Journal of Nematology 19(2):158–163. 1987.

Morphometric Analysis of *Anguina amsinckiae* from Three Host Species

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Abstract: *Amsinckia* species (fiddleneck) in the South Coast Ranges of California were surveyed to determine if any of the 12 different California species of *Amsinckia* are hosts of the nematode, *Anguina amsinckiae* (Steiner and Scott, 1935) Thorne, 1961. Previously only *Amsinckia intermedia* Fischer and Meyer was reported as a host of *Anguina amsinckiae*. The survey established that there are at least two additional hosts of *Anguina amsinckiae*: *Amsinckia lycopsoides* Lehmann and *Amsinckia gloriosa* Suksdorf. Seven sites containing nematode-infected *Amsinckia* plants were discovered. Every site contained two or more species of *Amsinckia*; however, only one site contained more than one species of *Amsinckia* that was galled. Nematode specimens from *A. intermedia, A. lycopsoides,* and *A. gloriosa* were used in a morphometric analysis of 14 morphological variables. Stepwise discriminant analysis of the variables to separate the populations by host were successful for females, and the pairwise *F*-tests showed all three populations to have different group means ($P < 0.05$). Males from the three hosts were not always separable, however, as only the nematodes from *Amsinckia gloriosa* had a different group mean ($P < 0.05$).

Key words: biological weed control, host–parasite relationships, *Amsinckia,* fiddleneck, discriminant analysis.

Fiddleneck (*Amsinckia* Lehmann) is a winter annual weed that is a serious pest in many crops, including oats, barley, wheat, and alfalfa, and in orchards and rangelands (11). In addition, fiddleneck is poisonous and presents a danger to livestock if eaten in sufficient quantities (7). Consumption of alfalfa hay infested with fiddleneck causes liver damage in hogs and cattle and "walking disease" in horses (6). No effective herbicides are registered to control fiddleneck in alfalfa (11). In 1980 Nagamine and Maggenti (10) proposed that *Anguina amsinckiae* (Steiner and Scott, 1935) Thorne, 1961 (spelling of specific epithet corrected) is a potential biocontrol agent of common fiddleneck (*Amsinckia intermedia* Fischer and Meyer).

Anguina amsinckiae was discovered near Winters, California (in the Sacramento Valley), in 1930 on the host plant *Amsinckia intermedia* (19). Subsequently, Steiner and Scott (16) described the nematode as a variety (var. *amsinckiae*) of *Anguillulina dipsaci.* In describing the life cycle of the nematode, Godfrey (8) referred to the nematode as *Ditylenchus dipsaci* var. *amsinckiae.* In 1961 Thorne (19) reclassified the nematode as a species of *Anguina.*

Statistical analyses of morphological measurements are becoming more commonly used in nematode taxonomy. In the absence of distinguishing features, morphometric methods are attractive in principle, but difficult to practice (3). Lima (9) measured 25 morphological characters from 76 populations of the *Xiphinema americanum* complex. A principal coordinates analysis and a cluster analysis grouped the populations into seven species including four previously undescribed species. In addition to identifying new species, morphometrics can be used to eliminate invalid classifications. Using the results of statistical analyses of morphological characteristics, Stynes and Bird (18) concluded that *Anguina funesta* Price, Fisher, and Kerr, 1979 was indistinguishable from *A. agrostis* (Steinbuch, 1799) Filipjev, 1936 and, therefore, a synonym for *A. agrostis.*

If *Anguina amsinckiae* is to be used successfully as an organism for biological weed control, the host range of the nematode must be determined. In addition, it should be established if there are different biological or geographical races of the nematode which are distinguished by host range or by morphological characteristics.

Received for publication 4 March 1986.
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Materials and Methods

Twelve different species of *Amsinckia* occur in California (12) with some being relatively rare (15). In March 1984, a survey of the South Coast Ranges of California, which are thought to be the phytogeographical center of the genus *Amsinckia* (12), was conducted to determine if species of *Amsinckia* other than *Amsinckia intermedia* are hosts of *Anguina amsinckiae.* Botanical collections of *Amsinckia* housed at the herbaria at the University of California and the California Academy of Science in San Francisco were consulted for the locations of the less common species. These sites were subsequently visited and nematode-galled plants were collected for all species of *Amsinckia* present. Galls, which replace flowers, are about 1 cm in diameter (11).

Specimens used for the morphometric analysis were taken from Site 2 (Santa Clara County; host—*Amsinckia intermedia*), Site 6 (San Luis Obispo County; host—*Amsinckia lycopsoides*), and Site 7 (San Luis Obispo County; host—*Amsinckia gloriosa*) (Fig. 1; Table 1). The nematodes were placed in Seinhorst's fixative (17) and transferred to glycerin using the De Grisse (5) method. For each of the three host plants, 30 females and 30 males were measured (n = 180). Ten variables were recorded for each specimen including total length, body width, tail length, esophageal length, anterior end to the center of the esophageal bulb, and to the excretory pore. Other measurements were, for females, the length

Fig. 1. Location in California of seven sites of *Anguina amsinckiae* nematosis (Table 1) surveyed in March 1984. Lines within the state represent county boundaries.

Table 1. Location and incidence of *Amsinckia* spp. from a survey of the South Coastal Ranges of California in March 1984.

Site	Location	Species present and (specimen number)
1	Solano County, 6.4 km south of Davis on the west side of Eggert Road.	*A. lycopsoides** (358), *A. intermedia* (359)
2	Santa Clara County, 3.2 km south of Morgan Hill on the west side of Monterey Road at Crowner Avenue.	*A. intermedia** (385), *A. lycopsoides* (386)
3	San Benito County, northwest of the intersection of Highway 25 and Live Oak Road.	*A. lycopsoides** (425), *A. intermedia** (426), *A. lycopsoides* × *intermedia** (428)
4	San Benito County, on Highway 25, 4.6 km south of intersection with Highway 146.	*A. lycopsoides** (475), *A. intermedia* (476)
5	San Luis Obispo County, intersection of Highway 58 and Shell Road.	*A. intermedia** (491), *A. menziesii* (492), *A. lycopsoides* (493), *A. gloriosa* (497)
6	San Luis Obispo County, 3.7 km east of Shull Road on Highway 58.	*A. lycopsoides** (501), *A. gloriosa* (502), *A. menziesii* (505)
7	San Luis Obispo County, 9.3 km east of Simmler on Highway 58.	*A. gloriosa** (563), *A. vernicosa* (566)

Voucher specimens deposited in the University of California, Davis, Department of Botany Herbarium (DAV).
* Galled by *Anguina amsinckiae.*

TABLE 2. Measurements of *Anguina amsinckiae* females from three different host species. Sample size is 30 nematodes for each host species.

Characteristic	*Amsinckia lycopsoides*	*Amsinckia intermedia*	*Amsinckia gloriosa*
Body length	1,332.9 (96.3) 1,199–1,535	1,385.9 (85.0) 1,198–1,525	1,286.6 (127.1) 1,106–1,581
Body width	45.0 (5.7) 30.8–60.8	45.9 (7.1) 36.1–65.4	49.9 (7.9) 35.4–69.2
Tail length	56.0 (9.2) 31.5–73.1	60.2 (9.4) 40.0–89.2	50.3 (10.1) 33.1–68.4
Esophageal length	172.5 (17.5) 144.6–221.5	157.0 (22.3) 107.7–195.3	140.2 (18.5) 92.3–169.2
Length from anterior to center of median bulb	60.5 (7.1) 45.4–79.2	61.5 (7.5) 38.5–81.5	59.0 (6.9) 46.1–80.7
Length from anterior to excretory pore	117.7 (11.6) 81.5–140.0	126.1 (16.9) 93.0–163.8	120.7 (13.4) 100.7–161.5
Post-uterine sac length	47.2 (10.0) 23.1–66.9	55.9 (8.2) 40.8–78.4	49.9 (10.0) 29.2–72.3
Vulva to anus distance	88.5 (11.1) 69.2–109.2	98.4 (19.8) 69.2–161.5	92.7 (14.1) 68.4–133.9
Post-uterine sac (%)	32.5 (7.1) 19.0–44.8	35.8 (4.9) 28.0–49.0	36.7 (5.6) 24.9–47.6
Vulva (%)	88.4 (1.6) 85.9–92.9	87.3 (1.8) 82.3–89.7	87.8 (1.7) 84.5–91.1

Mean measurement (μm or percentage) is followed by standard deviation in parentheses and range.

of the post-uterine sac, vulva to anus distance, percent post-uterine sac, and percent vulva, and for males, the length of spicule, gubernaculum, bursa, and testis. A stepwise discriminant analysis (1) of the morphological variables was performed for both sexes.

RESULTS

The survey determined that *Anguina amsinckiae* is not host specific to *Amsinckia intermedia*. *Amsinckia lycopsoides* Lehmann and *Amsinckia gloriosa* Suksdorf are also hosts of *Anguina amsinckiae*. Seven sites of infestation were located (Fig. 1; Table 1), and each contained more than one species of *Amsinckia*. With the exception of Site 3, only one species of *Amsinckia* was galled at each site (Table 1). At Site 1 both *Amsinckia intermedia* and *A. lycopsoides* were present, but only *A. lycopsoides* was galled, whereas at Site 2 both *A. intermedia* and *A. lycopsoides* were present, but only *A. intermedia* was galled. Hybrid-like plants containing floral characteristics of *A. intermedia* and *A. lycopsoides* were present at Site 3. At Site 2 virtually all the *A. intermedia* plants were galled, whereas a population of *A. intermedia* ¼ km to the north of Site 2 was free of nematode-galled plants.

Morphologically, all three nematode populations (Tables 2, 3) key to *Anguina amsinckiae* (4). Morphometric tests using stepwise discriminant analysis, which identify and combine diagnostic variables to separate the populations by host, were successful for females but not for all males.

The analysis for females chose the subset measurements of esophagus, postuterine sac, tail, and the "a" ratio as the canonical variables that best separated the populations. Canonical variables are derived from the above mentioned variables so as to best represent the separation of the groups in a graphical manner (1). The pairwise *F*-tests showed all three groups of females to be significantly different ($P < 0.05$). Plotting canonical variables showed much overlap of the female populations (Fig. 2). By using the classification it was possible to correctly identify the nematodes from *A. gloriosa* on 74.1% of the attempts. Nematodes from *A. intermedia* and *A. lycopsoides* were identified correctly on 53.3% and 44.4% of the at-

TABLE 3. Measurements of *Anguina amsinckiae* males from three different host species. Sample size is 30 nematodes for each host species.

Characteristic	*Amsinckia lycopsoides*	*Amsinckia intermedia*	*Amsinckia gloriosa*
Body length	1,187.1 (92.3) 1,005–1,425	1,286.4 (120.8) 972–1,459	1,158.7 (72.3) 1,012–1,272
Body width	39.2 (3.6) 35.4–53.8	38.2 (4.4) 30.8–46.9	37.1 (2.5) 31.5–44.6
Tail length	61.2 (9.1) 45.4–98.4	66.0 (7.1) 52.3–80.7	56.6 (7.2) 40.0–76.1
Esophageal length	170.6 (25.4) 123.0–208.4	172.6 (43.5) 116.9–250.7	149.1 (22.2) 110.0–225.3
Length from anterior to center of median bulb	60.7 (7.4) 39.2–73.8	67.2 (10.9) 46.1–105.4	62.5 (9.3) 43.1–99.2
Length from anterior to excretory pore	119.5 (14.4) 99.2–173.0	126.0 (19.8) 91.5–179.9	120.1 (19.6) 89.2–187.6
Spicule length	37.4 (2.9) 30.8–43.8	38.0 (3.2) 32.3–43.9	37.4 (3.7) 30.8–46.1
Gubernaculum length	10.6 (1.8) 7.7–14.6	11.3 (2.4) 7.7–15.4	11.9 (2.8) 7.7–20.7
Bursa length	82.5 (10.8)	84.1 (11.6)	74.5 (5.9)
Occupying length of testis	775.5 (130.3) 542.8–1,055.6	792.9 (142.7) 399.6–965.7	865.8 (79.2) 662.7–992.3

Mean measurement (μm) is followed by standard deviation in parentheses and range.

tempts, respectively. A correct classification of less than 33.3% would have no better discriminating power than a random assignment. Each group has individuals that are overlapping (Fig. 2). However, some individuals have unique orientation, segregating from the other two populations, and therefore have diagnostic morphology.

Males were not as separable as females. The discriminant analysis picked the classification that used tail length and the "T" ratio as the variables that best differentiate the nematode populations. The pairwise *F*-tests separated only the *A. gloriosa* male population ($P < 0.05$). Males from *A. intermedia* and *A. lycopsoides* were indistinguishable. Plotting of canonical variables illustrates the separation (Fig. 3). The classification correctly placed nematodes from *A. gloriosa* on 82.6% of the attempts; nematodes from *A. intermedia* and *A. lycopsoides* were correctly placed on 57.1% and 37.5% of the attempts, respectively. Again, as with females, there is much overlapping, yet the *A. gloriosa* male population is partially segregated from the other two. The *A. intermedia* and *A. lycopsoides* male populations appear to be closely situated.

DISCUSSION

Because the nematode galls were usually limited to only one host species at a given site, we suspected that even if other potential hosts were present there might be genetic differences among the nematode populations (i.e., biological races) that determine their host specificity. Moreover, genetic differences in different populations of the host plant might determine if the plant is a suitable host. For example, Godfrey (8) and Nagamine and Maggenti (10) reported that infections are always limited to small centers of nematosis. This pattern suggests that R-genes (genes resulting in host resistance) could be widespread in many populations of *Amsinckia* and that only certain populations of *Amsinckia* are suitable hosts for the nematode. *Amsinckia intermedia, A. lycopsoides,* and *A. gloriosa* are self-pollinating, and populations typically are morphologically and genetically uniform (13,14). Self-pollination can result in limited gene flow between different pop-

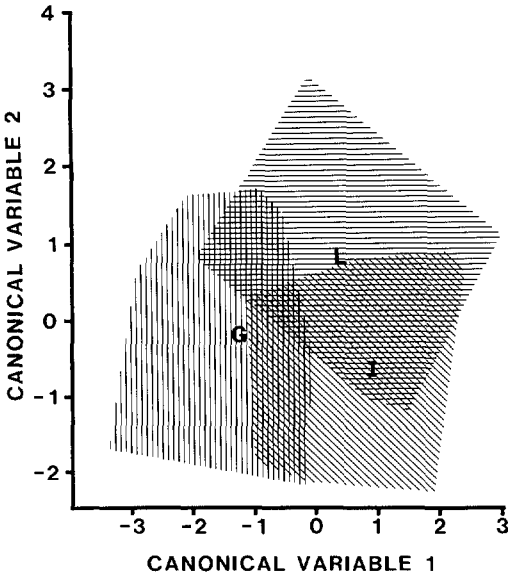

FIG. 2. Morphometric relationships for *Anguina amsinckiae* females assessed along two canonical variables. Esophageal, post-uterine sac and tail lengths, and "a" (total length divided by the greatest width) were selected to best separate the populations. Diagonal lines indicate the range for individual nematodes from *Amsinckia intermedia*, horizontal lines show the range for individual nematodes from *A. lycopsoides*, and vertical lines delimit the range for nematodes from *A. gloriosa*. I = the centroid (i.e., the mean of each of the canonical variables for the population) for nematodes from *A. intermedia*, L = the centroid for nematodes from *A. lycopsoides*, and G = the centroid for nematodes from *A. gloriosa*.

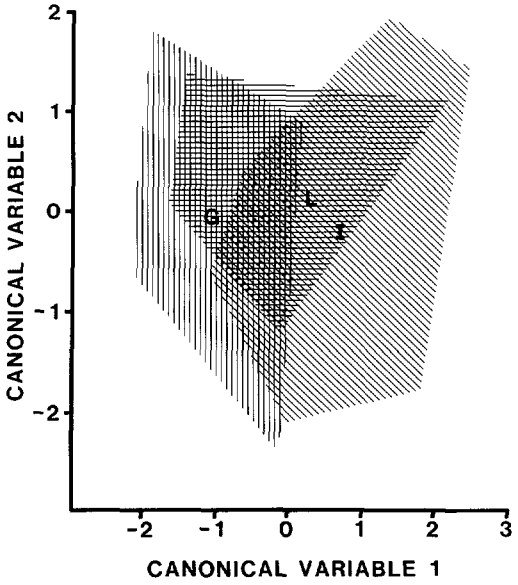

FIG. 3. Morphometric relationships for *Anguina amsinckiae* males assessed along two canonical variables. Tail length and the "T" ratio were selected to best separate the populations. Explanation as in Figure 2 legend.

ulations of *Amsinckia*. Genetic isolation of different populations of *Amsinckia*, resulting from infrequent cross-pollination, could help to explain the localization of nematode infection centers. At Site 3 many *A. lycopsoides* and several *A. intermedia* plants were galled; in addition, galled hybrid-like plants that were intermediate between *A. intermedia* and *A. lycopsoides* were present at Site 3. Natural hybrids between *A. intermedia* and *A. lycopsoides* are thought to occur (14). Interbreeding between *A. intermedia* and *A. lycopsoides* at Site 3 may account for the absence of stricter host specificities seen at the other six sites.

Differences in the morphometrics of *Anguina amsinckiae* from different hosts reflects to a certain extent the taxonomic relationships between the hosts. *Amsinckia intermedia* and *A. lycopsoides* are in the same section of the genus (Muricatae) and can hybridize (14); *A. gloriosa* is in a different section (the Tessellatae) (12) and is not known to hybridize with either of the two other host species (14). Correspondingly, *Anguina amsinckiae* individuals from *Amsinckia gloriosa* tended to segregate further from the other two nematode populations (Figs. 2, 3). Thus, it is possible that the degree of morphological similarity among the nematode populations is related to the phylogeny of the hosts.

Although differences in some of the morphological measurements of the three *Anguina amsinckiae* populations are statistically significant, the differences are not striking enough to merit describing new species, and there is considerable overlap among the three populations (Figs. 2, 3). Barnes (2) pointed out that there is a tendency for taxonomists to emphasize small morphological differences when describing new species found on previously unrecorded hosts. It is known that morphological variability within a nematode species can be influenced by availability of nutrients (20). Morphometrical differences

between *Anguina amsinckiae* populations from different host species may be nutritional related and may not reflect the existence of races or incipient species.

An understanding of the host–parasite interactions is necessary for the successful implementation of a biological weed control program. If a biocontrol agent is not specific and attacks economically desirable plants, dissemination of the organism would not be appropriate. Alternatively, if a biocontrol agent only reproduces on a few populations or biotypes within a host species, the agent may be too specific to be of practical use.

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