Seasonal Population Dynamics of the Plant-parasitic Nematode, *Anguina pacifica* on Golf Course Putting Greens in California

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Abstract: The plant-endoparasitic nematode *Anguina pacifica* is presently one of the most destructive pests of *Poa annua* in Northern California, causing stem galls at the base of tillers in this annual bluegrass, resulting in yellow or brown patches and irregular surfaces on golf course putting greens. The objective of the study was to investigate the population dynamics of *A. pacifica* on two golf course putting greens in California (‘Poppy Hills’ and ‘Olympic Club’) over ca. 16 months. For most of the sampling period, the number of galls and nematode eggs, juveniles and adults found at Olympic Club was higher than at Poppy Hills throughout the year. Seasonal fluctuations were observed in number of galls, eggs, adults, and juveniles within galls and soil. In general the number of galls and nematode populations (eggs, juveniles and adults) peaked from late-spring through midsummer, and then declined rapidly during late-summer (usually at the end of August) the same year. The nematode populations were correlated for most of the sampling period to the soil temperature. Our data suggest that the peak of infective juveniles that are released from the galls and search for a new host occurs during late spring and midsummer. A positive correlation was found between gall size and the nematode population within it. In light of these results, possible suggestions to improve the integrated pest management of *A. pacifica* are discussed.

Key words: *Anguina pacifica*, annual bluegrass, ecology, *Poa annua*, population dynamics.

The plant-endoparasitic nematode *Anguina pacifica* (Nematidae: Anguiniidae) causes stem galls at the base of tillers in annual bluegrass (*Poa annua* L., Poaceae), resulting in yellow or brown patches and irregular surfaces on Northern California golf course putting greens (Cid del Prado Vera and Maggenti, 1984). In general, *A. pacifica* juveniles penetrate plant tissues, creating bulb-like galls at the base of the *P. annua* stems. The nematode develops and lays eggs within the stem galls. However, up to this point, the life cycle of *A. pacifica* has not been completely studied and understood.

*Anguina pacifica* was described as a new species with a type locality as the Northern Pacific Coast of California (Cid del Prado Vera and Maggenti, 1984). All developmental stages (egg, juveniles, and adult) are found within a single gall. A typical gall contains several adults and hundreds of eggs or hundreds of juveniles. Adult females can lay up to 1,200 eggs during their life time (McClure et al., 2008). An unidentified bacterium may also be present inside the gall with the nematodes. There are three successive post-hatching juvenile stages (J2, J3, J4). After the J4 stage, the adults mate and reproduce. The J2 is the infective stage of this nematode (McClure et al., 2008). After burrowing out of a decayed plant gall, it invades new grass plants to continue development (Cid del Prado Vera and Maggenti, 1984; McClure et al., 2008). The ample moisture consistently supplied by overhead spray sprinklers in golf courses increases the likelihood of infection of new plants by juveniles released from decaying galls.

Northern California has approximately 400 golf courses and many of those in the coastal areas use the bluegrass turf species *Poa annua* for the putting green surface. The climate of this coastal region is characterized by cool summers with coastal fog and mild winters with an average temperature of 14 °C. Spring and autumn months have warm clear days and cool nights. The rainy season is between November and April. Superintendents of Northern California golf courses have historically preferred putting greens of creeping bentgrass (*Agrostis palustris* Huds.), a stoloniferous cool-season grass species, rather than bluegrass because it has rougher leaves, which decompose faster as a turf thatch layer. However, *P. annua*, which is self-seeding, can act as a weed by continuously invading creeping bent grass turf, disrupting its uniform appearance. Control of *P. annua* requires high cultural and chemical input and is difficult to achieve (Park et al., 2002). Although often considered a weed, *P. annua* has many characteristics of a high quality turf grass: high density, fine texture, consistency, year-round uniformity of surface and color, and cool weather and shade tolerance (Turgeon, 2002). Therefore, many golf course superintendents have accepted and adopted *P. annua* rather than try to control it. However, because *P. annua* is the only known host for *A. pacifica*, adopting this grass created a new nematode-pest problem.

Since 1978, *Poa annua* putting greens on the northern coast of California have shown increasingly severe symptoms of nematode damage (Cid del Prado Vera and Maggenti, 1984; McClure et al., 2008). This damage is unacceptable to golfers, whose performance and enjoyment depends on the consistency of the putting green. Registration of the post-plant nematicide Nemacur (fenamiphos) which was widely and effectively used on
turf grass in California (Winterlin et al., 1986) has been recently cancelled at the request of the registrant (Bayer, Kansas City, MO). With extended use, enhanced biodegradation of the product by bacteria had been encountered in some situations resulting in a loss of nematode control (Davis et al., 1995; Johnson, 1998).

Knowledge of nematode pest population cycle and activity over the year could facilitate development of effective treatment timing, and might provide insights helpful in future development of Integrated Pest Management (IPM) strategies.

The goal of this research was to monitor *A. pacifica* population fluctuations (within guts and in soil around roots) from two sites to achieve a better understanding of its life history and its seasonal population dynamics.

**Materials and Methods**

Two golf course sites, one at 'Poppy Hills' (PH) located in Pebble Beach, Monterey County, California (36°35'19"N, 121°56'22"W), and the second at 'Olympic Club' (OC) located south of San Francisco, San Mateo County, California (37°42'31"N, 122°29'39"W) were selected as the field sites for this research. Each site contained *Poa annua* turf infested with *Anguina pacifica*. Neither site had received Nemacur or other nematicide applications for at least a year prior to the start of the study. Golf course superintendents managed the two sites and collected turf samples for analysis approximately each second week. The sampling period was from June 2002 until October 2003.

Soil temperature was monitored hourly from September 2002 to August 2003 at OC, and December 2002 to October 2003 at PH, using Hobo data-loggers inserted 200 mm belowground (depth chosen to avoid damage by aeration equipment) (Onset Computer Corporation, Pocasset, MA). For the remainder of the sample period, soil temperature (minimum and maximum) was estimated using data collected at the nearby Santa Cruz weather station (data available at UC IPM Online: http://www.ipm.ucdavis.edu/; station name: ‘SantaCruz.A.’) using relationships developed by regression analysis \( r^2 \geq 0.81 \) for the period of concurrent measurement.

For sampling, each putting green site was divided into three sections. From each section, soil cores (108 mm diam.) were taken to a depth of 200 mm, and delivered via overnight shipment to the UC Davis Extension Laboratory for analysis. From each soil core, a sample (22 mm diam.) was tested. Shoots were examined for the presence of galls. Then, up to 21 galls per sample were randomly selected (usually 10 were selected) and dissected in deionized water to determine the number of nematodes and their stage of development: eggs, juveniles, and adults. Nematode counts were made with a dissecting microscope, (Bausch and Lomb). If a large number of juveniles and eggs were present, they were diluted and counted with a Hawsley slide. The size of galls (gall diam.) was measured using an ocular micrometer mounted in an 15X eye-piece on the dissecting microscope. In order to test the correlation between gall size and the nematode population within it, an additional random number of galls were tested each sampling period.

To evaluate the number of juvenile nematodes within the soil, nematodes were extracted from soil around roots via a 'Baermann funnel' (Ayoub, 1977). From each sample, 50 cm\(^3\) of soil was placed in the funnels for 48 hours, and extracted nematodes were counted under a dissection microscope.

Soil Moisture Content (SMC) was determined from approximately 30–40 g of soil from each sample and weighed on a digital scale (XD-4K Denver Instrument Company) before and after being dried in an oven at 65 °C for two days. SMC was monitored from October 2002 until September 2003. Once during the sampling period, a composite soil sample from each site was analyzed by the University of California DANR Analytical Laboratory for the following physical and chemical properties: pH, electrical conductivity (EC), organic matter (OM), and soil texture by percent of sand, silt and clay.

Spearman Rank Order Correlations were used to investigate relationships between mean soil temperature and nematode population size, mean number of galls, and between gall size and population sizes. Differences between means were compared using Mann-Whitney rank sum test (Zar, 1999). Statistical analyses were performed using SigmaStat, version 3.5 (SPSS Inc., Chicago, IL) and JMP version 7 (SAS Institute, Cary, NC).

**Results**

**Soil composition, moisture and temperature:** Both sites (PH and OC) had a sandy textured soil with very similar sand, silt, and clay content (Table 1). PH had about 3 times as much organic matter in the soil as did OC. The pH in both sites was also similar. The EC was approximately 5 times greater at PH than at OC (Table 1). For most of the sampling period, the SMC found at PH was higher than at OC. Only when the SMC at OC peaked (mid December 2002, and late January and April 2003) it was higher than at PH (Fig. 1).

High correlations were found between soil temperatures (daily minimum and maximum; ‘X’) in both sites and daily soil temperatures at the weather station in Santa Cruz (‘Y’) [daily minimum: \( r^2_{PH} = 0.86, (Y = 5.296 + 1.027X); r^2_{OC} = 0.81, (Y = 2.799 + 1.342X); \) daily maximum: \( r^2_{PH} = 0.85, (Y = -1.228 + 1.318X); r^2_{OC} = 0.81, (Y = 0.898 + 1.283X) \)]. On average, the temperatures at PH were higher by approximately 1.2 °C (daily minimum: \( U = 111593.0, P < 0.0001; \text{mean}_{PH} = 13.62, \text{mean}_{OC} = 12.54; \) daily maximum: \( U = 106707.5, P < 0.0001; \text{mean}_{PH} = 17.44, \text{mean}_{OC} = 16.29; \) Fig. 1).
Galls and Nematode populations: Both at OC and PH, the numbers of galls and the nematode population sizes fluctuated throughout the sampling period (Fig. 2). At OC, the highest number of galls was found on 25 April 2003 when there were 58.3 (se = 6.76) galls per sample, and the lowest number were found on 30 January 2003, when there were only 3.6 galls per sample (Figure 2a). The number of galls was not significantly correlated with soil temperature throughout the sampling period (\( r = 0.35, P = 0.099, n = 23 \)). However, it was correlated with soil temperature during January - August 2003 (\( r = 0.58, P = 0.028, n = 14 \)). At PH, the number of galls peaked at 59.3 (se = 11.89) per sample on 26 June 2003, ranged from 0 to 59.3 (Figure 2b), and was correlated with the soil temperature (\( r = 0.37, P = 0.042, n = 31 \)). A positive correlation, but with low r-value, was found between gall size and nematode population size within it (\( r = 0.19, P < 0.0001, n = 1255 \)). More specifically, gall size was correlated with the number of eggs (\( r = 0.14, P < 0.0001 \)) and adults (\( r = 0.24, P < 0.0001 \); Fig. 3), however not with the number of juveniles (\( r = 0.02, P = 0.512 \)).

At OC, the total population of nematodes within galls peaked during late April to late July 2003 with the highest record at the end of July (14,398.3 nematodes; se = 9148.8) (Fig. 2a). The population was not correlated to the soil temperature over the whole sampling period (\( r = 0.320, P = 0.134, n = 23 \)). However, during 2003 the total nematode population was significantly correlated to the soil temperature (\( r = 0.72, P = 0.003, n = 14 \)). Similarly at PH, the total nematode population peaked in early July 2003 (17,578.8 nematodes; se = 1407.1), then declined rapidly during August the same year (Fig. 2b). The total nematode population size was correlated with soil temperature (\( r = 0.44, P = 0.013, n = 31 \)).
The adult population at OC peaked during mid August 2002, late April 2003, and at the end of July 2003. The egg population peaked at the end of December 2002, end of April 2003, and at the end of July 2003 (Fig. 4a). The adult population was not correlated with the soil temperature (throughout the sampling period: \( r = 0.11, P = 0.61, n = 23 \); during 2003: \( r = -0.07, P = 0.81, n = 14 \)). The egg population was correlated with the soil temperature during 2003 only (\( r = 0.68, P = 0.007, n = 14 \)). A similar trend was observed at PH when both adult and egg populations peaked in early June 2002 and early-mid July 2003, then declined rapidly during August the same year (Fig. 4b). In this location, the adult population was marginally correlated to the soil temperature throughout the sampling period (\( r = 0.33, P = 0.068, n = 31 \)) and significantly correlated to the soil temperature during 2003 (\( r = 0.50, P = 0.028, n = 19 \)). The egg population was also correlated to the soil temperature (\( r = 0.57, P < 0.001, n = 31 \)).

The juvenile populations within galls and in the soil around the roots fluctuated throughout the year (Fig. 5). At OC, juvenile population within galls peaked in mid August and September 2002, and again at the end of May and mid August 2003 (Fig. 5a), and was correlated with the soil temperature (\( r = 0.58, P = 0.004, n = 23 \)). The juvenile population in the soil around the roots peaked in mid August 2002 and again in mid August 2003 (Fig. 5a), and was not correlated with the soil temperature (throughout the sampling period: \( r = 0.26, P = 0.219, n = 23 \); during 2003: \( r = 0.21, P = 0.463, n = 14 \)). At PH, juvenile population within galls peaked in early June 2002, mid July 2002, mid July 2003, and mid September 2003 (Fig. 5b), and was correlated with the soil temperature (\( r = 0.40, P = 0.026, n = 31 \)). In the soil, the juvenile population peaked in early June 2002, and again in early August 2003 (Fig. 5b), and was correlated with the soil temperature (\( r = 0.47, P = 0.012, n = 28 \)).

**DISCUSSION**

This study documents the seasonal population dynamics of the stem gall nematode, *Anguina pacifica* on golf course putting greens in California during the summer of 2002 through autumn 2003. *A. pacifica* populations varied between the two studied golf putting greens, but more importantly fluctuated throughout the year.

For most of the sampling period, the population sizes at OC were higher than at PH, and peaks were earlier (Fig. 2). These variations between the study sites may be explained by the difference between the soil’s physical characteristics, moisture and temperature differences, since these often have a strong impact on factors affecting nematode population growth (Thorne, 1961). On average, the temperature at PH was slightly (but statistically significantly) higher by approximately 1.2 °C than the temperature at OC. Therefore, soil temperature differences may not have accounted for the population differences between our study sites. In contrast, PH had about 3 times as much organic matter in the soil as did OC, and the electrolytic conductivity (EC) was approximately 5 times greater at PH than at OC (Table 1). Higher soil organic matter content and EC were
found to be positively correlated with plant-parasitic nematode population sizes, and have long been considered beneficial in managing plant-parasitic nematodes (e.g., Linford, 1937; Liu et al., 2006; Briar et al., 2007; Zhang et al., 2007). Another explanation may be the difference between the sites’ history. PH was opened for play in 1986 while OC has been in use since 1919. The difference in the age of the two locations has likely resulted in an older infestation at OC that could account for the generally higher populations present there.

Numerous studies have shown seasonal fluctuation in plant parasitic nematode populations. Some factors associated with these fluctuations are the soil seasonal moisture, pH level, temperature and, more directly, host plant quality (Yeates, 1973; Wallace, 1973; Robbins and Barker, 1974; Yeates and Risk, 1976; Siddiqui, 2007). In general, the nematode populations in our study were positively correlated with soil temperature and increased from late-spring through midsummer then declined rapidly during late-summer (usually at the end of August) the same year. The increase of A. pacificae populations may be a result of favorable temperature for nematode development and reproduction, and of host quality and availability (i.e. Poa annua shoots). On the contrary, the population decline may be caused by a decline in host quality and availability during late summer. Annual bluegrass, Poa annua, is a cool season turf grass showing bimodal shoot-growth pattern. Maximum shoot growth takes place in spring and again in early autumn. Shoot growth declines dramatically during summer and the spring inflorescences die. In autumn, additional shoot biomass is caused by new shoot growth from auxiliary buds and by seed germinations (Vargas and Turgeon, 2004). Settle et al. (2006) found a similar population dynamic of the lance nematode (Hoplolaimus galeatus) in creeping bentgrass (Agrostis canina L.) putting green. The lance nematode populations (in 2001 and 2002) peaked in midsummer and declined by September. Bekal and Becker (2000) also showed that in two infested bermuda grass (Cynodon dactylon L.) golf courses in California the populations of the sting nematode (Belonolaimus longicaudatus) declined rapidly in August. The authors suggested that the reduction in the nematode population was most likely due to a limitation of food supply.

The coastal climatic conditions at both putting green clubs allow development of A. pacificae throughout the year, and allow multiple generations to develop in plant galls. The data for OC show high numbers of galls occurring four times during the year, August and November 2002, April 2003, June 2003, and then again at the end of July 2003 (Figure 2a). Similarly at PH, four peaks of high gall numbers were recorded during 2003 in January, April, June and September. These data could be an indication of four successive generations, and suggest a possible scenario for the number of generations that may occur. McClure et al. (2008), who recently investigated the biology and distribution of A. pacificae, also proposed that an annual occurrence of three to four nematode generations is possible in the Californian Poa putting greens. Yet, additional studies are needed to confirm this speculative suggestion.

At OC, the high August 2002 gall count (12 August) is followed by a high egg count on 17 September 2002, which is followed by a high gall count on 19 November 2002, which in turn is followed by high numbers of eggs on 20 December the same year. In 2003, the first peak of gall count (together with egg peak) was observed on 25 April. This peak of galls is followed by high egg counts on 29 May and by a high gall count on 20 June. On 30 July, the last peaks of eggs and galls were counted (Fig. 2a and 4a). The egg population peaks (17 September 2002, 20 December 2002, 25 April 2003, 29 May 2003, and 30 July 2003) may suggest that development time from egg to egg takes about one to two months in the summer time (25 April – 29 May, and 29 May - 30 July), three months in the autumn (17 September – 20 December), and about four months in the winter to spring time (20 December – 25 April). This suggestion is in general agreement with the finding of McClure et al. (2008) according to which A. pacificae’s life cycle (within growth chambers) took 30 d, 36 d, and 46 d at average temperatures of 20, 16, and 12°C respectively. Yet, this is obviously only an estimate and additional controlled developmental studies are needed to confirm this speculative suggestion.

At OC four juvenile peaks per year were observed about a month after the egg peaks (12 August 2002, 17 September 2002, 30 January 2003, 27 March 2003, 29 May 2003 and 15 August 2003; Fig 5a). In this scenario, juveniles would be expected to be released from decaying galls around September, January, May, March, and August. Our OC juvenile population data found in the soil generally supports this prediction. This population peaked on 12 August 2002, 25 October 2002, 20 February 2003, 27 March 2003 and 15 August 2003 (Fig. 5a). If this is the case, these would be opportune times to apply non-systemic treatments to the soil in an effort to reduce the number of free moving juveniles. More specifically, early to midsummer is probably the best time to control free juveniles within the soil (Fig. 5). It makes sense that treatments that might be able to penetrate into galls should be applied at times of the year when high populations are present within the galls. If a product were known to be more effective on eggs, juveniles, or adults, the timing could be further refined to target times of the year when large numbers of specific stages are present. Because nematodes can be moved from one area to another on machinery, mechanical aerating at times when numbers of galls and nematodes are low might also help to reduce nematode spread. At PH, the juvenile population within galls also shows four main peaks per year: 7 June 2002, 17 July
2002, 23 January 2003, 13 June 2003, 11 July 2003 and the main peak on 11 September 2003 (Fig. 5b), followed by three peaks of juveniles per year in the soil: 7 June 2002, 2 January 2003, 30 May 2003, and 1 August 2003 (Fig. 5b). The generally low numbers of eggs and adult nematodes at PH could be obscuring the presence of the four peaks in this site.

We found a positive correlation between gall size, number of eggs, and adult nematodes. This finding suggests that the size of the galls depends on the degree of infection. These results are in agreement with other studies that showed that gall size is often correlated with the number of nematodes within it, and that gall size and nematode abundance may indicate host susceptibility. For example, galls created by the root knot nematode *Meloidogyne spp.* in many plant species are often correlated with the number of nematodes within them (McKenry and Roberts, 1985). Similarly, Anwar and Khan (1995) investigated the wheat seed gall nematode *Anguina tritici* and found that the number of *A. tritici* infective juveniles (J2) was correlated to the gall sizes. The authors used gall size as one parameter of wheat cultivar’s susceptibility to *A. tritici* infection.

In summary, we recorded dynamic temporal fluctuations in *Anguina pacifica* populations in two golf course putting greens in California. We have gathered valuable information about *A. pacifica* life stages and their population development for each site throughout the year. In general, the nematode populations increased from late-spring through midsummer, then declined rapidly during late-summer the same year. While our results suggest times at which nematicide treatments should be applied to these particular putting greens, it is probably necessary to either better define the optimal timing, or to establish broad periods of susceptibility on a regional scale.

**Literature Cited**


