectaries in *Polypodium* occur on each acroscopic lobe at the base of the individual segments of the leaf (Koptur, Smith, and Baker, 1982). Two species without nectaries, *Polypodium plesiosorum* Kunze and *P. furfuraceum* Schldl. & Cham., occurred at the same sites. While *P. furfuraceum* and *P. plesiosorum* had some new and developing leaves in January and February, new leaves were not abundant on the nectaried *Polypodium* species until March and April.

We did not observe nectar secretion on mature fronds in the *Polypodium* species. Mature vegetative fronds occasionally had black sooty mold growing on the frond in the area of the nectaries, perhaps indicating continued low-level secretion after the leaf matured, but this was not common. The major nectar production occurred while the leaf was expanding. There was no evidence of nectar secretion on mature reproductive (spore-bearing) fronds.

We found five species of nectar-drinking ants associated with nectaries of *Polypodium plebeium* and *P. lepidotrichum*: *Brachymyrmex* aff. *musculus,* two undetermined species of *Leptothorax,* an undetermined species of *Pheidole,* and *Solenopsis geminata.* At the Banderilla site where we conducted our ant-exclusion experiments, the nectaried *Polypodium plebeium* were associated with either *Brachymyrmex* aff. *musculus, Leptothorax* sp., or *Solenopsis geminata* (these were therefore the ant species involved in the experiment). Ants were not observed on any of the ferns without nectaries during the course of our biweekly observations. We regularly inspected both developing and mature fronds. *Polypodium* species with nectaries often had ants on the developing fronds (where nectaries are active); ants were not observed on mature fronds of those ferns (where sporangia are present, but nectaries are not active).

Ant-exclusion experiments—*Polypodium plebeium* fronds from which ants were excluded during their development suffered significantly greater damage than fronds with ants present (an average of 1.50 ± 1.56 cm^2 vs. 0.23 ± 0.35 cm^2 (means and standard deviations, respectively); $T = 4.004$, $P = 0.0008$, $N = 19$ pair; Fig. 1a). The same experiment on ferns without nectaries showed no significant difference between experimental and control fronds (1.78 ± 2.84 cm^2 vs. 3.20 ± 4.75 cm^2; $T = -1.212$, $P = 0.2442$, $N = 16$ pair; Fig. 1a). The control fronds on ferns without nectaries sustained substantially more damage than did control fronds on ferns with nectaries (3.20 ± 4.75 cm^2 vs. 0.23 ± 0.35 cm^2; $T = -2.73$, $P = 0.0099$, df = 34). Percentages of fronds damaged show the same patterns (Fig. 1b): *P. plebeium* fronds with ants excluded lost 3.9 ± 1.0% of their leaf area, and fronds with ants present lost only 0.4 ± 0.1%; $T = 3.39$, $P = 0.0017$. For ferns without nectaries, fronds with ants excluded lost 3.3 ± 1.2% of their leaf area, and fronds with ants present lost 7.5 ± 3% (not a significant difference, $T = -1.3$, $P = 0.2029$). Control fronds on *P. plebeium* lost substantially less leaf area than did control fronds on ferns without nectaries ($T = -2.48$, $P = 0.0179$).

Damage on the ferns in the experiment was due in most part to the feeding of sawfly larvae (Diprionidae), which were abundant during the month of the experiment. We collected and fed these larvae for a month, but our attempts to rear adults (for identification) were unsuccessful. Two of the fronds of *Polypodium plebeium* were damaged when they were incorporated into the cover for the chrysalis of a large moth, *Automeris tridens nopaltzin* Schaus (Saturniidae).

DISCUSSION

Our observations of *Polypodium* spp. in nature revealed the nectaries to be active during the early part of the life of the frond. Spores are not produced until after

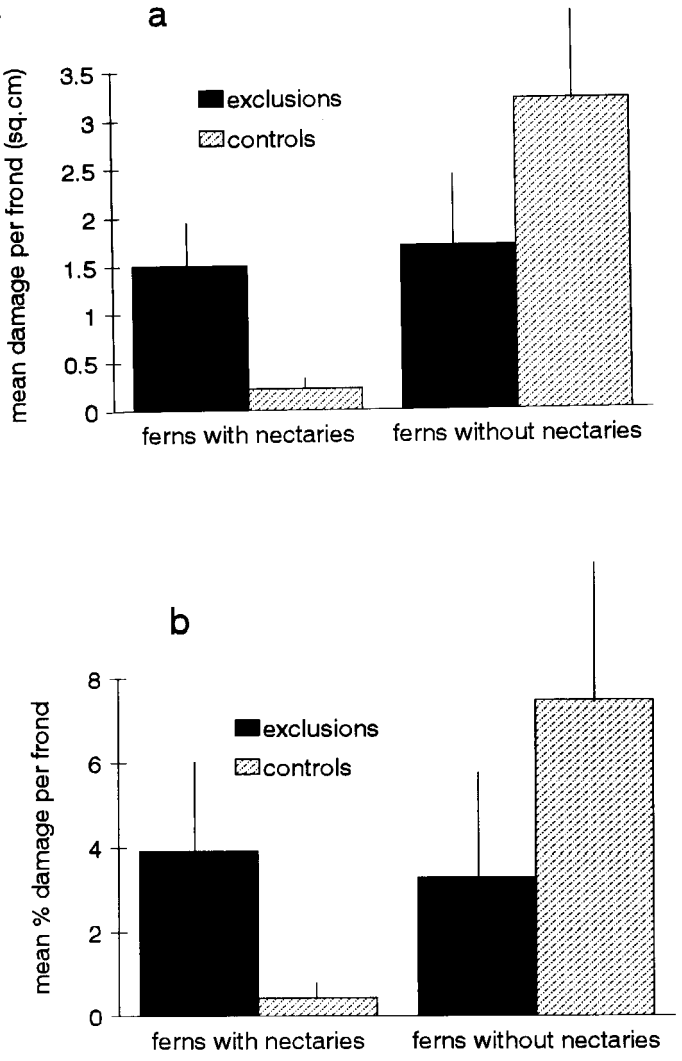

Fig. 1. (a). Ant-exclusion experiment results: mean damage sustained by each frond. Ferns with nectaries = *Polypodium plebeium* (N = 19 pair). Ferns without nectaries = *Polypodium furfuraceum, P. plesiosorum,* and *Phlebodium pseudoaureum* (combined N = 16 pairs). Error bars are 1 SE from the mean. (b) Ant-exclusion experiment results: mean percentage damage per frond. Species and sample sizes are as in (a). Error bars are 1 SE from the mean.

the leaf is fully expanded and mature; it does not appear, at least for the species we studied, that the function of the nectaries is to aid in the dispersal of the spores. We reject the spore dispersal hypothesis and turn our attention to ant protection.

The herbivore pressure from sawfly larvae provided us with a good situation in which to test the ant-protection hypothesis. When ants were excluded from fronds on ferns with nectaries, those fronds suffered significantly more damage than did control fronds on the same plants with ants freely visiting the nectaries. The evidence for ant protection in ferns with nectaries is strengthened by the lack of such evidence in similar ferns without nectaries; there was no significant difference in damage sustained between experimental and control fronds. Fronds to which ants had access (control fronds) had sustained much less damage in nectaried ferns than in ferns without nectaries, suggesting that ant protection is quite effective against the major defoliators of developing fronds active during the course of the experiment. We conclude that in *Polypodium plebeium* the nectaries active on the developing fronds serve to attract ants that protect the fronds against herbivores.

This is the first demonstration of reduction in herbivory via ant protection supported by nectaries in a pteridophyte, though our conclusions must be somewhat preliminary because our data are from only one year and our experiments at only one site. Studying bracken, Lawton and Heads (1984) suggested that although there appears to be no contemporary evidence for ant protection and the major herbivores of bracken are all counteradapted to ant defense, the nectaries on these ferns prevent colonization of the fern species by new herbivores. Ferns are ancient plants, and though many casual observers have given their long coevolutionary history as the reason for an elaborate chemical defense (Cooper-Driver, Finch, and Swain, 1977; Hendrix, 1977; Jones and Firn, 1979) and a perceived paucity of herbivores, ferns often receive substantial damage from certain herbivores adapted to a fern diet (Lawton, 1976; Balick, Furth, and Cooper-Driver, 1978; Cooper-Driver, 1978; Gerson, 1979; Hendrix, 1980; MacGarvin, Lawton, and Heads, 1986; Lawton, MacGarvin, and Heads, 1987; Shuter and Westoby, 1992).

The markedly seasonal production of new leaves in the nectaried epiphytic *Polypodium* make the newly flushed leaves an attractive resource to the Diprionidae (sawflies), adapted to eating ferns. These results are in contrast to those of Rashbrook, Compton, and Lawton (1992) where although they have evidence of protection in the laboratory (presenting bracken herbivores to ant colonies), bracken in the field does not benefit from ants because the ant levels are too low. Bracken nectaries are present and active on both young and mature fronds, whereas *Polypodium* nectaries are active only on the young fronds. The synchronous, seasonal production of nectar in the Mexican epiphytic *Polypodium* ferns provides an effective biotic defense against leaf-feeding larvae during the period of frond unfurling and maturation. It remains to be seen exactly how the ants accomplish this, and whether they are effective against any other guilds of *Polypodium* herbivores.

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