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H. Ferris, S. M. Schneider, and M.C. Stuth

Associate Nematologist and Research Associates, respectively. Department of Nematology, University of California, Riverside, CA 92521

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ABSTRACT

Phenology of the root-knot nematode *Meloidogyne arenaria* is influenced by host cultivar-specific penetration and infection processes which reflect host status. The relationships between number of nematodes penetrating or establishing infection sites in roots and physiological time (DD_{10}) was described by logistic functions for eleven grape cultivars. In three root-knot nematode-resistant rootstocks, a few larvae penetrated during the first 50 DD_{10} and then apparently left the root. Cultivars could be grouped according to their susceptibility to penetration and infection. Susceptible cultivars were Carignane, Barbera, French Colombard, Ruby Cabernet, Cabernet Sauvignon, Zinfandel, and the rootstocks AXR1 and St. George; moderately resistant were Tokay, Thompson Seedless and Perlette; and highly resistant or immune were Dog Ridge, Salt Creek and Harmony.

The behavior and interrelationships of organisms in an agroecosystem can be studied and appreciated through the analytical simplification of modeling and simulation. As with natural ecosystem models (10), it is useful to consider the agroecosystem as a series of interacting subsystems. Each subsystem can then be studied in isolation, coupling structures with other subsystems identified, and a series of hierarchical models developed at appropriate levels of resolution. The coupling structures between subsystems are specific to that interaction and may require extensive experimentation.

In the development of models of vineyards to allow exploration of crop and pest management possibilities, and to allow rational management decisions, models of the plant and various insect pests (8), powdery mildew (11), and root-knot nematodes (2,3) have been developed. To allow nematode subsystem coupling, the plant model has been extended by root phenology studies (9). The model of the root-knot nematode (*Meloidogyne arenaria*) subsystem adequately describes the relationship of the nematode and its abiotic environment, but the coupling process requires further quantification of the interaction with the host. Model generalization requires that these interactions be determined for a range of plant genotypes. Quantitative definition of the interaction must include the range of variability due to associated genetic and spatial influences (2,7).

The nematode simulation model, MELSIM (2,3), divides the interaction between plant-parasitic nematode and host into three general categories: 1) the penetration and infection process; 2) development of parasitic larvae to adulthood; and 3) egg production by adult females. The development process has been quantified in 14 grapevine cultivars or rootstocks, and some preliminary information has been obtained on egg production (5). The objectives of these studies are to quantify the probability of penetration and establishment of infection sites by *M. arenaria* in the roots of grapevine cultivars and rootstocks. The information allows for further strengthening and generalization of the coupling algorithms for models of nematode and plant subsystems.

MATERIALS AND METHODS

Two hundred single-bud hardwood cuttings of 11 cultivars and three rootstocks were rooted in 2.5 cm diam by 7.5 cm long plastic tubes containing steam-sterilized sand embedded in a moist, heated sand bed. After 2 months, 125 plants of each variety were selected, tubes removed from the propagating bed, and transplanted to larger tubes (4.2 × 15.5 cm) of sand standing on bricks in level galvanized pans. The bricks were subirrigated to a constant water level to generate uniform moisture conditions in the tubes by capillarity. After a one week establishment period, each tube was inoculated with 200 freshly-hatched second-stage juveniles of *M. arenaria* in 2 mL water. The larvae were inoculated throughout the root mass by using an automatic pipette and canula with plugged tip and holes drilled along the length.

Every day for 25 days, four plants of each variety, selected at random, were washed from the tubes and roots separated from soil by a water spray. Since determinants of the rate of nematode penetration into roots must include the amount of root tissue and infection sites present, and since the preferred infection sites are considered to be root tips, these two parameters of root

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size were measured. Roots were cut from the stem, blotted dry, and weighed. Nematodes within the root were stained by immersing the roots and boiling in acid-fuchsin lactophenol, rinsing, and destaining the plant tissue in clear lactophenol for at least 24 hours. Each root system was rinsed in water and spread in a film of glycerin between two glass plates (7.5 × 15 cm), one etched with a 1 × 1 cm grid. The glycerin improves optical qualities of the system, prevents drying, and adheres the plates together.

Nematodes within the roots and numbers of root tips were determined under a dissecting microscope. The syncytial feeding sites of *M. arenaria* are established within the vascular tissues, so penetrating larvae were categorized according to location in cortical or vascular tissues. The counting and analysis procedure was continued until it was obvious that there was no further penetration. Soil temperature was recorded throughout the period of exposure of plants to nematodes so that penetration rates could be based on physiological time, degree-days above a predetermined threshold of 10°C (DD_{10}) for *M. arenaria* (4,5).

Number of nematodes penetrating the root (total of cortical and vascular nematodes) or potentially establishing infection sites (vascular nematodes) were plotted relative to elapsed DD_{10} after inoculation. An appropriate model was selected which was visually compatible and biologically explanatory for each situation. When divided through by the maximum number of nematodes entering the root of a particular cultivar, the curves represent the cumulative probability of entry of a single age cohort over time. The derivative of the model allows prediction of the probability of root infection by the individuals remaining in the cohort over time, and is useful for simulation purposes.

In order to compare the susceptibility and pattern of infection among cultivars, it was necessary to convert nematode counts to a constant root size. Since root tips are the preferred infection site, numbers establishing infection sites per root tip was an appropriate measure. However, the plant system is dynamic; the number of root tips was increasing during the experiment. Accordingly, a function relating number of root tips to physiological time was developed for each variety. By dividing the expression for number of nematodes entering the root or establishing infection sites relative to DD_{10} by the expression for number of root tips relative to DD_{10}, a function was developed to describe the number of nematodes per root tip for each variety. The behavior of this function with time was studied so that its trends and maxima could be used as parameters of relative susceptibility of varieties to penetration and infection.

RESULTS

The number of nematodes penetrating into the cortical and vascular tissues of the root systems predictably increased to a maximum with time in most cases. This maximum was a function of the inherent level of resistance of the root system to penetration or infection, the size of the root system (number of available infection sites), and the energy level of the nematodes for penetration. The maximum penetration or infection would then form the limiting parameter for a logistic-type equation reflecting the biology of the process (Fig. 1, A-B). The apparent infection rate would be progressively modified through the experiment by the availability of infection sites and the declining energy levels of nematodes remaining in the soil and unable to feed. In three root-knot resistant rootstocks (Harmony, Salt Creek, Dog Ridge),

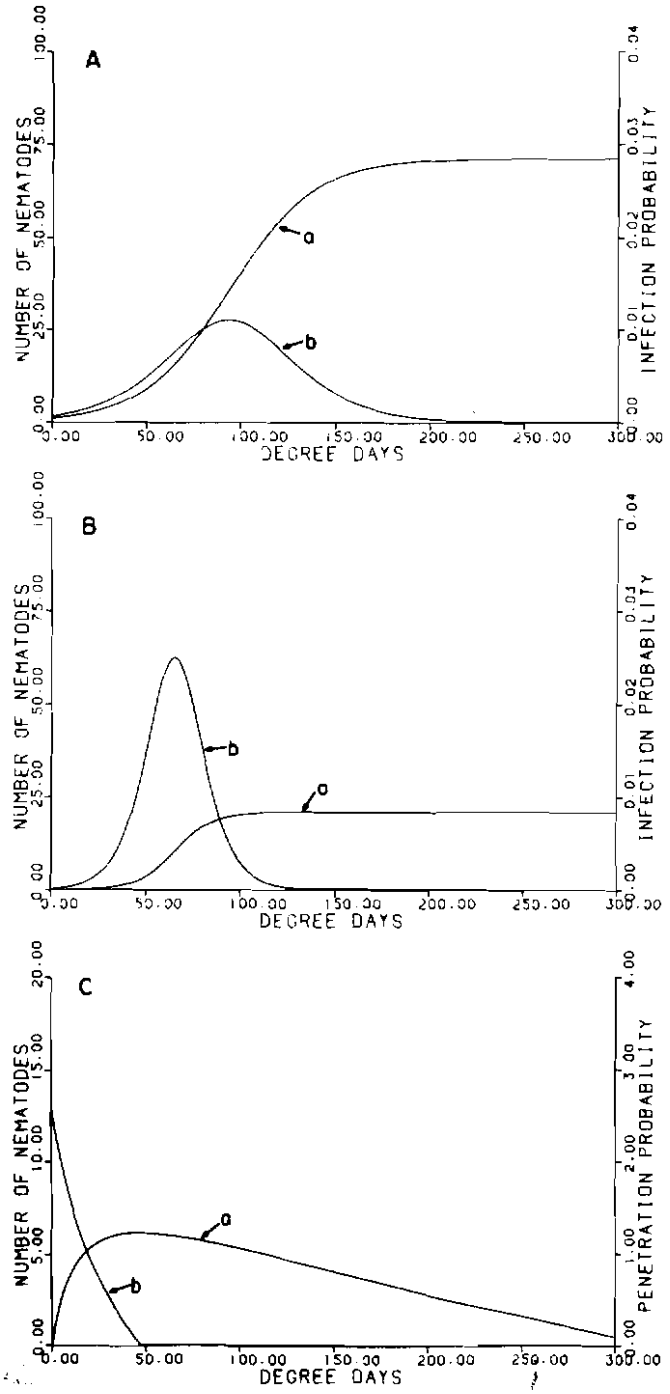

Figure 1. A-C. Cumulative number of nematodes (a) and probability of penetration (b) in the vascular region of grape roots relative to length of exposure (DD_{10}) to a single age cohort of *Meloidogyne arenaria* larvae. A. French Colombard, B. Thompson Seedless, C. Harmony.

there was initial movement into the root, hut infection sites were not established, and nematodes apparently moved out again. Quadratic functions relating the movement into and out of the root to elapsed DD_{10} were satisfactory descriptions in this case (Fig. 1-C).

As nearly as possible, the larval inoculum used in this experiment represented a single age cohort, and was exposed to the root system at one point in time. It is apparent, however, that all larvae did not enter the root system at the same time, either due to variability within the nematode and plant genotype, or to spatial heterogeneity of root distribution and infection site suitability (Fig. 1, A-C). The maximum number of nematodes entering each root system presumably represents those capable of infectivity. For any cultivar, if the equation for cumulative number of nematodes per root tip is divided by the maximum number of larvae penetrating or infecting, the function represents the cumulative probability of the process. The derivative of the function (Fig. 1, A-C) provides the probability of penetration at any point in time after exposure of a single age cohort to the root system. The form of the function in the susceptible varieties was $y = T/(1 + B \times (e^{(-S \times D)}))$ where D is number of DD_{10} after exposure to the root, T is the maximum penetration, B and S are constants determining curve shape, e is the base of natural logarithms. The appropriate values for the coefficients were calculated (Table 1). The expressions assume that the nematodes entering the root system for a particular cultivar were all of those with the capability, and uses the rate of their entry as an index of the probability of entry of a single age cohort under conditions of the experiment, on the respective cultivars (Fig. 1, A-C).

Table 1. Parameters of the logistic equation $y = T/(1 + B \times (e^{(-S \times DD_{10})}))$ describing the relationship between establishment of infection sites and elapsed DD_{10} exposure for grape cultivars susceptible to *Meloidogyne arenaria*.

	T	B	S[a]	Model r^2
AXR1	38.0	60.6	0.050	0.95
St. George	60.8	19.3	0.019	0.87
Tokay	20.0	419.0	0.090	0.89
Thompson Seedless	21.0	696.0	0.100	0.73
Perlette	24.0	164.0	0.075	0.68
Carignane	44.0	125.0	0.050	0.88
French Colombard	71.1	61.0	0.044	0.92
Ruby Cabernet	70.2	206.5	0.050	0.90
Cabernet Sauvignon	94.0	73.0	0.040	0.93
Barbera	68.0	76.0	0.040	0.91
Zinfandel	75.6	160.2	0.042	0.93

[a]T is the maximum number of nematodes penetrating, B and S are constants determining curve shape, e is the base of natural logarithims.

The maximum number of nematodes entering the root systems and establishing infection sites was determined for each variety using the appropriate model fit to the data by least squares methods (Table 2). In the resistant rootstocks, very few larvae entered the root system, and only about half of those entering progressed as far as the vascular tissues. By the end of the experiment, none of these nematodes were apparent in the root. In all other cultivars, the majority of the nematodes which entered the root system progressed to the vascular tissues. The period over which penetration occurred varied among cultivars, separating them into approximately three groups.

Table 2. Maximum number of larvae penetrating and establishing infection sites, and DD_{10} to achieve these maxima, for grape cultivars and rootstocks.

Variety Rootstock	(cortical & vascular) Max. penetration No.	DD_{10}	(vascular) Max. infection No.	DD_{10}
Dog Ridge	1.6	48	0.8	56
Salt Creek	4.7	42	2.1	57
Harmony	6.1	47	4.8	51
AXR1	43	210	42	210
St. George	62	300	61	300
Tokay	20	130	20	150
Thompson Seedless	22	135	21	145
Perlette	24	135	24	160
Carignane	44	215	44	225
French Colombard	71	215	71	230
Ruby Cabernet	75	230	70	235
Cabernet Sauvignon	94	255	94	255
Barbera	68	230	68	260
Zinfandel	76	250	76	260

Since root size and infection site number varied among cultivars and rootstocks and through the duration of the experiment, the relationship of root size to physiological time was determined for each cultivar and rootstock. In each case, the relationship was linear within the time limit of the experiment (Table 3). Functions for the number of nematodes per root tip were obtained by dividing the quadratic or logistic penetration functions by the linear root tip increase functions. This enabled comparison of susceptibility levels on a unit root size basis. The general form of the function for most cultivars (Fig. 2) was a rapid initial increase of larvae per root tip as larvae were fresh with high energy reserves, gradually declining as larvae penetration rate decreased and root tips increased, followed by a reduction as the root continued to grow and there was no further penetration. In the resistant rootstocks, of the few nematodes entering, the greatest numbers per root tip occurred shortly after the initial exposure and then decreased as larvae moved from the root and root tip increase continued.

Table 3. Parameters of linear models relating numbers of root tips to DD_{10} for rootings of grapevine cultivars and rootstocks over a 300 DD_{10} time period.

Variety/Rootstock	Intercept	Slope	r^2
Harmony	297.98	0.14	.93
Salt Creek	142.12	0.72	.86
Dog Ridge	127.22	0.33	.85
Carignane	136.48	1.23	.94
St. George	158.66	0.62	.88
Zinfandel	187.06	0.50	.85
Barbera	219.79	0.73	.93
Tokay	220.45	0.33	.86
Ruby Cabernet	274.43	0.71	.88
AXR1	230.19	0.50	.83
Thompson Seedless	223.88	0.08	.68
Perlette	152.00	0.11	.82
Cabernet Sauvignon	308.80	0.26	.87
French Colombard	254.70	1.20	.96

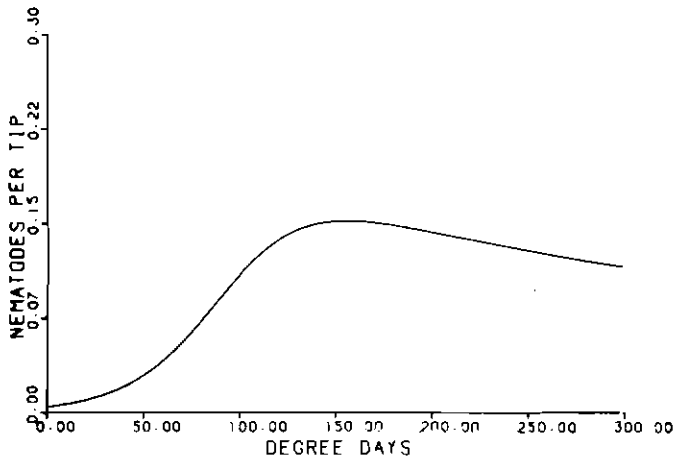

Figure 2. Number of nematodes per root tip in the vascular region of French Colombard roots relative to length of exposure (DD_{10}) to a single age cohort of *Meloidogyne arenaria* larvae.

The maximum number of nematodes per root tip during the increase and decrease cycle, and the physiological time interval after inoculation at which maxima were achieved (Table 4), represents an index of relative susceptibility to penetration and infection. Three main groupings occur: 1) susceptible (Carignane, Barbera, French Colombard, Ruby Cabernet, Cabernet Sauvignon, Zinfandel, and the rootstocks AXR1 and St. George) represented by Fig. 1A; 2) moderately resistant (Tokay, Thompson Seedless, Perlette) represented by Fig. 1B; 3) highly resistant or immune (Dog Ridge, Salt Creek, Harmony) represented by Fig. 1C.

Table 4. Maximum number of nematodes per root tip and physiological time of occurrence (DD_{10}) after exposure.

Variety	Total Penetration		Total Infection	
	DD	Max/tip	DD	Max/tip
Dog Ridge				
Salt Creek				
Harmony				
Tokay	220	.07	220	.07
Thompson Seedless	259	.09	259	.09
Perlette	176	.06	173	.06
AXR1	177	.13	179	.13
Carignane	155	.12	164	.12
St. George	127	.25	130	.24
Barbera	188	.18	166	.19
French Colombard	154	.15	155	.15
Ruby Cabernet	196	.18	194	.16
Zinfandel	189	.26	187	.26
Cabernet Sauvignon	190	.26	191	.26

DISCUSSION

Primary objectives of this study were to develop model parameters for the quantitative coupling structures between nematode and grape plant models. Previous work (5) quantified the development of established infection of single age cohorts of *M. arenaria* larvae in grape cultivars. These studies examined the rates and variability in the infection process. An initial point of investigation was whether differences in resistance or susceptibility among cultivars and rootstocks were expressed at the epidermal level (reduced penetration) or at the vascular level (fewer infection sites established). Consequently, nematodes in the roots were distinguished according to spatial location (Table 2). Apart from the resistant rootstocks, all nematodes entering the root system eventually migrated to the vascular tissues and presumably established infection sites. Population levels were low enough so that competition for sites was minimal. In the resistant rootstocks, where nematodes moved out of the root, only about half of the few nematodes which entered were seen in the vascular tissues. In all cases it appears that any resistance (either horizontal or vertical) is expressed as a lack of, or reduced, penetration of the root system primarily, with failure to establish infection sites or induction of host biochemical defenses occurring in resistant rootstocks.

The cultivars and rootstocks fell into three categories when larval penetration per root tip (Fig. 2) was considered. Of the cultivars tested, Tokay, Thompson Seedless and Perlette showed a level of resistance to penetration. It is interesting that the groupings for infection were the same as those in the development studies (5) and can be related to the geographic and genetic origin of the cultivars. French Colombard, Cabernet Sauvignon, Carignane and Barbera are European in origin, while Ruby Cabernet is the product of a cross between Carignane and Cabernet Sauvignon. Zinfandel is of uncertain origin, while Tokay, Thompson Seedless and Perlette are Mideastern (1,12). Again, the cultivars of probably Mideastern origin exhibited a greater degree of horizontal resistance than those of European origin. Apparently the resistance is expressed throughout the period of interaction of plant and nematode. The consequences of these differing levels of resistance to *M. arenaria* in attempting to establish and maintain productivity of various cultivars in sandy soils of inland regions of California (root-knot favorable areas) have been previously discussed (5). The cultivars with moderate resistance have proved adaptable and productive in the sandy to loam soils of the inland valleys of California where root-knot problems are likely. Introduction of the more susceptible cultivars in these areas may be disappointing, especially in sandy soils. Fortunately, the use of resistant rootstocks is a viable alternative and should be considered in areas and soils where root-knot nematodes may be a constraint to production of certain cultivars. Investigation is warranted of the use of cultivars with some resistance or tolerance to root-knot nematodes as rootstocks with suitable horticultural characteristics for susceptible cultivars in less sandy soils.

Besides the number of nematodes entering per root tip, the amplitude of the penetration probability curve (Fig. 1, A-C) might be considered another parameter of the level of resistance. Consider that the uniform cohort of larval nematodes used as inoculum probably had the same level of energy reserves, and that a certain energy level is required to penetrate roots of different cultivars.

As the energy level of nematodes remaining in the soil declined with time through lack of feeding, so they would drop below the minimum energy status necessary to penetrate a specific cultivar. Penetration continued for up to about 250 DD_{10} in the susceptible cultivars, 135 DD_{10} in the horizontally resistant, and 50 DD_{10} in the resistant (Table 2, Fig. 1, A-C), indicative of the energy level necessary to achieve penetration in the various varieties.

An appreciation of the variation associated with rate processes is necessary for the development of realistic simulation models (4,7). Basing population development in simulators on average or maximum rates results in prediction of synchronous development of age cohorts of organisms. In contrast, consideration of the probability of an event occurring results in distributed development of the population with time (Fig. 3) and more closely emulates reality. In this illustrative simulation, the developmental progress of one age cohort of infective larvae to adulthood for two varieties is predicted, considering variation in the infection process and in development (5). The susceptible French Colombard cultivar, exposed to a single age cohort of infective larvae sustained about 36% penetration and development of infection sites as opposed to 11% in Thompson Seedless, although the available root tips at the start of the experiment were similar — 255 and 224, respectively (Table 3). The distributed delay of penetration is compounded by a further distributed delay in development. Reproductive maturity is attained from 440 to 800 DD_{10} in French Colombard and from 500 to 900 DD_{10} in Thompson Seedless after exposure to the root. These development patterns are further extenuated by repeated exposure of new infection cohorts to the root as a result of egg hatch. In interpretation of the seasonal dynamics of a nematode population in the vineyard, it is valuable to consider that the population as a whole is not cycling on a 4 week generation amplitude and that the host-, parasite-, and environment-mediated distributed delays can result in seasonal patterns observed through repeated sampling (6).

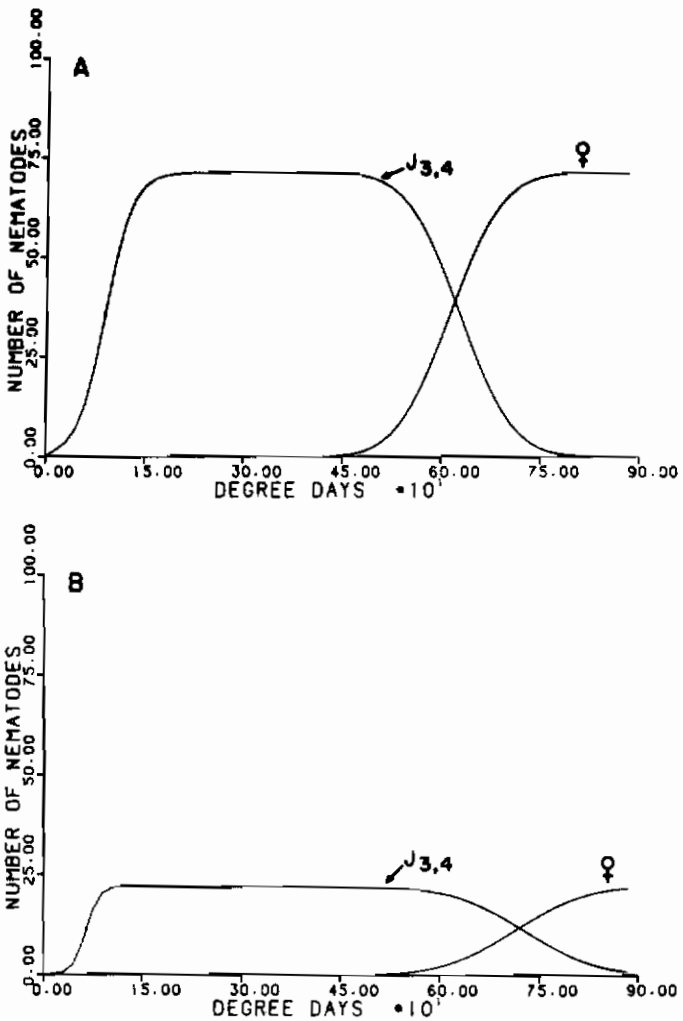

Figure 3. A-B. Simulated number of parasitic larvae in the root ($J_{3,4}$) and egg-producing adult females ($♀_R$) of adult females relative to length of exposure (DD_{10}) to a single age cohort of *Meloidogyne arenaria* larvae. A. French Colombard, B. Thompson Seedless.

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