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Subanguina calamagrostis and Its Biology in *Calamagrostis* spp. in Iowa, Ohio, and Wisconsin[1]

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Goto and Gibler (5) reported that nematodes in leaf galls on *Calamagrostis canadensis* (Michx.) Beauv. near St. Paul, Minnesota, resembled *Anguillulina graminophila* [= *Subanguina graminophila* (T. Goodey, 1933) Brzeski, 1981]. Wu (10) described *Anguina calamagrostis* Wu, 1967, later renamed *Subanguina calamagrostis* (Wu, 1967) Brzeski, 1981 (3), as occurring on *C. canadensis* in Ontario and Quebec, Canada.

Calamagrostis canadensis is a common grass in low wet prairies of the central United States. *Calamagrostis inexpansa* A. Gray occurs in similar habitats, but is less prevalent. During 1982–85, we found *S. calamagrostis* in *C. canadensis* galls collected from the Bergman, Crossman, Hayden, Mark Sand, Steele, Stinson, and Williams prairies in six Iowa counties, and in *C. expansa* galls from Dickenson County, Iowa. *C. canadensis* galls containing juvenile and adult nematodes were also collected at the Irwin Prairie just west of Toledo, Lucas County, Ohio, and near Port Wing, Bayfield County, Wisconsin. None of the locations had ever been cultivated.

Fifty galls from 1 to 12 mm long (averaging 4.1 mm) and resembling the elongated and furrowed galls described by Goto and Gibler (5) and Wu (10) occurred on abaxial or adaxial leaf surfaces (Fig. 1). Color varied from light yellowish-green to tan in young galls to dark brown in old galls. Pycnidia of the fungus *Dilophospora alopecuri* (Fr.) Fr. often made old galls appear blackened. New leaf growth often occurred in axils when galls were near nodes. Large galls often caused leaves to be twisted and contorted. Examination of several galls from all Iowa collections indicated that the life cycle was completed in June or July when adults, eggs, and juveniles were found in new grass growth. Most adults had deteriorated by mid-August. Young green galls found in early September were less than 2 mm long and contained juveniles but not adults or eggs.

Thirty galls in overwintered debris from

FIG. 1. Galls incited by *Subanguina calamagrostis* in *Calamagrostis canadensis,* Steele Prairie, Iowa, 1982.

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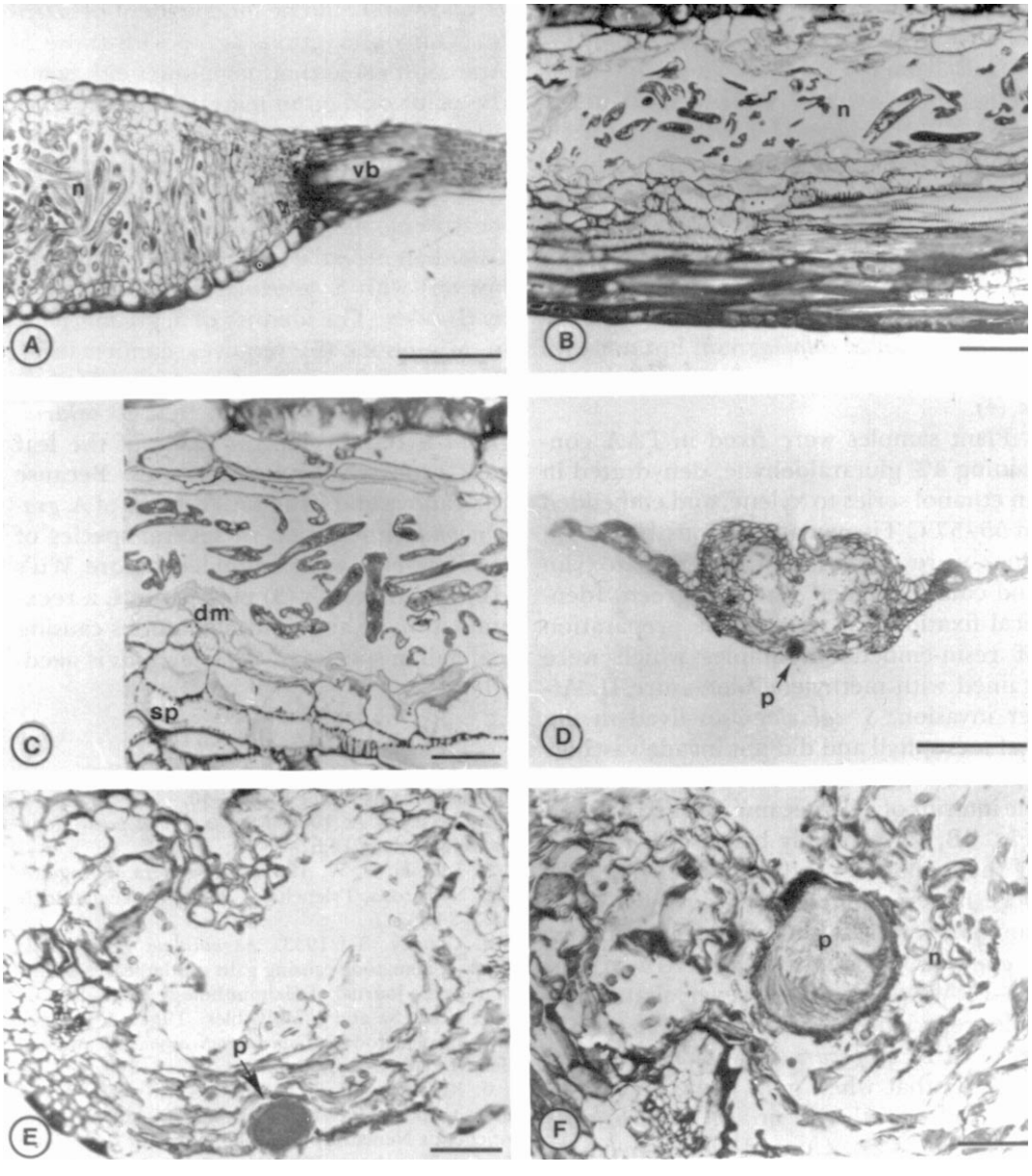

FIG. 2. Histological sections of *Calamagrostis canadensis* leaves infested with *Subanguina calamagrostis* and *Dilophospora alopecuri*. A) Longitudinal section of portion of a gall showing nematodes and nonenlarged normal tissue containing vascular bundles. Bar = 0.2 mm. B) Longitudinal section showing many nematodes in a gall that did not contain *Dilophospora alopecuri*. Bar = 0.1 mm. C) Longitudinal section of a portion of a gall showing nematodes, sclerified parenchyma, and deteriorating mesophyll cells bordering the cavity. Bar = 0.05 mm. D) Cross section of *C. canadensis* leaf showing enlarged gall bordered by normal tissue. Note pycnidium of *D. alopecuri*. Bar = 0.5 mm. E) Cross section of a gall containing a pycnidium of *D. alopecuri* and few nematodes in the surrounding tissue. Bar = 0.1 mm. F) Detail of pycnidium of *D. alopecuri*. Bar = 0.05 mm.

Abbreviations: dm = disintegrating mesophyll, n = nematode, p = pycnidium, sp = sclerified parenchyma, vb = vascular bundle.

the Williams Prairie were collected in April 1985 and examined. Eighteen contained *D. alopecuri* but no nematode eggs and few or no juveniles; the other 12 galls contained few to no pycnidia and many nematode juveniles but no eggs or adults. When

juveniles were immersed in water, most became active within 2 to 12 hours.

Galls from the Hayden, Steele, and Bergman prairies were dissected, and nematodes were preserved by Seinhorst's method (8). Galls often contained 3–4 females and males each, but one gall contained 11 females. One gall collected in September 1983 contained 2,960 juveniles. Measurement of 12 females, 8 males, 20 juveniles, and 34 eggs agreed with Wu's (10) measurements for *S. calamagrostis* but not with Goodey's measurements for *S. graminophila* (4).

Plant samples were fixed in FAA containing 4% glutaraldehyde, dehydrated in an ethanol series to xylene, and embedded in 55–57 C Tissuemat. Sections 10–12 μm thick were stained with iron-hematoxylin and counterstained with fast green. Identical fixation was used in the preparation of resin-embedded samples which were stained with methylene blue-asure II. After invasion, *S. calamagrostis* lived in the leaf mesophyll and did not invade vascular bundles (Fig. 2A, B). Mesophyll cells lining the interior of galls became crushed or lysed (Fig. 2B, C), probably because of feeding by the nematodes. Most parenchyma cells were hypertrophied, but there was occasional hyperplasia. As galls aged, a fungus, *D. alopecuri*, frequently colonized them (Fig. 2D–F). We observed, however, that nematodes reproduced and developed in the absence of *D. alopecuri*. It was observed repeatedly that when galls contained many nematodes, little or no *D. alopecuri* occurred (Fig. 2B, C), and where pycnidia were common, nematodes were few (Fig. 2D–F). This fungus can parasitize *Anguina agrostis* (A. F. Bird and A. McKay, pers. comm.), and such a phenomenon could be operative in our observations. The presence of *D. alopecuri* in galls has led to conflicting reports. Bessey (2), Goodey (4), and we found the fungus and anguinid nematodes in the same gall, but Sprague (9) did not. Atanasoff (1) believed that in wheat the fungus could develop only in the presence of *Anguina tritici*. Schaffnit and Wieben (7) demonstrated that leaf infection by *D. alopecuri* could be independent of *A. tritici*. Our observations agree with those of Atanasoff (1) in that nematodes either left the plant or died in material heavily colonized by the fungus.

Galls from Canada, Iowa, Minnesota, and Wisconsin were physically similar and the measurements of nematodes from Iowa and Canadian material agreed with each other but not with *S. graminophila* as described by Goodey. The identity of *S. graminophila* in Minnesota (5) requires confirmation. Because of the host restrictions of many anguinids, it is probable that *S. calamagrostis* is responsible for many of the leaf galls reported on *C. canadensis*. Because Kir'yanova and Krall's (6) reports of *A. graminophila* infestation of several species of *Calamagrostis* were compiled before Wu's (10) and Brzeski's (3) publications, a reexamination of anguinid nematodes causing leaf gall in species of *Calamagrostis* is needed.

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