

## Overwintering Stages of *Meloidogyne incognita* in *Vitis vinifera*

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**Abstract:** The overwintering of *Meloidogyne incognita* in and around *Vitis vinifera* cv. French Colombard roots was studied in a naturally infested vineyard at the Kearney Agricultural Center, in a growth chamber, in inoculated vines in microplots at the University of California, Davis, and in a greenhouse. Infected roots were sampled at intervals from onset of vine dormancy until plants accumulated about 800 degree days (DD—base 10 C). Embryogenesis within eggs, classified as less than or more than 16 cells and fully differentiated, and numbers of juveniles (second to fourth stage) and preovipositional and mature (egg-laying) adult stages in roots were determined. All stages were present at the onset of dormancy. Juveniles and immature females were not recovered during the dormant period. Mature females and eggs were always present in roots, although the number of mature females generally decreased with time after onset of dormancy. In contrast, in a greenhouse experiment that accumulated comparable DD without the host plant going through dormancy, the number of mature females increased. After bud break, the number of eggs per female increased and all nematode stages were found in host roots. Eggs in all stages of embryogenesis were observed at all times of sampling, indicating that females overwinter and are capable of laying eggs when conditions improve in the spring and need to be considered in nematode management decisions.

**Key words:** degree days, *Meloidogyne incognita*, nematode management, overwintering, root-knot nematode, *Vitis vinifera*.

In discussing predictive models for root-knot nematode management, Ferris et al. (3) introduced a mechanistic description of the relationship between host and parasite, incorporating nematode population energy demand and the impact on host physiological efficiency. Life-table based nematode population models, especially of obligate sedentary endoparasites with only one infective stage, require determination of the age structure of the initializing population. In annual crops, the overwintering population of sedentary endoparasites such as root-knot nematodes consists of eggs and second-stage juveniles, whereas in perennials such as clover all stages of *Meloidogyne incognita* and *M. hapla* can survive freezing temperatures and continue to develop (13). In perennial crops that undergo dormancy, however, it is not known whether root-

knot nematode populations overwinter solely as second-stage juveniles and eggs in soil, or whether females and other stages in the roots remain viable during the winter dormancy period of the crop. Further, it is not known whether the overwintering stages are viable or whether females are able to reinitiate egg production when conditions improve in the spring. Population fluctuations in serial samples removed from the field provide mixed indications on this point, and usually the data are confounded with sampling error (3). Preliminary microscopic examination of *M. incognita* (Kofoid and White) Chitwood eggs extracted from *Vitis vinifera* L. cv. French Colombard roots during the spring of 1986, when plants had accumulated 252–461 degree days (DD—base 10 C) after the onset of dormancy, showed different stages of cell differentiation. Since about 200 DD are required for a juvenile to develop and hatch (3), these observations indicated that adult and (or) other stages of root-knot nematodes might be capable of surviving in the roots over the dormant season and producing eggs the following spring. The objectives of this study were to determine the age structure of overwintering *M. incognita* populations in and around *V. vinifera* cv.

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French Colombard roots and whether the surviving stages reach maturity and produce eggs in the spring.

#### MATERIALS AND METHODS

Experiments were conducted in the field, growth chamber, microplot, and greenhouse.

*Field experiment:* A 10-year-old French Colombard vineyard infected with *M. incognita* was selected at the Kearney Agricultural Center, Parlier, California. Daily soil temperature data were collected at a depth of 15 cm around plant roots. Root and soil samples from 15 to 20 cm deep around five different plants on each date were collected at 2–4-week intervals from the time the plants were completely dormant (1 December 1986) until they accumulated 803 DD. A plant was considered dormant when it was completely defoliated and to have emerged from dormancy at onset of bud break. Roots were washed free of soil and bleached in 10–60% NaOCl for 5 minutes (8). Nematode development from acid-fuchsin lactic-acid (8) stained roots and embryogenesis from eggs collected from the bleach was determined. Nematodes in 1 g of randomly selected root were counted and classified as juveniles (second to fourth stage), preovipositional females, and mature (egg-laying) adult females (7). Mature females that were either parasitized or decaying were not counted. At each sampling date, the stage of embryogenesis of 100–150 eggs per plant was microscopically determined and classified as less than 16 cells, more than 16 cells, and fully differentiated juveniles. Eggs and juveniles from 500-cm<sup>3</sup> soil samples were also counted after elutriation (1).

*Growth chamber experiment:* French Colombard green cuttings (two-leaf stage) were propagated (11). Eighty plants potted in 414-ml styrofoam cups were each inoculated on 15 July 1986 with 3,000 single-age cohort second-stage *M. incognita* juveniles by pipetting into the soil 2–3 cm around the plant. Juvenile inoculum was prepared by blending roots in 0.5% bleach for 30 seconds and collecting eggs and

hatching them in a mist chamber on a modified Baermann and funnel method. To ensure age uniformity, all plants were washed free of soil 1 week later, repotted as before, and kept under greenhouse conditions. Soil temperature data were collected continually using a Radio Shack TRS-80 model 100 portable computer. After 521 DD were accumulated (26 August 1986), 50 infected plants were placed in a growth chamber where light and temperature conditions were gradually decreased to 9 C night and 11 C day over a 2-week period. Plants entered dormancy about 12 weeks later, by which time they had accumulated an additional 90 DD. Three months later temperature and light periods were gradually increased to break dormancy, and the experiment was concluded after an additional 765 DD had accumulated. Root and soil samples from 6–12 plants were collected three times from the onset of dormancy and processed as described for the field samples.

*Microplot experiment:* Plants were propagated and inoculated as in the growth chamber, but the microplot experiment was started ca. 2 months later. Plants were placed in 15-cm-d clay pots which were embedded in soil in microplots at the University of California (UC), Davis, so that dormancy could occur naturally. Soil temperature data were collected from a UC Davis weather station. A total of 519 DD had accumulated from the time of inoculation to the onset of dormancy. The experiment was terminated 630 DD after dormancy ended, during which time root and soil samples were collected three times and processed as in the field experiment.

*Greenhouse experiment:* The purpose of this experiment was to maintain plants in a nondormant condition for comparison with those of the microplot experiment. Infested plants were established as described for the microplot experiment and maintained in a greenhouse until comparable DD were accumulated. Six to twelve plants were harvested at 330, 720, 1,039, and 1,465 DD. Root and soil samples were processed as before, and the numbers of

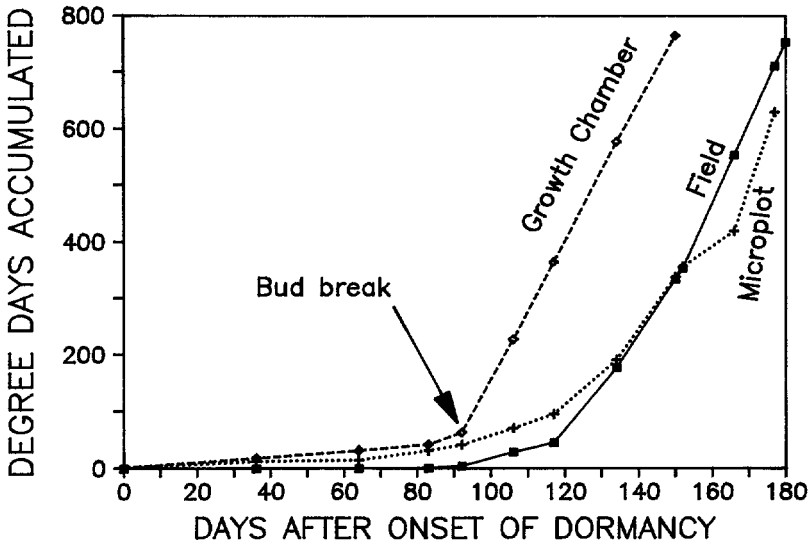


FIG. 1. Number of DD accumulated from onset of complete dormancy to end of experiment in field, microplots, and growth chamber. Onset of dormancy for field and microplot experiments was 1 December 1986. At time of dormancy, 519 and 611 DD had accumulated in microplots and growth chamber experiments, respectively.

females were compared with those of the microplot experiment.

*Data analysis:* Data sets in each experiment had two or three components, representing biologically distinct processes. An initial period of decline of some or all life stages following onset of dormancy involved biological and physiological processes different from those involved in stage-specific increases at or following the completion of host dormancy. Consequently, breakpoints relative to host dormancy were selected to reflect population fluctuation during different sections of the experimental time span and were fitted by separate regression models.

## RESULTS

From the time of complete dormancy to the end of the experiments, plants accumulated 630, 765, and 803 DD in the microplot, growth chamber, and field experiments, respectively (Fig. 1). In each experiment, dormancy lasted about 3 months, during which no more than 100 DD were accumulated.

A few weeks after onset of dormancy of the field vines, the number of juvenile stages ( $y = 180.961 - 3.212x$ ,  $r^2 = 0.58$ )

in roots declined ( $P = 0.01$ ) to zero, and none were recovered in roots ( $y = 2.621 - 0.032x$ ,  $r^2 = 0.11$ ) or soil for about 4 weeks (Fig. 2A). As the temperature increased and root development and bud break commenced, their numbers ( $y = -38.275 + 0.519x$ ,  $r^2 = 0.20$ ) significantly ( $P = 0.01$ ) increased. Equations describing the population of the preovipositional females ( $y = 26.356 - 0.216x$ ,  $r^2 = 0.22$ ) show a decline ( $P = 0.01$ ) followed by a period of no change ( $y = -2.491 + 0.023x$ ,  $r^2 = 0.12$ ) and then an increase ( $y = -88.297 + 0.615x$ ,  $r^2 = 0.47$ ,  $P = 0.01$ ), a trend that is similar to number of juveniles. Mature females were always present, although their numbers ( $y = 144.174 - 0.605x$ ,  $r^2 = 0.43$ ) decreased significantly ( $P = 0.01$ ) with time (Fig. 2A).

In the dormant period of the microplot experiment, where less than 100 DD were accumulated, the numbers of juveniles ( $y = 31.144 - 0.273x$ ,  $r^2 = 0.65$ ) and preovipositional females ( $y = 39.567 - 0.347x$ ,  $r^2 = 0.68$ ) declined ( $P = 0.01$ ) to zero and then significantly ( $P = 0.05$ ) increased with time ( $y = -60.619 + 0.532x$ ,  $r^2 = 0.34$ , and  $y = -24.429 + 0.214x$ ,  $r^2 = 0.29$ , respectively) (Fig. 2B). The mature fe-

males, however, declined ( $y = 87.202 - 0.192x$ ,  $r^2 = 0.11$ ) by about 36% over the duration of the experiment.

The population of mature females ( $y = 49.004 - 0.139x$ ,  $r^2 = 0.08$ ) in the growth chamber study was similar to that in the microplot experiment. There were few juveniles ( $y = 2.209 - 0.017x$ ,  $r^2 = 0.10$ ) and preovipositional females ( $y = 4.473 - 0.037x$ ,  $r^2 = 0.32$ ,  $P = 0.05$ ) when the host was dormant, but their numbers significantly ( $P = 0.01$ ) increased in the spring ( $y = -175.387 + 1.597x$ ,  $r^2 = 0.37$ , and  $y = -97.164 + 0.887x$ ,  $r^2 = 0.39$ , respectively) (Fig. 2C).

Unlike the plants that went through dormancy, the total number of females from the greenhouse experiment increased with time (Fig. 3). The number of eggs per female did not change during the dormant period ( $y = 6.897 + 0.048x$ ,  $r^2 = 0.05$ ), but it significantly ( $P = 0.01$ ) increased ( $y = -76.356 + 0.736x$ ,  $r^2 = 0.98$ ) after bud break in the field experiment (Fig. 4). The number of eggs per gram of root followed a trend similar to that of number of eggs per female.

In the field study, the number of eggs with less than 16 cells ( $y = -2.222 + 0.273x$ ,  $r^2 = 0.74$ ) and the nondifferentiated stages ( $y = 16.554 + 0.407x$ ,  $r^2 = 0.99$ ) in the eggs increased ( $P = 0.05$ ) initially, whereas the differentiated stages ( $y = 89.256 - 0.738x$ ,  $r^2 = 0.92$ ) decreased (Fig. 5A). Subsequently, the number of eggs with less than 16 cells ( $y = 29.345 - 0.154x$ ,  $r^2 = 0.76$ ,  $P = 0.05$ ) declined, followed by an increase ( $y = -60.818 + 0.524x$ ,  $r^2 = 0.41$ ) in the spring, whereas the nondifferentiated stages ( $y = 54.15 - 0.151x$ ,  $r^2 = 0.46$ ,  $P = 0.05$ ) and differentiated stages ( $y = 45.516 - 0.127x$ ,  $r^2 = 0.25$ ) continued to decrease (Fig. 5A). In the microplot ( $y = 18.226 + 0.081x$ ,  $r^2 = 0.38$ ,  $P = 0.01$ ) (Fig. 5B) and growth chamber ( $y = 14.926 + 0.101x$ ,  $r^2 = 0.15$ ) (Fig. 5C) studies, the number of eggs with fully differentiated juveniles increased slightly throughout the duration of dormancy. However, the numbers of less than 16 cells ( $y = 39.509 - 0.105x$ ,  $r^2 = 0.54$ , and  $y = 37.672 - 0.189x$ ,

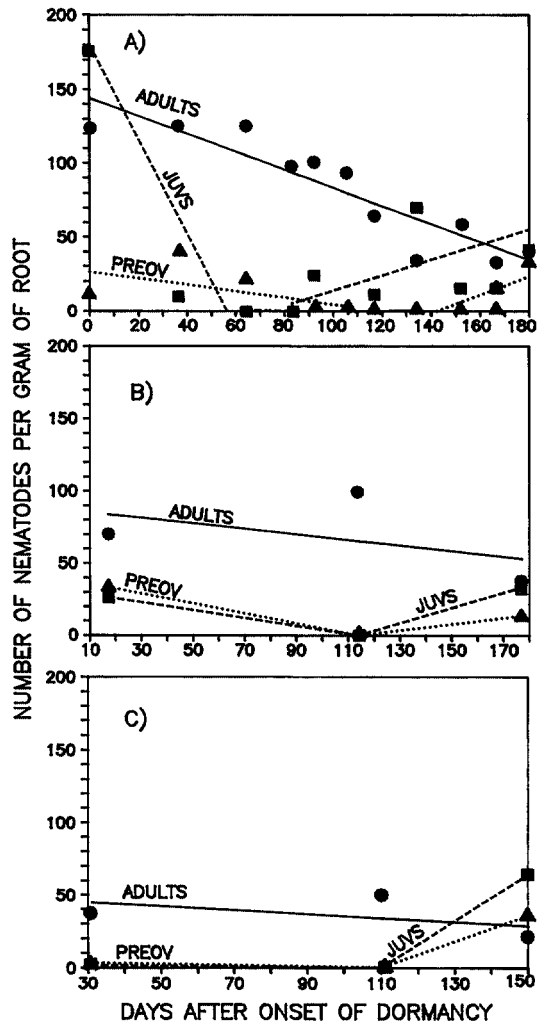


FIG. 2. Presence of juvenile stages (JUVS), preovipositional (PREOV), and mature females (ADULTS) of *Meloidogyne incognita* per gram of French Colombard roots from the onset of dormancy to the end of the experiment. A) Field. B) Microplot. C) Growth chamber. Data points represent means of 5-12 replications.

$r^2 = 0.43$ , respectively) and the nondifferentiated stages ( $y = 45.857 - 0.106x$ ,  $r^2 = 0.68$ , and  $y = 42.001 - 0.039x$ ,  $r^2 = 0.17$ ) in the microplot and growth chamber studies, respectively, decreased ( $P = 0.05$ ) with time (Fig. 5B, C).

### DISCUSSION

Plant-parasitic nematodes differ considerably in their ability to tolerate temperature extremes, and their tolerable thermal ranges vary with geographic region

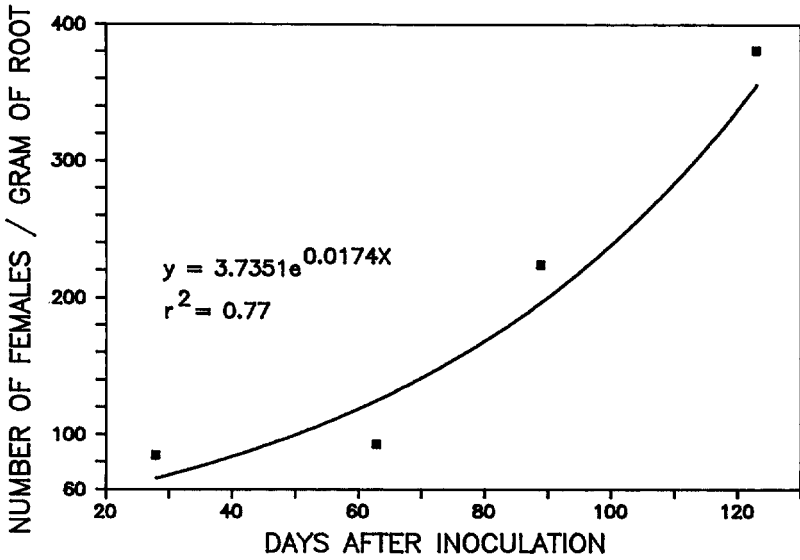


FIG. 3. Total number of *Meloidogyne incognita* females per gram of French Colombard roots conducted under greenhouse conditions for comparison with microplot experiment. Data points are means of five replications.

(14). Studies have shown that *Heterodera cruciferae* can develop in temperatures as low as 5 C (9). In some root-knot nematode species, juvenile development in eggs is generally limited at temperatures below 10 C, and about 200 DD is required for the juveniles to emerge from the egg and another 550 DD to reach the mature adult stages (3-6).

In this study, adult females of *M. incognita* in grape roots and eggs in roots and soil survived during dormancy, whereas the juvenile stages and immature females did not. This is contrary to the findings of Vrain et al. (13) and probably is related to host phenology. The absence of the juveniles when the grapes were dormant excludes the possibility of diapause. Rather,

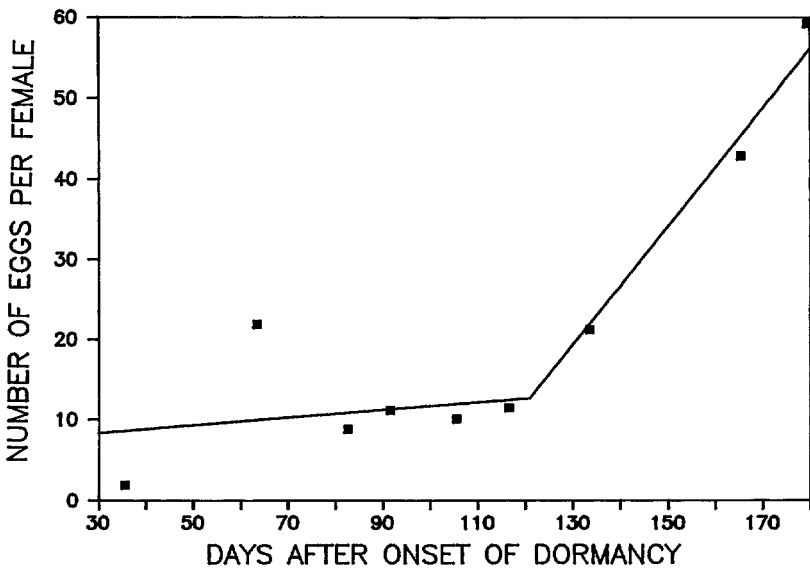


FIG. 4. Number of *Meloidogyne incognita* eggs per adult female recovered from French Colombard roots in the field study over the duration of the experiment. Data points are means of five replications.

the inability of juvenile stages to survive or develop into the adult stage in the dormant season of the host when less than 100 DD were accumulated indicates that these stages are either not maintaining the link with the giant cells or that they are less able to tolerate the low temperature. Minimum temperatures required for survival and development of root-knot nematodes may differ inside and outside a host root. Further, the study supports findings (12) that the sensitivity of exposure to minimum temperature may be different for different stages and may also be influenced by the host status.

Number of females surviving through the dormant period of the host declined by about 36–65% (Fig. 2), whereas those in the nondormant host (Fig. 3) and with comparable DD accumulation increased. This indicates a decline in health of the nematode, possibly caused by fungal parasitism (Gaspard, unpubl.), biochemical changes in plant tissue, or nematode age. The negative correlation, however, between the number of mature females and eggs per female with increasing DD in the spring indicates that egg production per female increased even though the total number of mature females decreased. This demonstrates that they are capable of laying eggs after overwintering in the host. After accumulating about 200 DD in the spring, only fully differentiated root-knot nematode juveniles should have been present in the eggs (3–5), but the presence of all stages of embryogenesis within eggs at all sampling days indicates that mature females continued to lay eggs as spring soil temperatures increased. The range of developmental stages in the egg results from the progression from one stage to the next and is reflected by the increasing numbers of juveniles and preovipositional stages in the spring, indicating initiation of a new generation.

There is some phloem and root activity before bud break or new root formation (2). At this time, the energy demand of the surviving females may be particularly significant. If females are producing eggs, we

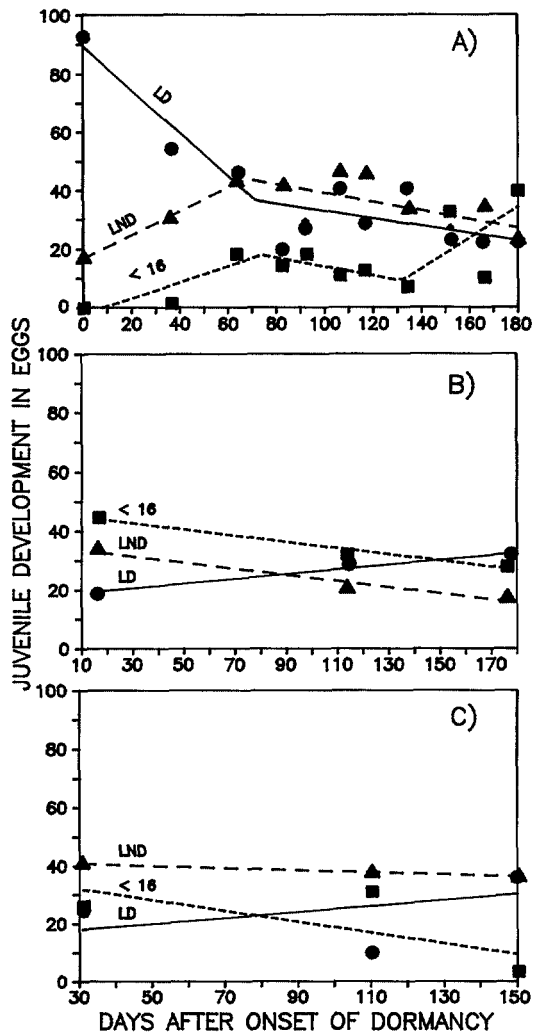


FIG. 5. Numbers of eggs with less than 16 cells (< 16), more than 16 cells (LND), and differentiated (LD) *Meloidogyne incognita* juveniles within eggs collected from French Colombard roots. A) Field. B) Microplot. C) Growth chamber. Data points represent means of 5–12 replications.

hypothesize that they must be feeding, and thus they constitute a drain on the host's energy reserve. We calculate that nematode population levels of the magnitude of those observed in the field study may reduce the total energy in 1 g of root by 80 calories. Such a population may be not only a considerable drain on energy for the existing and new flush of roots, but the nematode's ability to produce eggs can affect population estimates for the coming season.

Current root-knot nematode models for grapes (and other perennials with similar phenology) are initiated with the assumption that eggs and second-stage juveniles are the life stages constituting the initial population in the spring. However, the models need to include the overwintering adult females that introduce offspring early in the growing season, thus affect the age structure of consecutive generations.

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