Biology of Anguina plantaginis Parasitic on Plantago aristata[1]

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Abstract: Among 17 species and cultivars of plants exposed to *Anguina plantaginis*, only *Plantago aristata* (bracted plantain) was a host. Larvae penetrated the emerging apical meristem; reproduced and migrated progressively; caused twisting and galling of leaves, looping and spiraling of peduncles, and transformation of floral structures into galls. Extreme infections caused stunting and death of entire plants. Hypertrophy and hyperplasia of leaf mesophyll, cell separation and disintegration, and xylem wall thickening in older galls occurred. Only third-stage larvae were infective, and they exhibited cryptobiosis under adverse conditions. *Key words:* histology, life cycle.

A new *Anguina* sp. *(Anguina plantaginis)* from bracted plantain, *Plantago aristata* Michx, was first reported from Lee County, North Carolina, in 1963 (5). Subsequently, it has been collected from Edgecombe, Stanley, Johnston, Granville, Wake, and Bladen Counties in North Carolina; Union and Pickens Counties in South Carolina; and Birmingham County in Alabama. The description and observations on the postembryogenesis of *A. plantaginis* are in preparation by H. Hirschmann *(personal communication).*

Other *Anguina* spp. have been reported to have attacked different plants (1, 2, 3, 4, 7, 8, 10). Goodey (2) described galls in leaves of *Agrostis tenuis* Sibth. caused by *Anguina graminophila* (Goodey) Christiei and reported that cell hypertrophy and hyperplasia involved epidermal, mesophyll, and vascular tissues. He also discovered that fourth-stage larvae from old galls entered young leaves still in the leaf sheaths. The fourth-stage of *A. balsamophila* (Thorne) Filipj. is also infective, but in *A. tritici* (Steinbuch) Chitwood, *A. agrostis* (Steinbuch) Filipj., and *A. graminis* (Hardy) Filipj. the second stage is infective.

Because of the destructive nature of this nematode on bracted plantain, its biology and its potential as a pest of other plant species (including economic crops grown in North Carolina) was investigated.

We studied the following: (i) host range, (ii) host penetration, (iii) morphological and histological symptoms of the disease, (iv) effects of temperature and moisture on disease development, (v) life cycle of the nematode, and (vi) longevity of the nematodes in soil under different moisture conditions. A preliminary report of the biology of this nematode has been published (9).

MATERIALS AND METHODS

Nematodes initially collected in summer, 1964, from galled *Plantago aristata* in Bladen and Wake Counties, North Carolina were cultured on *P. aristata* in the greenhouse. For inoculum, infected plants were washed, dried, and stored in paper bags at room temperature. Large numbers of nematodes were obtained simultaneously by pre-soaking dried galled plants 1 h, comminuting them with water for 8 min in a blender, and extracting the nematodes in Baermann funnels for 24 h. They were concentrated by settling and decanting from fresh tap water and then stored at 4 C until needed. Approximately 100 *A. plantaginis* were placed around the seeds at the time of planting.

Unless otherwise noted, all experiments contained 15 single plant replicates per treatment. Plants were grown in a greenhouse at 25-30 C in steam-sterilized soil-sand mixture (3:1, v/v) in 7.5-cm diam clay pots.

Plants to be visually inspected for nematodes were fixed 2 days in a mixture of glacial acetic acid and 95% ethyl alcohol (1:1, v/v), cleared 8-10 days in saturated chloral hydrate, and stained with acid fuchsin (3 μg/ml acid fuchsin in distilled water

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saturated with chloral hydrate) until the nematodes were sufficiently stained to be visible. Stained material was stored in small open vials containing 5% lactic acid in glycerin and kept in a desiccator at room temperature in the dark to minimize photolysis of the stain.

Host-range studies were conducted on two groups of plants. Each contained 17 species or cultivars (bermudagrass, *Cynodon dactylon* (L.) Pers.; fescue, *Festuca elatior* L.; soybean, *Glycine* max (L.) Merr. 'Lee'; barley, *Hordeum vulgare* L.; Italian ryegrass, *Lolium multiflorum* Lam.; alfalfa, *Medicago sativa* L. 'Cherokee', 'Atlantic', and 'Lahontan'; tobacco, *Nicotiana tabacum* L. 'Dixie Bright', and 'NC95'; Guinea grass, *Panicum maximum* Jacq.; Pearl millet, *Pennisetum glaucum* (L.) R. Bv.; English plantain, *P. lanceolata* L.; common plantain, *P. major* L.; plantain, *P. rugelii* Dcne.; and wheat, *Triticum vulgare* Vill. 'Atlas'). Each treatment was replicated eight times. The first of these groups of plants was harvested, killed, cleared, and stained for observation of nematodes in the tissues 15 days after inoculation. The second group received a supplemental 100-nematode inoculum on the 15th day after inoculation and was maintained in the greenhouse for a total of 4 months. During that time, all plants were observed twice monthly for development of galls or epinastic growth patterns.

To determine exactly where *Anguina plantaginis* invades *P. aristata*, the following techniques were used: (i) an open plastic sleeve 1.5-cm diam x 3.5 cm long was placed over each test seedling of *P. aristata* growing in 7.5-cm clay pots and filled with soil leaving only the leaf tip exposed above the tube, and 50 larvae were placed on the soil in the tube; (ii) seeds were germinated on filter paper in a small watch-glass, and 50 larvae were added in a small amount of water and kept covered with another small watch-glass to prevent drying; and (iii) 30 nematode larvae concentrated in a droplet of water were placed on the young leaves of *P. aristata* seedlings and protected from evaporation by a beaker inverted over the pot. The bud region was inspected 2, 6, 10, 14, 18, 24, 72, and 120 h after inoculation to determine approximate time and site of nematode entry into the plant.

Leaf segments containing nematode galls of various sizes and shapes were collected for the histopathological investigations. Nongalled leaf segments from inoculated plants of the same age were also collected and studied for comparison. The histological samples were prepared and stained with safranin-fast green as specified in Johansen (6).

Disease development at various temperatures was estimated in terms of the number of galls produced on the leaves.

Two methods were used to study the effect of moisture on disease development: (i) high humidity was maintained at the leaf surface by placing 400-ml beakers over each inoculated plant after watering for either 0, 5, or 9 h; (ii) inoculated plants kept in a mist chamber, in which the plants were exposed to a fine mist every 5 min, were compared with similarly inoculated plants kept on a bench in the greenhouse with only the soil watered.

Galls from plants inoculated at time of seeding were collected and dissected under water at 5-day intervals over a 2-month period. The nematodes which floated out were mounted on slides for detailed studies of size and stage of development.

To determine the longevity of the nematodes in moist soil, one flower gall, one leaf gall, and 100 active free larvae (taken from air-dried, infected *P. aristata* which had been stored in the laboratory for approximately 1 year) were placed in soil in separate pots, watered daily, and seeded with *P. aristata* after 1, 3, 6, 9, and 12 months. In a control series, water was withheld until time of seeding.

RESULTS

Only *P. aristata* showed symptoms of infection by *A. plantaginis*. In a very few cases, one or two nematodes penetrated the bud region of *P. lanceolata* but did not develop or cause leaf galls.

Nematode larvae penetrated *P. aristata* through the young tissues of the bud region as early as 2 h after inoculation. As new shoots emerged over a period of several months, they in turn became infected. All aboveground parts eventually became infected; nematodes were found in the developing leaf, peduncle, and flower tissues.

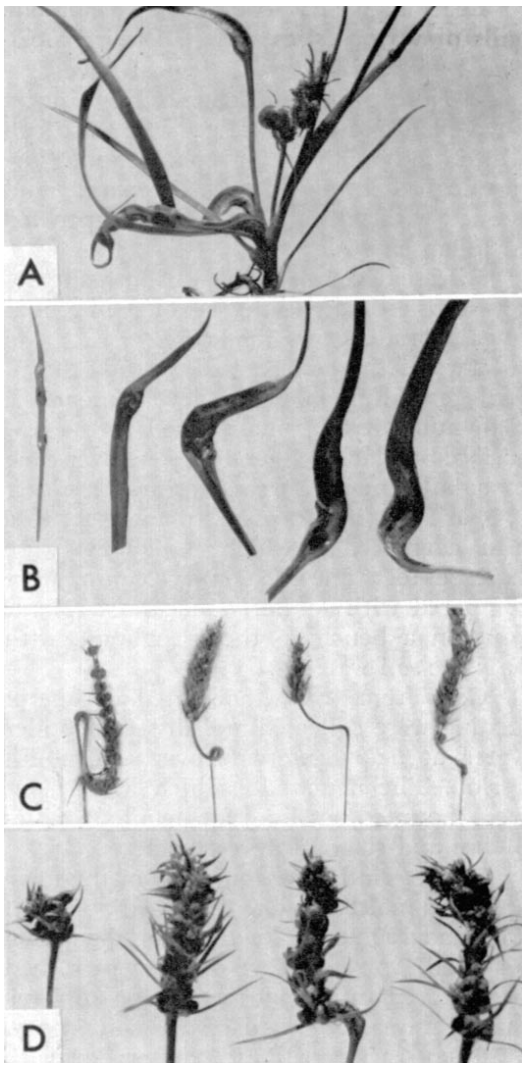

FIG. 1-(A to D). *Plantago aristata* infected with *Anguina plantaginis: A)* infected leaf, peduncle and flower tissues; *B)* leaf galls; *C)* galls, loops, and spirals on peduncles; *D)* galled inflorescences.

Anguina plantaginis caused formation of hard, globular, or oval (0.5-5.0 mm in diam) galls on leaves, peduncles and inflorescences of plantain (Fig. 1-A). The galls, which turn from yellow to deep purple with age, occurred anywhere on the leaves (except on the mid-rib) and more commonly upon lower than upper surfaces. Affected leaves were distorted or twisted, especially at gall sites (Fig. 1-B). In some cases, galls, loops, and spirals were also observed in the peduncles (Fig. 1-C). Microscopic examination of the inflorescences (Fig. 1-D) showed that floral structures such as the bracts, sepals, and the petals may be transformed into galls containing hundreds of nematodes. The dry, shriveled, galled seeds were not viable.

In lightly-infected plants, leaf galls tended to occur between the veins. In heavily infected plants, however, the galls encompassed the entire leaf, and the veins became part of the general swelling. The mesophyll was most severely affected. Where infection was heavy, the palisade mesophyll was disrupted. Central mesophyll cells were enlarged, and in some cases, became either cylindrical and arranged in rows radiating below the vascular bundle or assumed a more spherical form. Enlarging cells tended to break away from one another, which increased intercellular space and added to swelling of the gall. The nematodes fed and reproduced in the intracellular spaces (Fig. 2-A). In some cases, however, several individuals were inside the enlarged cells (Fig. 2-B). In old leaf galls, the cell wall of xylem cells was thickened by tyloses, and the tissues adjacent to the gall appeared shrunken (Fig. 2-C). Occasionally, leaf galls containing either none or only one nematode were found (Fig. 2-D), but most contained many nematodes in various stages of development (Fig. 2-E).

Temperature and moisture conditions did not significantly affect the rate of gall development.

Only third-stage larvae survived in dried tissues of *P. aristata*. These revived when temperature and moisture were again favorable, entered the leaves of young seedlings, developed into fourth-stage larvae within 2 days, and became adults after an additional 5 days. Mating took place in the galls, and the females laid large numbers of eggs. The first molt occurred within the egg, and second-stage larvae hatched about 10 days after infection and started feeding. For an additional 10 days they continued development into third- and fourth-stage larvae and second-generation adults. By this time, the adults of the first generation were dead. A high percentage of the larvae of the second generation developed into infective larvae. The infective third-stage larvae had smaller gonads and denser body contents than the fully developed third-stage larvae.

Nematodes freed from galls survived desiccation in a dormant state for 6 mo in

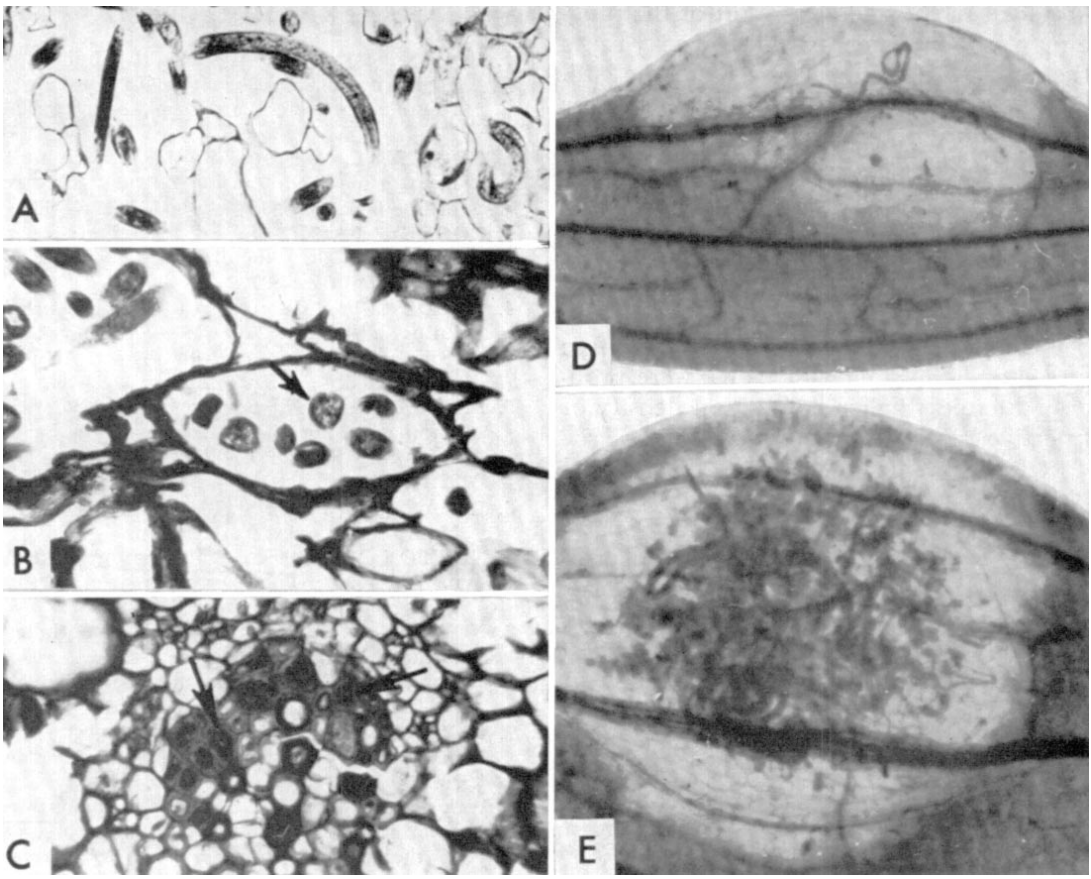

FIG. 2-(A to E). Cross sections of *Plantago aristata* leaf galls caused by *Anguina plantaginis: A)* mesophyll and palisade tissue containing nematodes and showing widened cells; *B)* Nematodes located in an enlarged mesophyll cell (arrow); and *C)* old leaf gall showing thickened cell walls of xylem cells (arrow). (D-E). Stained leaf galls in *P. aristata* induced by *A. plantaginis: D)* gall containing one nematode; and *E)* gall containing many nematodes in different stages of development.

fallow soil and were still infective to plantain. Larvae contained in leaf and flower galls survived for 12 mo. Leaf infection increased progressively, and reached its peak at 75 days after inoculation. Highest number of leaf infections occurred when flower galls were used as inoculum, and survival was always better when the soil was dry than when it was wet (Fig. 3).

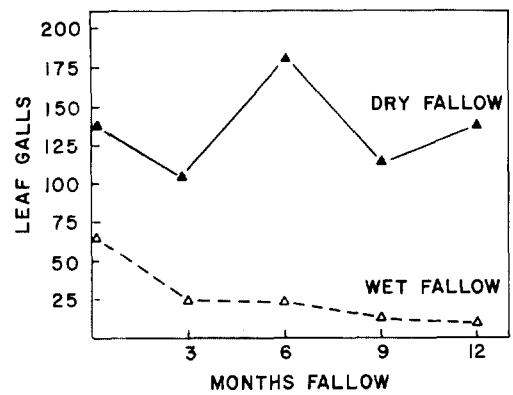

FIG. 3. Development of leaf galls on *Plantago aristata* infected with *Anguina plantaginis* as influenced by flower-gall inoculum, length of fallow, and moisture conditions under which inoculum was maintained in soil. Data represent the total of six plants for each treatment.

DISCUSSION AND CONCLUSIONS

These investigations constitute the first extensive report on the biology of *Anguina plantaginis* parasitic on *P. aristata*. Nematodes were not always found in galls, a fact suggesting either that the gall was incited by ectoparasitic feeding or that the nematode entered the leaf, incited gall formation, and then migrated out of the leaf. In severe infections, water transport may be

occluded in the gall as a result of the plugging of xylem elements, and this may cause the collapse of tissues adjacent to the gall.

Anguina plantaginis survives in soil for several months, certainly long enough to enable the nematodes to survive from one planting season to the next. Moist soil breaks dormancy, and nematodes active in the soil rapidly deplete stored food in the body. The higher rates of infection which resulted when flower galls were used as inoculum, compared with the 100 active free larvae and the single leaf galls, were probably due to a higher population density of infective larvae.

Of the plants tested, only the bracted plantain *(P. aristata)* was attacked by *Anguina plantaginis*. This high degree of parasitic specialization suggests that the association between the nematode and host has evolved over a long period of time. It should be indicated, however, that only two populations of the nematode were used in these studies. With the use of additional populations, it may be found that other *Plantago* spp. and crop plant species are hosts for this nematode.

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