Journal of Nematology 26(3):315–323. 1994.

Epidemiology of *Anguina agrostis* on Highland Colonial Bentgrass

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Abstract: The epidemiology of *Anguina agrostis* was investigated in field plots of Colonial bentgrass (cv. Highland), *Agrostis tenuis,* near Corvallis, Oregon. Each October from 1990–92, nylon mesh pouches, each containing 10 galls, were buried in the field or placed on the soil surface in microplots. Pouches were collected monthly or bimonthly between December and June and nematodes per gall counted. Nematode egression from galls began in late March and was completed by mid-May, corresponding to the period of floral initiation in bentgrass. In 1991 and 1992, 0.09-m^2 plots were inoculated with 0, 1, 5, 15, 50, 120, or 200 galls/plot. The disease severity (number of galls) and disease incidence (% seed heads with galls) increased linearly at inoculum densities below 50 galls/plot. At higher inoculum densities, disease increase approached an asymptote. In 1991, plots were established to determine the characteristics of disease spread. Disease foci were established by placing 0, 5, 50, or 500 galls along 30-cm sections of row in the fall. In July 1992, seed heads were harvested at 30 and 60 cm from each focus within and across plant rows. Most infestations were found within 30 cm of foci at all inoculum levels. At high inoculum densities, the distribution of galls was aggregated with the majority of galls located on less than 10% of the seed heads. These disease spread and incidence data suggest populations of *A. agrostis* increase slowly in bentgrass in Oregon.

Key words: Anguina agrostis, Agrostis tenuis, bentgrass, crop loss, epidemiology, nematode, seed-gall nematode, population dynamics.

The seed-gall nematode, *Anguina agrostis* (Steinbuch, 1799) Filipjev, 1936, has been reported from a range of graminaceous hosts (9,16). The discrepancies reported in the host status of *A. agrostis* have been resolved by electrophoresis, which identified differences among morphologically identical populations of *Anguina* spp. collected from different grass species (15). Two morphologically indistinguishable *Anguina* species, *A. funesta* Price, Fisher & Kerr, 1979 and *A. agrostis,* are economically important on ryegrass, *Lolium* spp., and bentgrass, *Agrostis* spp., respectively. In Australia, the impact of annual ryegrass toxicity, a fatal poisoning of livestock caused by *Clavibacter toxicus* Riley & Ophel, which is vectored by *A. funesta* (3,12,16,19), has been the impetus for extensive research on the biology of *Anguina.* On bentgrass, *Agrostis tenuis,* grown for seed in the Pacific northwest region of the United States, *A. agrostis* was found in 3–9% of fields surveyed (1,2) and was reported to cause 50–75% yield loss (8). In addition to direct loss of yield, bentgrass seed contaminated with galls is restricted from export to some international markets.

Anguina agrostis and *A. funesta* have similar life cycles and complete one generation per year (8,12). Galls containing second-stage (J2) dauer juveniles develop and drop to the ground as the seed heads mature during the summer. After the fall rains begin, the dauer juveniles transform to active J2 and leave the galls. In Australia, J2 egression from ryegrass galls started within 4–6 weeks of the first rain and corresponded to the physical and biological breakdown of the gall tissue (12). In Oregon, however, the relationship between gall decomposition and nematode egression is unknown. Once in the plant crown, J2 remain between leaf sheaths or rhizomes until spring, when floral primordia form (8,10,12). Upon invading the floret ovaries, J2 rapidly develop to adults and reproduce. Juveniles molt once in the eggs, hatch quickly as J2, and become resistant dauer J2 before the galls dry (4,5,6). The life cycle requires 3–4 weeks and is often completed before the influorescence opens (8). The infested floral primordia

Received for publication 3 March 1994.

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We thank Kristin Freitag and Patrica Guild for technical assistance.

develop into thick-walled galls enclosed in the lemma and palea.

The epidemiology of *A. agrostis* on bentgrass has not been elucidated. The objectives of this research were to i) determine the egression pattern of juveniles from overwintering galls in relation to infection of Highland Colonial bentgrass plants, ii) document spatial and temporal characteristics of disease progress, and iii) investigate the population dynamics of *A. agostis.*

Materials and Methods

Egression study: Prior to seed harvest in July of 1990, 1991, and 1992, *Anguina agrostis* galls were collected from Colonial bentgrass fields and research plots. Ten galls selected at random were sealed in 1–2 cm^2 pouches constructed from 100-μ mesh nylon fabric. In October, prior to the start of fall rains each year, pouches were placed in a field at Hyslop Research Farm, Corvallis, Oregon. Gall pouches were covered with 0.5–1.0 cm of soil in a nonfumigated, fallowed area in October 1990 and placed on the soil surface of 15-cm-d microplots, each with one Highland Colonial bentgrass plant in October 1991 and 1992. Galls used as controls were stored under dry conditions in sealed vials at room temperature.

Each year, five samples were collected monthly from December through February and semi-monthly from March through June. Galls were removed from pouches, soaked overnight in sterile water, and teased apart in water to free the nematodes. At each date, 10 control galls also were soaked overnight and teased open. The number of active and quiescent J2 were quantified for each gall by observing the J2 in 25% of the surface of gridded petri dishes. Quiescent J2 had straight bodies and did not respond to the touch of a needle. The percentage of nematodes remaining in the galls at each date was estimated by dividing the number of nematodes per exposed gall by the mean number of nematodes in the control galls over all sampling dates. In July 1992 and 1993, all bentgrass heads were collected from each microplot and galls were quantified.

Population dynamics studies: Plots were established in a Woodburn silt loam soil at the Hyslop Research Farm. The site was fumigated with methyl bromide at 350 kg per ha in August 1990 and in September seeded with Highland Colonial bentgrass at 3.2 kg/ha in rows 30 cm apart. Twenty-five wooden frames, 1.5 × 1.5 m (plots), were strung with wire spaced at 30-cm intervals to produce 16 subplots each with a 30 × 30 cm opening. Plant rows were centered in each subplot. In October 1990, subplots were infected with 0, 1, 5, 15, or 50 galls by shaking galls from an envelope over the area. A 30 × 30 × 60 cm high plastic box with open ends was placed in each subplot during inoculation to ensure that galls were confined to the subplot area. The soil surface was covered with a 3–5 mm layer of sand to prevent movement of galls. Following the July 1991 harvest, plots were thoroughly cleaned with an industrial vacuum cleaner to remove all loose debris, including galls. Subplots were reinfested at the same gall densities in October 1991 and additional plots were infested at 120 and 200 galls per subplot. J2 population densities per gall of inoculum were 201 and 856 in 1990 and 1991, respectively.

Each July, when galls were mature, but before seed heads reached a stage where they easily shattered, heads were collected by hand from each subplot, placed in paper bags, and allowed to air dry. A random number table was used to select one-half of the 16 subplots from each plot for evaluation. The number of heads per subplot was determined and heads were evaluated for the mean number of galls, galls per head, and percentage of infected heads per plot.

Galls from all plots were bulked and used for egression studies the following year. The population of nematodes at the end of the season was based on the number of active J2 in 200 control galls assayed in the egression study the next season.

Disease spread study: Twenty-four plots, 3 × 3 m, were established at Hyslop Re-

search Farm in a 1-year-old bentgrass field. In October 1991, two rows (subplots), either 1 m north or south of the center of each plot, were inoculated by dispersing 5, 50, or 500 galls on to the plants in 30-cm sections of the rows that ran east and west. Inoculum had a mean of 856 active J2 per gall, which resulted in inoculum densities of approximately 15, 150, and 1,500 J2 per seed head at the foci. Inoculated and noninoculated plots were replicated six times.

In July 1992, 50 heads were collected in 30-cm segments of row at the foci and in the row 0–30 cm and 30–60 cm to the east and west of the foci. Samples were also collected from a 30-cm section of the rows 30 cm (one row) and 60 cm (two rows) north and south of the foci. Heads were inspected, galls on each head counted, and percentage of heads infected and galls per head calculated.

Statistical methods: Proportional data were normalized using an arcsine square root transformation prior to analysis. For the egression study, ANOVA was used to evaluate the relationship between sampling date and the percentage of active J2 in galls at each date (18). J2 egression data for all dates sampled over 3 years was fitted to a second-order polynomial model. Similarly, the relationship between infestion of plants and P_i also was examined by fitting the population dynamics data to linear regression or second-order polynomial models. Means are presented with standard deviations. Correlations were computed between the number of flower heads per plot and the infestation rate at each P_i in 1990 and 1991.

RESULTS

Egression: The percentage of active J2 recovered from galls placed in the field ranged from 50–87% during the 3 years. There were no differences ($P < 0.05$) in the percentage of active J2 in galls among sampling dates from December through April and control galls each season.

The percentage of nematodes remaining in galls was stable until mid-March, when the percentage dropped rapidly (Fig. 1A). By mid-May the galls contained no active *A. agrostis*. A similar pattern of nematode activity was evident from plant infestation data (Fig. 1B). No galls were found in plants from microplots when gall pouches were removed before April 1, whereas plants were infected when galls were left in plots after April 1. Egression from the galls buried in 1990–91 and placed on the soil surface in 1991–92 and 1992–93 followed a similar pattern. When the galls were collected, both buried pouches and those placed on the soil surface were infused with soil.

Annual weather patterns during the 3-year study were similar (20). Monthly

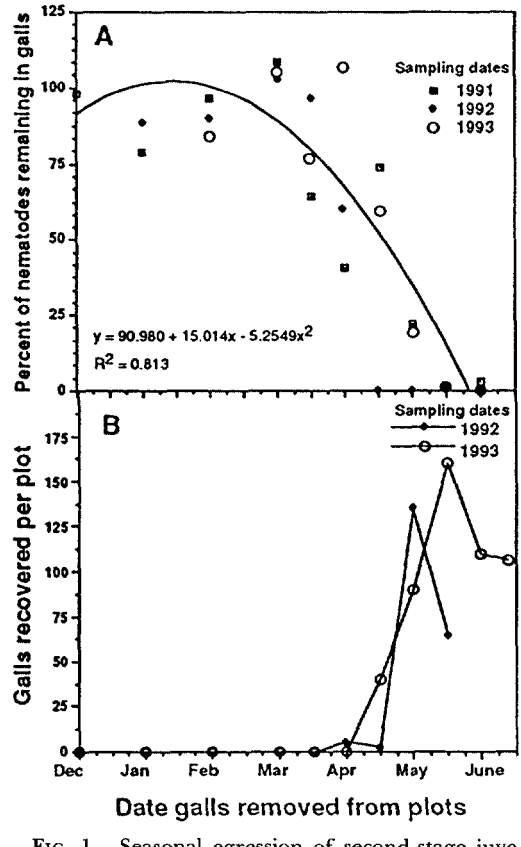

FIG. 1. Seasonal egression of second-stage juveniles (J2) of *Anguina agrostis* from Highland Colonial bentgrass galls placed in a field at Hyslop Research Farm, Corvallis, Oregon. A) Percentage of J2 remaining in galls at each sampling date. B) Number of new galls recovered in plots from which inoculum galls were removed at each sampling date.

temperatures were warmer during 1991–92 than during the other two years (Fig. 2A). The number and distribution of days with measurable precipitation between 1 October and 1 April were similar each year (104 days in the first and third year and 98 days in 1991–92); however, monthly distribution differed each year (Fig. 2B), and no precipitation was recorded in May 1992.

Population dynamics: The number of galls per head and percentage infestation increased linearly with inoculum density in 1991 (Fig. 3A,B). A similar trend was observed in 1992 at gall densities between 0–50, but the rate of infestation approached an asymptote at 200 galls per plot (Fig. 3D,E). Gall production increased linearly with increased inoculum density in both years (Fig. 3C,F). In the plot inoculated with 120 galls, the plant stand was poor, causing the poor fit of these data to the regression models. When adjusted for the number of nematodes per gall (201 and 856 in 1991 and 1992, respectively), the relation between the number of galls and nematode inoculum density in each year was similar. There were twice as many heads per plot the second year, doubling from 127 in 1991 to 258 in 1992. However, the number of seed heads per plot was not correlated with percentage of heads infested and galls per head in either year or at any initial population (P_i) (r = 0.011–0.626, $P \leq 0.05$).

The relationship between P_i and final population (P_f) in plots was linear with a slope just above 1.0 ($r > 0.97$) in the two years (Fig. 4A,B), indicating a slight population increase. The P_i and P_f were 201 and 856 J2 per gall in the first season and 856 and 486 J2 per gall in the second season, respectively.

Disease spread: The incidence (percentage of heads infected) and disease severity (galls per head) at the foci in the infested rows increased with increasing inoculum density (Fig. 5). However, the effect of inoculum density was not detected 30 cm from the disease foci. Expansion of disease foci was limited to 60 cm or less at all inoculum densities, with most infections within and across rows occurring <30 cm from the focus.

As with percentage infestation in the population dynamics plots, an asymptote was approached near 46% at P_i 500 per seed, the highest inoculum density (Fig. 6). Compared to the population dynamics study, the number of galls per head in the disease spread study was higher at P_i 50 and continued to increase to P_i 500, although the rate of increase declined.

As the P_i was increased from 5 to 500, the percentage of heads infested increased and the galls became more aggregated (Fig. 7). At P_i 5, the majority of infested heads contained 5 or fewer galls, while at P_i 50 22% of the infested heads contained 2–25 galls. At P_i 500, the large number of galls in plots was accounted for by 10% of the heads that had 26 or more galls, with a maximum of 330 galls observed in one head.

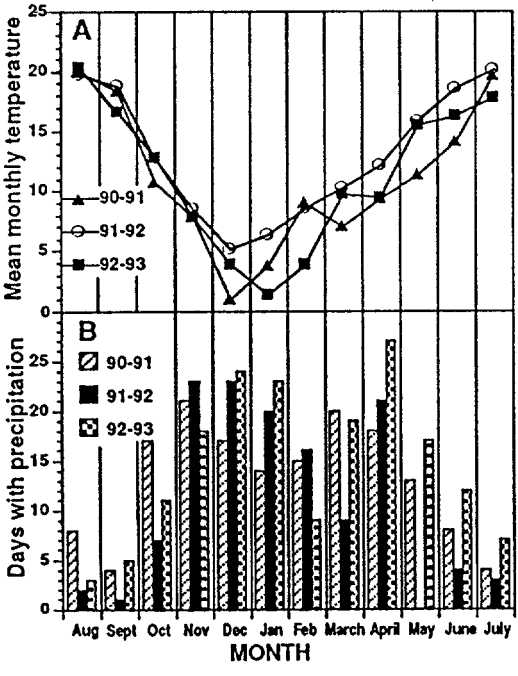

FIG. 2. Weather data collected at Hyslop Research Farm, Corvallis, Oregon, during *Anguina agrostis* studies from 1990 through 1993. A) Mean monthly air temperature. B) Seasonal distribution of precipitation.

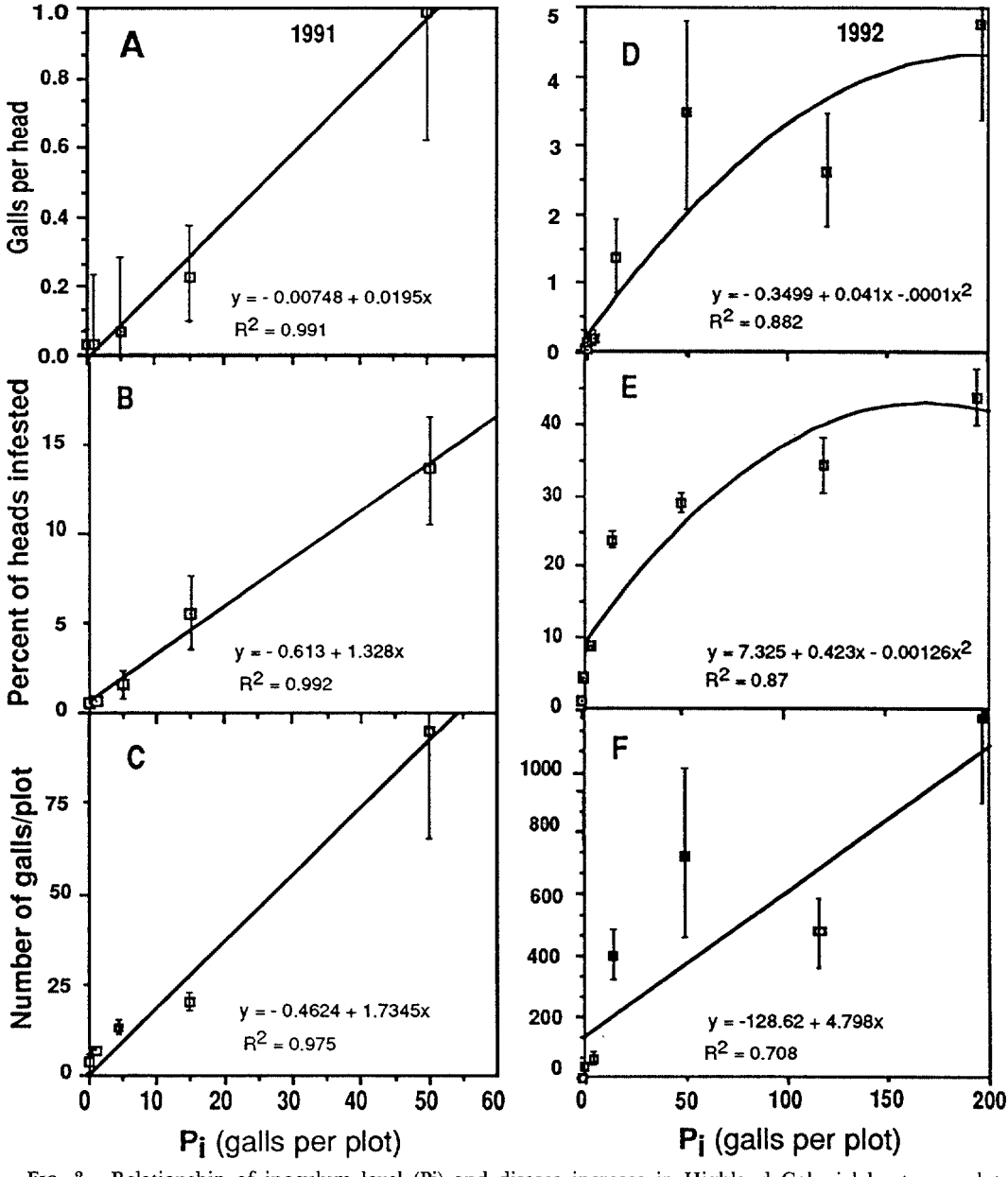

FIG. 3. Relationship of inoculum level (Pi) and disease increase in Highland Colonial bentgrass plots infested with seed galls of *Anguina agrostis*. Disease parameters measured in 1991 and 1992, respectively, were as follows: A,D) Galls per head. B,E) Percentage of heads infected. C,F) number of galls per plot. Error bars are the standard deviation.

DISCUSSION

During the 3-year study, egression of J2 from the galls corresponded with the development of floral primordia in the early spring. Although J2 were not assayed in the crown of plants, data suggests that egression was minimal from October through February based on J2 content in galls and infection timing in microplots. These data, however, do not agree with an earlier study in which J2 egressed from As-

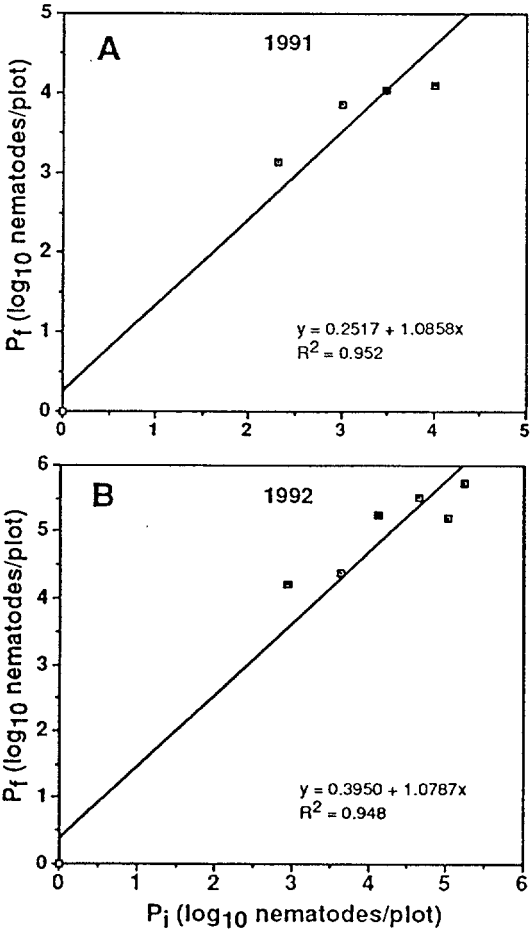

FIG. 4. Relationship between initial (Pi) and final (Pf) population densities of second-stage juveniles of Anguina agrostis in Highland Colonial bentgrass plots after 9 months. A) 1991. B) 1992.

toria Colonial bentgrass galls in the fall and spent the winter in leaf sheaths (8). Experimental and environmental conditions in these studies may explain the differences observed. In the earlier study, the bentgrass fields examined were located in or near tidal lands of the Pacific coast where soils are saturated much of the year, whereas our plots were established on a well-drained soil in the Willamette Valley. While we introduced galls into newly planted microplots each year, the previous study was conducted in infested bentgrass fields. Because not all J2 egress from galls in one year (12), the early fall infestion observed in the previous study may have come from a reservoir of old galls.

Since the mode of egression is unknown in bentgrass, two mechanisms proposed for egression of *A. funesta* J2 from ryegrass galls may be applicable. In a physical model, the gall rind is softened in the autumn rains, the mucoprotein matrix in the gall swells, rupturing the rind and liberating the J2 (7). In a biological model, microbial degradation of the gall rind allows J2 egression (12). This model was based on colonization of galls by bacteria and fungi, decay of rind, and the corresponding egression of J2. The process started with the first autumn rains, with J2 egression starting after 4–6 weeks and completed by 14 weeks. In these studies, the pattern of egression differed from our experiments in which J2 egression began in early spring. Since galls in our study were exposed to soil microflora and were wet for at least 104 days before egression was detected, the rind of bentgrass galls must be more durable than that of ryegrass galls or another mechanism must be operating. There should be selection pressure to synchronize J2 egression with floral initiation, because J2 experience substantial overwinter mortality in the plant crown and infection is greatest when J2 egression coincides with floral development (10). Our current research is attempting to elucidate the relationship of rind integrity in bentgrass galls, plant phenology, and *A. agrostis* egression.

Seed gall in Willamette Valley bentgrass fields is generally found in a low percentage of heads in discrete foci (1,2), suggesting a low nematode reproductive rate. This was supported by our study in which the nematode reproductive rate was less than 1.1. Based on disease progress data (galls), *Anguina* populations appear to be self-limiting as nematode densities increase. We found that multiplication was greatest at Pi $\leq$ 50 galls/plot and approached an asymptote at Pi of 200 gall/plot. Population increase was probably not limited by number of flowers at the highest Pi, since 40% of the heads were infected with a mean of only four flowers infected per head. In the disease spread study, the

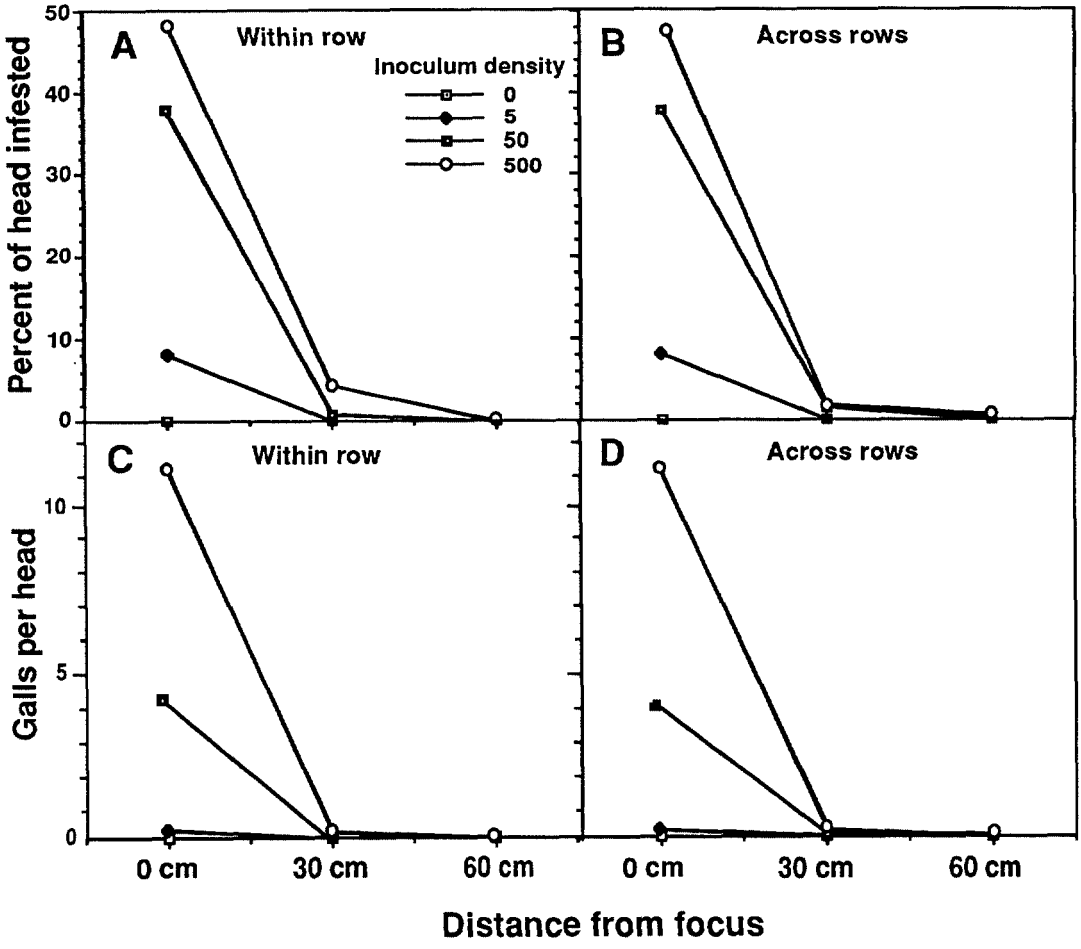

FIG. 5. Annual expansion of disease foci in Highland Colonial bentgrass plots inoculated with seed galls of *Anguina agrostis* at four inoculum densities; 0, 5, 50, and 500 galls per focus. A,B) Incidence of infected heads within and across plant rows, respectively. C,D) Disease severity measured by galls per head within and across rows, respectively.

multiplication rate declined above Pi 50, but the rate of decline was less than in the population dynamics plots. The difference in placement of inoculum, broadcast over the entire plot in the population dynamics study or distributed only in the plant rows in the disease spread study, may have resulted in a higher proportion of J2 reaching floral primordia and a higher rate of gall formation in the disease spread study. A similar trend was observed with the multiplication of *A. funesta* on ryegrass in Australia (11). As Pi (galls/m^2) increased, the multiplication rate (number of galls per head) declined, and the infection efficiency increased with plant density as head density increased from ln (natural log) 4 to 8 heads/m^2. In our study, however, head densities in plots only doubled by the second year and had no significant effect on nematode multiplication and disease progress.

We deduce that nematodes do not randomly infect plants, since high Pi produce aggregates of galls on a small percentage of seed heads. Such behavior should enhance reproductive success for amphimitic nematodes. Multiple infestion of flowers is common with *A. agrostis* (8) and *A. funesta* (12), in which up to seven adults per gall have been reported. However, at high inoculum densities, the competition for flow-

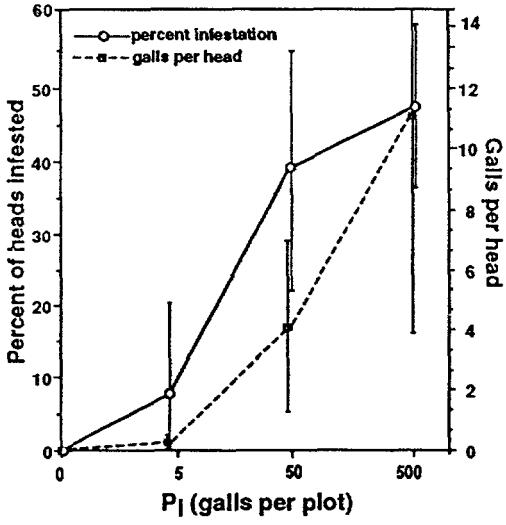

FIG. 6. Relationship of three initial densities (Pi) of *Anguina agrostis* galls and disease increase in Highland Colonial bentgrass. Error bars are the standard deviation.

ers and aggregation of J2 in single flowers could act to reduce the rate of gall production, as observed in our two studies.

The mechanism of nematode aggregation has yet to be elucidated. *A funesta* J2 have been shown to be differentially attracted to suitable hosts, suggesting nematode orientation to the concentration of attractive compounds produced by different grasses (14). However, *A. funesta* did not show strong chemotaxis in in vitro experiments (13). One hypothesis for the aggregation pattern observed in our studies is the orientation of J2 to an aggregation pheromone. A second explanation for aggregation could by synchrony of egression with the floral development of a proportion of heads, such that a cohort of heads is highly infested.

The high variability in our study may have resulted from differing viability among nematodes in galls, environmental conditions (10), and condition of the host. During our experiments, variation in weather conditions may have caused variation in disease and population dynamics. The spring of 1992 was warmer and drier than 1991 or 1993, resulting in early plant development and conditions less favorable for nematode egression. In our disease

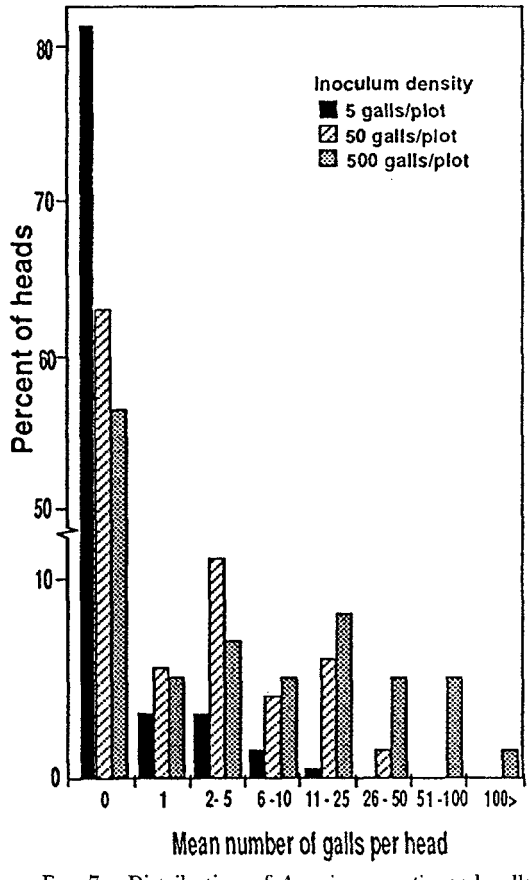

FIG. 7. Distribution of *Anguina agrostis* seed galls in Highland Colonial Bentgrass heads inoculated at three inoculum densities of galls per plot.

progress study, the number of J2 per gall was approximately four times greater in the second than in the first year. When gall Pi is adjusted for nematodes per gall, the disease progress was similar at gall Pi evaluated in both years. The J2 density in galls collected from plots at the end of the second year (Pf) was 50% of mean J2 density in Pi. It is apparent that gall data are not sufficient for modeling disease progress in bentgrass.

In addition to population increase, the distribution and expansion of seed-gall foci is a function of gall and nematode dispersal. Our data demonstrated that expansion of foci is limited under field conditions. Nematode dispersal was $\leq$60 cm/year in the established bentgrass field. If plants in our plots were allowed to fully mature and seed heads shatter, galls could

have been dispersed an additional 30–80 cm from plants that lodged. Because natural dispersal processes are limited, the expansion and establishment of foci in fields is best explained by redistribution of galls by threshing machines during harvest (8). Newly planted fields also could be infested by seed stock contaminated with galls. The low incidence and rate of spread of seed gall in Willamette Valley bentgrass fields of Oregon suggest that *A. agrostis* will remain a minor pest.

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