Quantitative Aspects of the Development of *Meloidogyne arenaria* Larvae in Grapevine Varieties and Rootstocks

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Abstract: The development and productivity of parasitic stages of *Meloidogyne arenaria* were quantitatively defined in 14 varieties or rootstocks of grapevine. Mean development to maturity was related linearly to the number of degree-hours above 10 C temperature experienced from the time of penetration in all cultivars in which nematode adulthood was achieved. Averaged across varieties, 13,142 heat units were required for development of the mean individual to maturity. The standard deviation of the developing individuals about the mean, expressed as a proportion with 1 representing adulthood, did not differ with time or among varieties after 7,000 degree-hours had elapsed. Earliest egg production was observed after 7,662 degree-hours, averaged across varieties, considerably before mean development to maturity. Varieties were also ranked relative to the number of larvae establishing infection sites, and the rate of egg production per adult female. Varieties could be grouped according to their levels of horizontal resistance. *Key Words: Vitis vinifera,* pest management, modeling, root-knot nematode.

Improving and prolonging vineyard productivity involves cultural manipulations based on understanding of the relationship of plant-parasitic nematodes and grapevines to their environment and their interaction with each other. Optimization of crop and pest management approaches may require systems-analysis techniques to allow detailed consideration of complex agroecosystems. Simulations with the computer simulator MELSIM, which models the population dynamics of *Meloidogyne arenaria* on grapevines (3, 4), have indicated that further quantification is needed of the parameters of interaction between the nematode and its host.

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Grapevine varieties and rootstocks vary in nematode host status through a range of levels of horizontal resistance (7, 8), but quantitative appreciation of these phenomena is inadequate for purposes of modeling and pest management. Descriptive algorithms of the interaction must include the range of variability to be expected (3, 6). This range is due to variation among individuals and differences in the suitability of infection sites. MELSIM has been used to investigate the importance of this range of variability and has shown that definitive quantitative data are required on the development of parasitic larvae and on vine varietal differences in their reaction with nematodes.

The present study was undertaken to quantify the parameters of interaction for parasitic larval stages of nematode life cycles with various grapevine varieties and rootstocks.

MATERIALS AND METHODS

A greenhouse system was developed for growing grape rootings under constant and uniform conditions of soil moisture and texture. Plants were grown in blow sand in open-ended 3.8-cm-ID PVC tubes standing on bricks. The bricks stand in a galvanized pan in which water is maintained at a constant level. As water is lost from the tubes, by direct evaporation or by evapotranspiration through the plant, it is simultaneously replaced by capillarity from the constant reservoir.

Cuttings of 14 grapevine varieties or rootstocks listed in Table 1 were rooted in 2.5-cm-diameter tubes embedded in sand in a heated rooting bed, moisture was supplied to the bed, and the humidity was maintained by an aerial mist system. All cuttings used in the experiment originated as virus-free stock from collections at the University of California, Davis. Cuttings were monitored for the development of roots. When root development was satisfactory, freshly hatched second-stage *M. arenaria* larvae were inoculated into each rooting tube with a Cornwall pipette. After 24 h, plants were removed from the rooting tubes, and adhering soil particles were washed from the roots so that any larvae within the roots had entered within the same 24-h period and would constitute an inoculum cohort of the same age. Plants with the uniform-age infection were transplanted into the PVC tube system and placed on bricks in the galvanized pans. Soil temperature in the growth tubes was monitored continually so that physiological development time could be calculated in degree-hours above 10 C (heat units) (5). Preliminary experiments had shown that initial egg production by females could be expected when 7,000 heat units had accumulated after larval penetration. Six thousand heat units after penetration, four plants of each variety or rootstock, selected at random, were washed from the soil in the tubes at daily intervals. *Meloidogyne* eggs were extracted from roots (2) and egg production was used as the criterion for the end of the development period. For plants harvested between 7,000 and 11,000 heat units after penetration, the roots salvaged after extraction of eggs were stained with acid fuchsin-lactophenol and examined microscopically for the numbers and development stages of nematodes in the roots. These microscopic analyses provided data on variability in development as adulthood was approached. To visually assess nematode development in the roots, the parasitic phase of the nematode life cycle was divided into five classes based on the morphology of the stained nematodes. The classes were L_{2A}, second-stage larvae from penetration to cigar-shape; L_{2B}, from cigar-shape to sub-pyriform; $L_{3/4}$, from sub-pyriform to pyriform; $L_{4/A}$, pyriform individuals with no indication of eggs or aperture to root exterior; adults, with presence of eggs or aperture to root exterior. A development scale was assigned to each class on the basis of data from Tyler (9). Tyler used a heat-unit system to assess the length of development in various life-cycle stages of *Meloidogyne* in tomato, and according to her data, our development classes respectively corresponded to 0–3,000, 3,001–7,050, 7,051–7,400, 7,401–7,750, and >7,750 heat units after root penetration. We expressed the development at each stage as a proportion of the number of heat units to complete the parasitic stage of the life cycle. The proportional development spanned by each class was then 0–0.19, 0.20–0.65, 0.66–0.93, 0.94–0.98, and 0.99–1.0. At each sampling date, the number of individuals in each development category was multiplied

by the median proportional development for that category. The mean development on each day or at each heat unit period was determined by dividing the sum of these products by the total number of individuals counted in that root system.

This weighted development method using the Tyler scale of heat units for relating mean development to the numbers of elapsed degree-hours appeared more rational than averaging techniques which do not take into account the relative development stage of various categories assessed. Regression techniques which forced the regression line through the origin were used to analyze the data and correlate the mean development to the number of elapsed heat units. Data from the experiment also yielded information on the numbers of individuals which had penetrated and established infection sites in each variety after the 24 h of exposure to the inoculum, and on the variability in the pattern of development in each variety. By relating the number of eggs determined by the egg extraction technique to the number of females counted in the root system, we were able to develop information on egg production per female in the various varieties and rootstocks.

To investigate any relationship between rate of development or susceptibility to infection and size of the root system, we measured the root system of each vine by two methods. Relative length of the root system was obtained by determining the number of times it intersected a 1-cm-sq grid. Dry weight of representative root systems from each variety or rootstock was determined and expressed relative to the number of intersections counted for that system.

RESULTS

Mean rate of development. Forcing the regression line through the origin provided a significant linear relationship between the development and elapsed degree-hours in each variety (Table 1). Significant relationships were also obtained on the unforced raw data, but a zero intercept was more acceptable biologically. Since there were very few individuals in the root systems of the rootstocks originating from *Vitis champini* and *V. rupestris* (Harmony, Salt Creek and Dog Ridge), both resistant to *Meloidogyne* spp., they are omitted from the analyses in Table 1. From the linear regression coefficients established, we predicted the number of degree-hours above 10 C required for mean development to egg-laying maturity (Table 1). In most varieties tested, the mean individual achieved maturity after approximately 13,000 heat units. The development period was somewhat longer in Thompson Seedless and shorter in Cabernet Sauvignon, Zinfandel, and other wine varieties. Since there were no obvious differences among the development rates, the mean rate was calculated for all varieties except the resistant rootstocks. This mean (13,142 heat units) allows generalized predictions and simulations on rates of development of *Meloidogyne* in vine roots where assessments on individual varieties are not required. Although there were low numbers of parasitic larvae in some root systems of individual vines, none of the nematodes in the resistant rootstocks reached productive maturity as determined by the production of eggs. Apparently, the resistance is expressed primarily to penetration and development of infection sites, and secondarily as a resistance against development to maturity. The number of degree-hours until first egg production was observed (Table 1) is considerably lower

TABLE 1. Relationship between mean development and degree-hours above 10 C for *Meloidogyne arenaria* in grapevine varieties and rootstocks. Regression lines forced through origin.

Variety/rootstock	Linear-regression coeff. ($\times 10^5$)	DH for mean development to maturity	DH for first egg production
French Colombard	7.83	12,765	7,849
Barbera	7.67	13,041	8,280
Carignane	7.84	12,759	7,436
Ruby Cabernet	8.01	12,479	7,436
Salt Creek	N.S.		None
Dog Ridge	N.S.		None
St. George	7.69	13,003	7,849
Harmony	N.S.		None
Thompson Seedless	6.36	15,728	7,849
Perlette	7.22	13,842	7,436
Emperor	7.13	14,031	7,849
Tokay	7.31	13,689	7,849
Cabernet Sauvignon	8.13	12,296	7,436
Zinfandel	8.51	11,755	7,009
Mean	7.61	13,142	7,662

than that for development of the mean individual to maturity. A dangerous bias would occur in population simulations if uniform development of all individuals was assumed.

Variability in development. To quantify the variability in development, the standard deviation among individuals assigned to each development class at each succeeding degree-hour interval was determined for each variety. For the critical development range, when individuals were approaching maturity, there were no significant differences in the standard deviation of development stage within a variety. In other words, the standard deviation was not related to or did not vary with elapsed degree-hours. No data are available outside the critical period during which direct observation techniques were used. On the basis of these studies, a mean standard deviation of development was ascertained for each variety (Table 2). Except in the case of the rootstocks Dog Ridge, Salt Creek, and Harmony, each standard deviation was based on data from at least 30 vine root systems and on an average of 2,011 developing individuals in each variety. These mean standard deviations are based on 10 heat-unit periods in each case, and the precision and reliability of each mean is indicated by the standard deviation among each set of 10 estimates (Table 2).

The standard deviations were used to describe the variability in nematode development quantitatively in each variety by making the assumptions of normal distribution of the data, so that at any point in physiological time, the development stage of 67% of individuals would be within 1 standard deviation above or below the mean development at that time, and 96% would be within 2 standard deviations. On the basis of the assumption of the normal curve, at each degree-hour period, the mean development of the infection cohort was predicted from the development equations (Table 1); the distance of each development category, in standard deviations, from that mean was calculated, and the proportion of individuals in each category at that time was predicted. The data generated are probably expressed most usefully in terms of cumulative numbers of individuals reaching adulthood relative to elapsed heat units. To simplify presentation, the mean rate of development for all varieties except the resistant rootstocks and the mean standard deviation for the same varieties were used to produce a cumulative curve (Fig. 1). To illustrate differences among individual varieties, the numbers of mature egg-laying females are predicted at 8,000, 12,000, and 16,000 degree-hours (Table 3). In most varieties it took about 20,000 degree-hours

TABLE 2. Standard deviation of development of individuals about the mean development for *M. arenaria* in grapevine varieties and rootstocks.

Variety/rootstock	[a]Mean standard deviation	[b]Standard deviation
French Colombard	0.19	0.03
Barbera	0.19	0.05
Carignane	0.17	0.06
Ruby Cabernet	0.15	0.03
Salt Creek		
Dog Ridge		
St. George	0.13	0.04
Harmony		
Thompson Seedless	0.22	0.05
Perlette	0.21	0.05
Emperor	0.21	0.07
Tokay	0.20	0.04
Cabernet Sauvignon	0.17	0.03
Zinfandel	0.17	0.02
Mean	0.18	

[a]Mean of observations at 10-DH intervals.
[b]Standard deviation of the calculated mean.

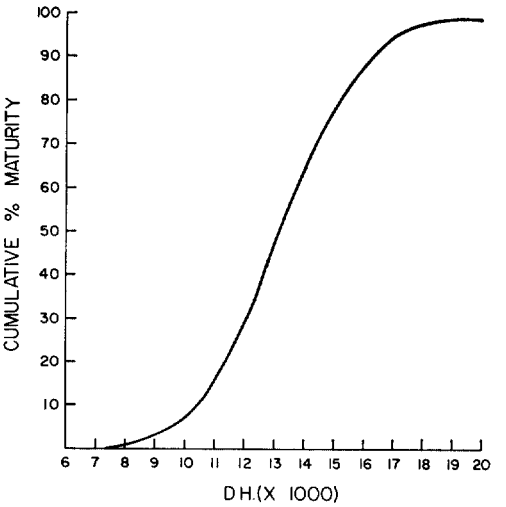

FIG. 1. Cumulative percentage achieving reproductive maturity of a single infection cohort of *M. arenaria* larvae with increasing degree-hours of development.

TABLE 3. Predicted percentage of penetrated *M. arenaria* larvae reaching maturity after various degree-hour intervals.

Variety/rootstock	DH of development		
	8,000	12,000	16,000
French Colombard	2	36	90
Barbera	1	31	88
Carignane	1	34	93
Ruby Cabernet	1	39	97
Salt Creek	0	0	0
Dog Ridge	0	0	0
St. George	0	26	96
Harmony	0	0	0
Thompson Seedless	1	11	53
Perlette	1	22	77
Emperor	1	21	74
Tokay	1	23	80
Cabernet Sauvignon	1	43	97
Zinfandel	2	55	98

for all of the individuals capable of reaching maturity to do so. There is some danger in using such predictions since the standard-deviation estimates on which they are based were developed from the degree-hour data range between 7,000 and 11,000. Strictly, they should be used only for predictions within the range of this data base. Nevertheless, it is of interest to project to 100% development to maturity, and it is perhaps justifiable on the basis of the precision of the standard-deviation estimates for development (Table 2).

Numbers penetrating, establishing infection sites, and developing. Besides their importance as fundamental parameters for the simulation model, both the rates of development and the numbers of larvae developing constitute an expression of the host status or horizontal resistance of the vine variety to the nematode. In these studies, each variety was initially exposed to the same number of second-stage *Meloidogyne* larvae (730 ± 192) for a 24-h period. The number of larvae in the root is a measure of the susceptibility of that root system to infection. Interpretation of those data is somewhat difficult, however, since the root systems of the different varieties differed in size at inoculation. Unfortunately, we have no measure of the relative size of the root system at the time of inoculation, although we can attempt to adjust larval counts relative to root size as measured in arbitrary length units or dry weight. Measurements taken between 7,000 and 11,000 degree-hours after inoculation (Table 4) indicate that the numbers of individuals within roots at various times during that period did not vary significantly for individual varieties. It is presumed that those counts reflect the number of nematodes which were able to establish infection sites within the roots, and that at this level of population stress all such individuals have the capacity of achieving maturity.

TABLE 4. Number of *M. arenaria* larvae establishing infection sites in grapevine roots after 24 h of exposure to an initial inoculum of 730 ± 192 larvae.

Variety/rootstock	No. per root system[a]	No. per root system per constant length[a]	No. per 0.1 g root[b]
Ruby Cabernet	142.2 V	311.8 T	70.8
French Colombard	69.9 W	203.1 TUVW	52.4
Cabernet Sauvignon	65.6 W	165.7 UVWX	38.6
Barbera	65.1 W	147.4 UVWXY	43.4
Zinfandel	50.5 WX	118.0 VWXYZ	18.7
Carignane	48.9 WX	240.7 TU	69.7
St. George	35.6 XY	220.9 TUV	60.4
Perlette	34.5 XY	66.7 XYZ	11.7
Emperor	23.4 Y	92.6 WXYZ	18.5
Tokay	22.8 Y	60.1 XYZ	11.8
Thompson Seedless	15.0 YZ	30.3 YZ	5.1
Harmony	1.9 Z	3.1 Z	1.9
Salt Creek	1.7 Z	7.9 Z	2.6
Dog Ridge	1.2 Z	3.5 Z	1.2
LSD 0.05	19.4	105.8	

[a]Means based on counts from 30–37 root systems. Means with letters under the same subgroup are not significantly different.

[b]Based on measurements of dry weight of root systems of known length.

There were significant differences between varieties in the number of larvae establishing infection sites when either the raw data were considered or when root size was adjusted to a constant length (Table 4). From those counts, the varieties could fall into three main groups: 1) the rootstocks (Harmony, Salt Creek, and Dog Ridge) in which infection and establishment of infection sites were extremely low; 2) varieties such as Thompson Seedless, Perlette, Emperor, and Tokay, which are largely of Mideastern origin (1, 10) and which had significantly fewer larvae per root either with the raw data or adjusted data than did the remainder of the varieties; 3) wine varieties, including Ruby Cabernet, which appeared to be most susceptible to penetration. When nematode counts are per unit of root system, it is apparent that the wine varieties are generally less resistant than the others tested.

Egg production. Although this experiment was designed primarily to determine the length and variability of development for each age cohort of parasitic larvae in different grapevine varieties, there are some data generated on egg production per female, since egg counts were made and numbers of females determined. In calculating those rates of egg production, subjective error is introduced in the visual decision of the number of individuals in a root system which are sufficiently mature to be capable of egg production. Since the number of eggs produced will be related to the number of heat units during the production period (5), egg data are expressed as eggs per female per 1,000 degree-hours (Table 5). The mean egg production per female per 1,000 degree-hours, with its standard deviation, is shown for each variety. Only plants from which eggs were recovered were considered in those calculations.

As with the number of larvae, a consideration with these data is the size and vigor of the host root system from which the eggs are being produced. Consequently, egg data were also corrected to constant root size (Table 5). Note that there was no egg production in the resistant rootstocks where the few parasitic larvae did not achieve maturity, and these varieties were not considered in the egg calculations or in the analysis of variance. There were no differences in egg production per female per 1,000 degree-hours in the non-rootstock varieties either with the raw egg data or the egg data adjusted to constant root size. The lack of significant differences in these data reflects the extreme variability in egg counts between vines. There are considerable differences in the mean number of eggs per female per 1,000 degree-hours within the individual varieties. Note that Carignane, Ruby Cabernet, St. George, Thompson Seedless, and Tokay have extremely high egg production, whereas French Colombard, Perlette, and Zinfandel have much lower production of eggs per female. Such differences would have marked effects on the population dynamics of the nematodes in the different varieties. More work is necessary here to quantify the egg-production parameters.

TABLE 5. Egg production per *M. arenaria* female per 1,000 degree-hours in grapevine varieties and rootstocks.

Variety/rootstock	Eggs/♀/1,000 DH[a] Mean	Std. dev.	Eggs/♀/1,000 DH (const. root length)[a] Mean	Std. dev.
French Colombard	4.1	6.8	7.1	10.7
Barbera	5.2	5.1	17.7	24.4
Carignane	9.4	18.6	24.6	48.3
Ruby Cabernet	23.5	46.2	32.0	66.0
Salt Creek	0.0		0.0	
Dog Ridge	0.0		0.0	
St. George	6.9	12.5	24.9	66.9
Harmony	0.0		0.0	
Thompson Seedless	20.3	39.9	38.0	91.3
Perlette	7.5	12.6	10.9	23.7
Emperor	7.0	4.7	19.1	22.7
Tokay	13.9	24.9	27.7	46.5
Cabernet Sauvignon	7.7	8.6	18.2	25.7
Zinfandel	7.3	10.6	10.8	17.2
Mean	10.3		22.7	

[a]Differences between varieties were not significant. Rootstocks in which eggs were not produced were not included in analysis of variance or overall means.

DISCUSSION

On the basis of the results on the infection and establishment capabilities of *Meloidogyne* larvae in grapevine roots, on the rates of variability of development to

maturity, and on the egg production of mature females, we are able to use simulation techniques to predict the number of productive females and eggs produced after definite periods of physiological time. The parameters established can be incorporated into simulation models such as MELSIM for predictive and pest management purposes. They can be used also to compare the host status of the grapevine varieties and rootstocks (Table 6). Here, we predict the numbers of adult females and eggs produced 13,000 degree-hours after vine root systems of uniform size are exposed to 1,000 *Meloidogyne* larvae. In generating these data we have used parameters adjusted to constant root length for larval penetration and egg production. We express greater confidence in the reliability of the data presented for larval penetration and development than in those for egg production.

It is interesting to relate the geographic and genetic origin of the grapevine varieties to the data presented. French Colombard, Cabernet Sauvignon, and Carignane are French in origin, Barbera is Italian, while Ruby Cabernet is the product of a cross between Carignane and Cabernet Sauvignon; the origin of Zinfandel is uncertain (1, 10). These varieties have similar quantitative relationships with *M. arenaria* (Table 6). Tokay, Thompson Seedless, Perlette, and possibly Emperor are Mideastern in origin and exhibit a greater degree of horizontal resistance. It is perhaps significant that these varieties have proved adaptable and productive in the sandy soils of the San Joaquin and Coachella Valleys, where problems are likely from *Meloidogyne* spp., including *M. arenaria*. Attempts to introduce varieties such as Ruby Cabernet in those areas may be disappointing unless resistant rootstocks or effective nematode control practices are used. Although root-knot-resistant rootstocks are not commonly used in the San Joaquin Valley, these studies suggest that they may be necessary for wine grape varieties in that area. Note that the data attest only to the host status of the plant to nematodes and do not reflect the reaction of the host to the nematode in terms of its level of tolerance. Further work is necessary in this area to translate numbers of nematodes into expected yield loss or reduction in plant growth.

TABLE 6. Predicted numbers of adult females and eggs produced 13,000 degree-hours after exposure to 1,000 larvae of *M. arenaria* for grapevine varieties and rootstocks. Data adjusted to constant root length.

Variety/rootstock	Females	Eggs
French Colombard	70	1,157
Barbera	44	1,776
Carignane	82	4,385
Ruby Cabernet	116	7,791
St. George	69	3,064
Thompson Seedless	4	288
Perlette	14	363
Emperor	20	884
Tokay	15	949
Cabernet Sauvignon	63	2,677
Zinfandel	51	1,412

LITERATURE CITED

1. BROOKS, R. M., and H. P. OLMO. 1972. Register of new fruit and nut varieties. University of California Press, Berkeley.
2. BYRD, D. W., JR., H. FERRIS, and C. J. NUSBAUM. 1972. A method for estimating numbers of eggs of Meloidogyne spp. in soil. J. Nematol. 4:266-269.
3. FERRIS, H. 1978. Modification of a computer simulation model for a plant-nematode system. J. Nematol. 10:198-201.
4. FERRIS, H. 1976. Development of a computer simulation model for a plant-nematode system. J. Nematol. 8:255-263.
5. FERRIS, H., and S. D. VAN GUNDY, 1978. Meloidogyne ecology and host interrelationships. In: Proc. Int. Meloidogyne Symposium, 1977. (in press).
6. FERRIS, H., H. S. DUVERNAY, and R. H. SMALL. 1978. Development of a data base on the effects of soil temperature on Meloidogyne arenaria eggs for a simulation model. J. Nematol. 10:39-42.
7. HACKNEY, R. W., and H. FERRIS. 1975. Infection, development and reproduction of Meloidogyne incognita in eight grapevine cultivars. J. Nematol. 7:323. (Abstr.).
8. RASKI, D. J., W. H. HART, and A. N. KASIMATIS. 1973. Nematodes and their control in vineyards. Calif. Agric. Exp. Stn. Circ. 533 (Revised). 20 p.
9. TYLER, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:391-415.
10. WINKLER, A. J., J. A. COOK, W. M. KLIEWER, and L. A. LIDER. 1974. General Viticulture. University of California Press, Berkeley. 710 p.