The Reproduction and Life Cycle of a South African Population of *Xiphinema index*

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The seasonal fluctuation of a South African *Xiphinema index* population, the influence of four hosts on the length of the life cycle and the reproductive rate with fig as host at three different temperatures, were studied. Reproduction was highest during early summer (November and December) when the mean maximum dayly air temperature was between 25 and 30°C. Under controlled conditions at 25±2°C the duration of the life cycle of *X. index* was between 75 and 85 days with the grapevine rootstock Fairy as host, between 55 and 65 days with Jacquez rootstock and between 45 and 55 days with fig and Paulsen 775 rootstock. Using fig as host, the highest reproductive rate of the nematode was at 28°C, with a 216-fold increase and the lowest at 18°C, with no adults after 85 days.

In the South African Plant Certification Scheme for Wine Grapes, which falls under the Plant improvement act (Act no. 53 of 1976), the elimination of virus diseases is of major importance. One of the Scheme's requirements for sites, in which grapevines are established and propagated, is the absence of vectors of grapevine fanleaf virus (GFLV) in the soil. GFLV is the only longidorid-vectored virus known to occur on grapevine in South Africa (Gorter, 1977). As a South African population of *Xiphinema index* was shown to be a vector of GFLV (Malan & Meyer, 1992), the risk of re-infection of virus free material by these nematodes must be seriously considered.

Before the establishment of virus free plant material, soil analysis to establish the absence of *X. index* is the only practical method to reduce the risk of re-infection of grapevine with GFLV. Low levels of infestation may remain undetected and therefore soil samples should be taken during the period when the highest number of females occurs.

This study was undertaken to establish the optimum soil sampling time for the detection of *X. index* in the vineyards of the Western Cape Province. At present samples are usually taken during September, based mainly on the population fluctuation of *Xiphinema americanum* as found by Smith (1967). Nematode numbers were monitored under field conditions, supplemented with laboratory experiments.

MATERIALS AND METHODS

Seasonal population fluctuation: Five grapevine blocks on the farm La Bonne Vigne near Robertson, in the Western Cape Province, were chosen for the study. The average minimum and maximum air temperature and rainfall during a two-year period were obtained from the Robertson weather station. The soil fractions of the different blocks were analysed.

Ten soil samples were randomly drawn from each block and sampling was repeated at the same vines at monthly intervals for two years. Samples were taken to a depth of 60 cm between the roots of the vines using a soil auger. These were pooled into two composite samples for each block. Nematodes were extracted by suspending 500 mL soil in water which was then sieved twice through three stacked 150 µm sieves (Flegg, 1967). Residues on sieves were washed into a 500 mL beaker and cleared for 24 h on a modified Baermann funnel, fitted with a 142 µm aperture nylon sieve. The number of *X. index* and *X. americanum* present in each sample was recorded.

The effect of different hosts: *In vitro* cultured plantlets of the grapevine rootstocks Fairy and Paulsen 775, as well as fig (*Ficus carica*) and Jacquez rootstock seedlings were used as hosts for *X. index*. Plants were planted in 250 mL plastic pots. After they reached a height of approximately 10cm, 20 non-gravid *X. index* females from a glasshouse fig culture were used to inoculate each plant. They were subsequently kept in a growth chamber at 25 ±2°C. All the soil from six plants of each host was used for the extraction of nematodes after 25 days and thereafter every 10 days. The nematode life cycle was considered as complete when the number of adults recovered exceeded the initial inoculum (Cohn & Mordechai, 1970). The day/degree (day°) requirement for the production of an egg above a daily threshold of 10°C and 14°C, was calculated at 55 days for the four hosts (Brown & Coiro, 1985; Coiro *et al.*, 1991).

The effect of temperature: Fig seedlings (54 plants) were used as host to determine the effect of temperature on the length of the life cycle and reproduction of *X. index*. The plants were placed in growth chambers at temperatures of 18 ±1°C, 23 ±23°C and 28 ±2°C. Each plant was inoculated with 20 non-gravid females of *X. index*. After a period of 45, 65 and 85 days, the soil from six plants of each treatment was removed and the number of nematodes recorded. The day° requirement above a daily thresh-

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old of 10°C and 14°C for the production of an egg was calculated at 45 days for the three temperatures.

RESULTS

Seasonal population fluctuation: Low numbers of *X. index* were found in vineyard soils from in block 1, 2 and 3, while higher numbers occurred in blocks 4 and 5 (Figs. 1 and 2). In 33% of the monthly samples taken from block 1, 42% from block 2 and 8% from block 3, no *X. index* were found.

The average maximum air temperature increased to above 20°C during August. From December till the end of February it was above 30°C and during March it again dropped to below 20°C (Fig. 2.). Blocks 4 and 5 in which R99 was the rootstock, both had a high percentage of clay and loam (Table 1). The mean number of *X. index* from blocks 4 and 5 stayed the same from July to November (Fig. 2). During December it increased sharply with a slight decrease in January and February. A second increase occurred during March and April, with a sharp decline during May and June. Numbers of *X. americanum* increased during August and decreased in November. The population remained constant during summer. During March it again increased, but declined during winter (Fig. 2, block 3).

The effect of different hosts: More adults than the initial inoculum were found after 85 days on the rootstock Fairy, after 65 days on Jacquez and after 55 days on fig and Paulsen 775. The highest reproductive rate was on the rootstock Paulsen 775 and the lowest on Fairy (Fig. 3). The length of the life cycle was estimated as being between 45 and 55 days on Paulsen 775 and fig, between 55 and 56 days on Jacquez and between 75 and 85 days on Fairy. Root feeding symptoms were visible from day 25 on Paulsen 775 while no feeding symptoms were observed after 85 days on Fairy. Egg development using 10° and 14°C as thresholds, was 76,7 and 56,0 day° respectively on Fairy and 14,6 and

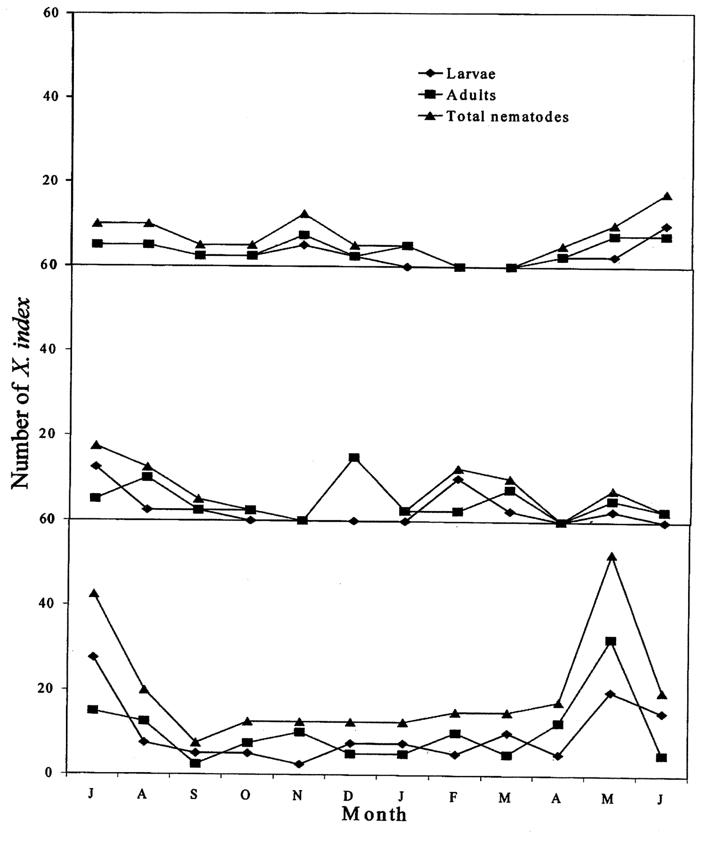

FIGURE 1
The seasonal fluctuation of *Xiphinema index* in grapevine soil (number of nematodes/500 mL soil over a two-year period at three sampling sites.

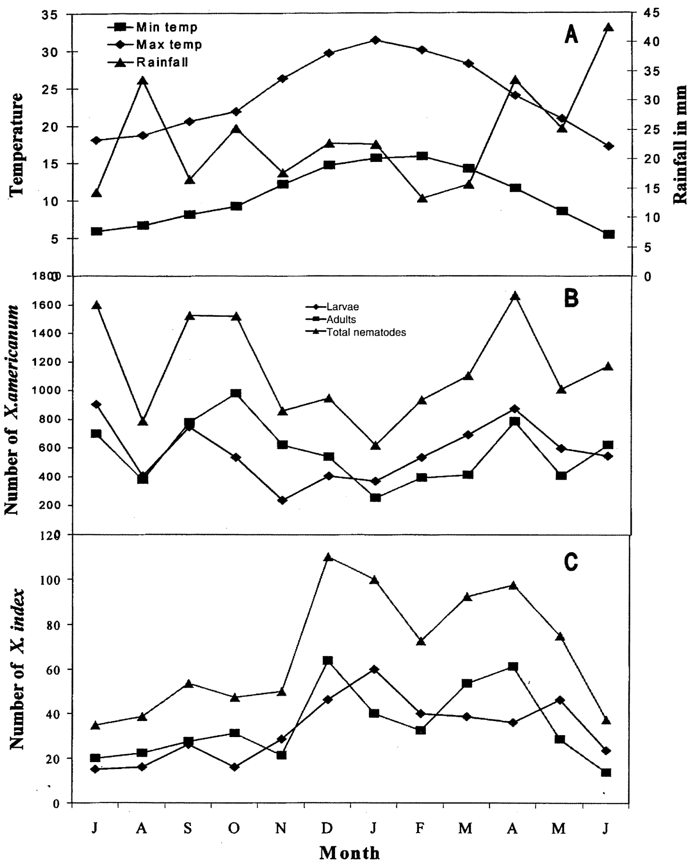

FIGURE 2
A. Minimum and maximum air temperure and rainfall for the experimental vineyards at Robertson. **B.** Seasonal fluctuation of *X. americanum* in one of the sites. **C.** Mean seasonal fluctuation of *Xiphinema index* in two sites Mean numbers over a period of two years (numbers of nematodes/500 mL soil).

TABLE 1
Rootstock cultivar and soil fractions of experimental vineyards used for seasonal population studies.

Block	Root-stock	% Coarse Sand	% Medium Sand	% Fine Sand	% Loam	%Clay
1	R110	22,6	18,47	40,93	13,0	5,0
2	R99	9,81	21,24	44,94	19,0	5,0
3	101-14 Mgt	7,87	17,23	59,90	14,0	3,0
4	R99	13,01	10,33	34,66	24,0	13,0
5	R99	13,01	20,95	24,04	25,0	17,0

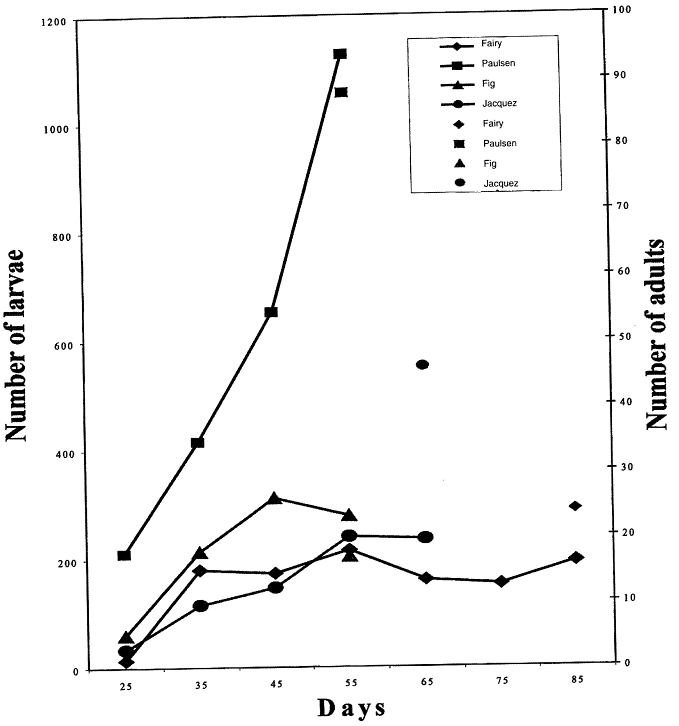

FIGURE 3
The reproductive rate of 20 *Xiphinema index* on four different hosts.

TABLE 2
The reproductive rate of 20 *Xiphinema index* at three different temperatures with fig as host.

Days	18°C Larvae	18°C Adults	23°C Larvae	23°C Adults	28°C Larvae	28°C Adults
45	0	–	205	–	408	–
65	10	–	355	38	827	248
85	13	–	368	96	4017	310

TABLE 3
The number of day° above a daily threshold of 10° and 14° required for the proudction of eggs of female *Xiphinema index* with different hosts and at different temperatures.

Different hosts at 25°C	Day°/egg at a threshold of 10°C	Day°/egg at a threshold of 14°C
Fairy	76,7	56,3
Jacquez	68,8	50,4
Fig	59,4	43,5
Paulsen 775	14,6	10,7
Different temperatures with fig as host	**Day°/egg at a threshold of 10°C**	**Day°/egg at a threshold of 14°C**
28°	39,7	30,9
23°	61,5	43,9
18°	–	–

10,7 day ° respectively on Paulsen 775 (Table 3).

The effect of temperature: More adults than the initial inoculum were found after 65 days at 23° and 28°C and no adults were found after 85 days at 18°C. The highest reproductive rate occurred at 28°C (Table 2). A 216-fold increase of nematode numbers occurred at 28°C in comparison with a 23-fold increase at 23°C after 85 days (Table 3). The best visual root development took place at 23°C, with thinner and darker roots at 28°C. The estimated number of day° at the two thresholds for egg development on fig at 18°C was 39,7 and 30,9 respectively.

DISCUSSION

Since the vineyards were irrigated, soil moisture was not expected to have had a marked effect on the nematode population. In blocks 4 and 5, with sandy loam soils and high fine sand complement, the number of *X. index* was higher than in block 1 and 2 with sandy soils. In a previous report Sultan & Ferris (1991) found the increase of *X. index* was highest in sandy loam soils and in fine sands. This would appear to confirm that *X. index* is more prevalent in soils with a higher fine particle complement. Cotton *et al.* (1970) showed a definite seasonal fluctuation in populations of *X. index* with higher populations at higher temperatures. In a study on the population fluctuation of *X. index* in Italy (Amici, 1965) a seasonal fluctuation pattern emerged, with maximum numbers of nematodes in the summer months, even though the populations were very small. Temperature fluctuation seems to be an important factor contributing to population numbers of *X. index*. Only Cohn (1969) could not find a definite pattern in the seasonal fluctuation in a total of seven species of *Xiphinema* and two species of *Longidorus*.

In this study the number of *X. index* stayed relatively constant at low temperatures (<25°C) during winter. During November and December, when the temperature was above 25°C, an increase in nematode numbers occurred, but when the temperature reached 30°C or above during January, the numbers declined. This indicates an optimum reproductive temperature for the South African population of 25-30°C, which occurs in the Western Cape during early summer and early atutumn.

A different pattern emerged from *X. americanum*. In this case populations increased fastest during spring and autumn at temperatures between 20-25°C. These results are comparable with those of Smith (1967) who suggested sampling for *Xiphinema* spp. during spring.

Under optimum glasshouse conditions (24°C) Radewald (1962) found that egg to adult development of *X. index* took 22-37 days, whilst under more variable glasshouse conditions Taylor (1963) reported a period of 36 days for development from second stage larvae to adults. The rates of population increase of between 2 000 and 3 000 fold over 4-18 months obtained in a growth chamber with fig or grapevine as host (Taylor, 1963), also indicated a short life cycle for this species. Coiro & Agostinelli (1991) reported that 70 days were required for *X. index* to complete a full developmental cycle at 23 ±1°C on *Vitis vinifera* cv. Mission, while Coiro *et al.* (1990) estimated the life cycle to be 55 days at 22°C or 29°C for a California population of this species. Brown & Cairo (1985), using fig as host at 18°C, found the day° for an Italian population of *X. index* to be 24, and that of a USA population to be 26 at a threshold of 10°C for the production of each egg. For the South African population of *X. index* with fig as host, 59,4 day° were estimated for the production of an egg. This indicates that the South African population of *X. index* has a slower reproductive rate than other populations when using fig as host.

Xiphinema index had a much higher reproductive rate on Paulsen 775 than on fig, but had a similar life cycle length on the two hosts. Similar reproductive rates were obtained on fig, where the nematode has a life cycle of 55 days, and on the nematode tolerant rootstock Fairy (Malan & Meyer, 1993), on which the nematode had a life cycle of 85 days. With Paulsen 775 and Fairy as hosts, the day° for the development of an egg was estimated as 14,6 and 76,7 respectively (Table 3). This demonstrates the profound effect of different grapevine rootstocks on egg production. The results indicate that the variable nematode numbers found on different hosts in previous studies (Malan & Meyer, 1993), were not only due to the difference in reproductive rates of *X. index* on the hosts, but to the length of the life cycle and the day° requirement of the nematode on different hosts.

Cohn & Mordechai (1970) found population increase of *X. index* fastest at 28°C and estimated the life cycle to be completed in 3-6 months. This study also indicated the reproductive rate to be highest at 28°C. This is in conflict with results of Coiro *et al.* (1990) who found reproduction for a Californian and Italian population of *X. index* to be lower at 29°C than at 22°C. Almost no reproduction took place at 18°C on fig. The higher reproduction at 28°C in this study occurred in spite of better root development at 23°C. This is contrary to the common belief that better root growth should result in a higher reproduction of nematodes.

Coiro *et al.* (1990) assumed a minimum temperature of 14°C below which reproduction did not occur, since little reproduction occurred at 15°C. They estimated the day° for egg production in a Californian population of *X. index* to be lower (6,6 and 12,6 per egg at 22°C and 29°C respectively) than the Italian population (45,7 and 84 per egg at 22°C and 29°C respectively) with fig as host. The day° requirement for the production of an egg for the South African population of *X. index* was estimated as 57,1 which is much higher than that of the Californian population and lower than the Italian popultion (39,7 day° at 28°C). At a constant temperature of 28°C the second generation of adults already produced larvae after 85 days, shortening the life cycle to less than 42,5 days with fig as host.

The reproductive length of the life cycle of *X. index* are separate parameters to be investigated in population dynamics. They appear to be separately influenced by soil type, the origin of the population, temperature and host plant. Grapevine soil samples for the South African Plant Certification Scheme for Wine Grapes are normally taken in September. In the Western Cape reproduction was highest during early summer (November and December) when maximum temperatures were between 25 and 30°C, indicating that this is the best period for sampling. In blocks known to be infested with *X. index,* there would be a 28% chance if not detecting *X. index*, particularly where populations are small (Fig. 1). This accentuates the role of temperature related seasonal fluctuation of nematode numbers and the importance of continued periodic soil sampling of units after planting in the South African Plant Certification Scheme for Wine Grapes to confirm the absence of *X. index.*

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