Journal of Nematology 21(1):74–80. 1989.

Impact of *Meloidogyne incognita* on Physiological Efficiency of *Vitis vinifera*

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Abstract: Four-week-old French Colombard plants rooted from green cuttings were inoculated with 0, 1,000, 2,000, 4,000, or 8,000 *Meloidogyne incognita* second-stage juveniles and maintained at 25 C night and 30 C day. Leaf area and dry weight and the rates of photosynthesis, stomatal conductance, and internal leaf CO_2 concentration were measured at intervals up to 59 days after inoculation. Nematode stress dosage, measured as the product of cumulative number of juveniles and females and their total energy (calories) demand, was up to 3.4 kcal and accounted for up to 15% of the energy assimilated by the plants. There was a decline in the rate of leaf area expansion and leaf, stem, shoot, root (excluding nematode weight), and total plant dry weight with increasing nematode stress. Root weight including nematodes was not affected. Total respiration, plant photosynthesis, energy assimilated into plant tissue and respiration, and gross production efficiency decreased significantly with nematode stress. Photosynthetic rate, transpiration rate, stomatal conductance, and internal CO_2 concentration were not affected. This study demonstrates that the energy demand for growth and reproduction of *M. incognita* accounts for a significant portion of the total energy entering the plant system. As a result, less energy is partitioned into leaf area expansion which, in turn, affects the energy entering the system and results in decreased productivity of nematode-infected grape vines.

Key words: assimilation, energy partitioning, leaf area expansion, nematode energy demand, *Meloidogyne incognita,* photosynthesis, respiration, *Vitis vinifera.*

Root-knot nematodes can directly or indirectly affect host physiological processes and productivity. Their high reproduction capacity and obligate parasitism diverts a significant proportion of the photosynthate material from the host (11). Their ability to disrupt the vascular tissue alters the translocation of water and solutes to the shoots. These processes can be classified as direct effects. This parasitism indirectly influences the mechanisms of the photosynthetic apparatus and other physiological processes such as CO_2 exchange which, in turn, affect the total energy available to the plant and the partitioning of photosynthate into leaves, stems, and roots. Several studies have shown the effects of nematodes on photosynthesis, nutrient uptake, and related physiological processes and the consequent impact on host productivity of annual crops (10,14,18). In an earlier paper (13) we addressed growth, reproduction, and energy requirements of *Meloidogyne incognita* in grape vines. In a host–parasite interaction, however, it is necessary to understand the relationship between the nematode energy demand and the efficiency of host physiological processes in compensating for this demand. Furthermore, such physiological information may be used for application in nematode management (2). The objectives of this study were to determine the effect of *M. incognita* (Kofoid and White) Chitwood on 1) total energy entering the plant system, 2) partitioning of the energy within the plant, and 3) the relationship between nematode energy demand and the impact on the productivity of a susceptible grape, *Vitis vinifera* L. cv. French Colombard.

Materials and Methods

French Colombard green cuttings (two-leaf-stage) were rooted in a 1:1 vermiculite : sponge rock mixture in a mist chamber. Four weeks later 80 plants were assessed for uniformity of growth, leaf area, and photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate (collectively referred to as physiological parameters). Plants were

Received for publication 15 September 1987.

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The authors thank Foundation Seed and Plant Materials Service (UC, Davis) for supplying the grape cuttings, the UC Integrated Pest Management Grape Commodity Group for financial support, and Dr. Bruce A. Jaffee for reviewing the manuscript.

inoculated with 0 (control), 1,000, 2,000, 4,000, or 8,000 *M. incognita* second-stage juveniles pipetted into the soil and randomly placed in a growth chamber set at 30 C for 12 hours at light (400 $\mu E \cdot m^{-2} \cdot sec^{-1}$; 700–400 nm) and at 25 C for 12-hour dark period. Treatments were replicated four times. Plants were watered to saturation twice a week with normal strength Hoagland solution, otherwise daily with distilled water.

Leaf area and plant dry weight of 20 plants (four replications × five treatments) were measured at 11, 30, 44, and 59 days after inoculation. The rates of leaf area and plant dry weight increase over 59 days were determined for each treatment and treatment totals were compared.

Nematode population and developmental stages (3) were determined from 1-g samples of randomly selected roots stained in acid-fuchsin lactic-acid (5) at each harvest date. Nematode fresh weights were calculated (13), dry weights obtained by assuming 25% of the total nematode weight (20), and nematode dry weights were subtracted from the root dry weights at each harvest time. Nematode stress dosage, defined as the number of juveniles and females multiplied by the total energy consumption (in calories) by each developmental stage, was calculated (13). The total energy demand was 0.00228 calories for a second-stage juvenile, 0.00934 calories for a third–fourth-stage juvenile, and up to 1.186 calories for a female depending on age. The total plant energy consumed by the nematodes was subtracted from the total energy assimilated by the plants at the end of the study.

Physiological parameters were simultaneously measured on a 10–15-cm^2 area of single leaves using a LiCor 6000 portable IR CO_2 gas analyzer. Two or three measurements per plant (on different leaves) were taken during a time period starting 2 hours after dawn and ending 5 hours before dusk. During measurement, growth chamber doors were open to increase ventilation and each plant was placed in the same position. Opening growth chamber doors during measurements resulted in a 30.1% decrease in the rate of photosynthesis compared with having the doors closed. To reflect ontogenetic changes between older and younger leaves, the physiological measurements were made on primary leaves (present before the plants were rooted) at 0, 11, 16, and 23 days, and on primary and secondary leaves (that grew after rooting) at 30, 44, 52, and 59 days after nematode inoculation.

In order to estimate the partitioning of energy within the plant, it is necessary to determine the total CO_2 that enters the plant system. As there was no apparent difference in the rates of photosynthesis between treatments or time, we assumed a uniform daily photosynthetic rate. The photosynthetic rate for the days on which measurements were not made was the mean of the rates for measured days on either side. After adjusting the daily photosynthetic rates to the level of closed doors, total CO_2 fixed by each plant over the duration of the experiment was calculated as follows:

$$DPP = R \times cf \times LA$$

where DPP = daily plant photosynthesis in mg CO_2, R = rate in mg CO_2 dm^{-2} hr^{-1}, cf = daily photoperiod (12 hours) and LA = leaf area. Daily rates were summed to give the total CO_2 fixed over the duration of the experiment.

Total respiration (TR) was calculated using equations derived from other studies (6,7,19) and incorporated into the grape plant growth model (4) installed on the University of California's Integrated Pest Management Computer System. The appropriate formulae are

$$TR\ (g\ CO_2) = (MR + GR) \times 0.682$$

where MR = maintenance respiration in grams glucose per gram dry matter (DM)/hr, GR = growth respiration in grams glucose per gram DM and 0.682 is a conversion factor to CO_2.

$$MR = B_1 \times 1.7^{(T_2 - T_1)/10} \times DM$$

where B_1 is the base for leaves (0.001757),

stems (0.00064), or roots (0.00064), 1.7 is a constant, and T_2 and T_1 are the experimental temperature and the temperature at which standard measurements are taken, respectively.

$$GR = B_2 \times DM$$

where B_2 is the base for leaves (0.24), stems (0.24), or roots (0.51).

An energy budget (kcal) for each treatment was calculated as:

$$TCF = A_1 + NC + UN$$
$$A_2 = A_1 + NC$$

where TCF = total CO_2 fixed, A_1 = energy assimilated into plant tissue (P) and respiration (R), NC = energy consumed by nematodes, UN = unaccounted for (photorespired or cost of transporting the photosynthate to the nematode or both), and A_2 = total assimilation. TCF was converted into dry weight equivalent using 1.88 g CO_2 to 1.0 gram plant dry weight (17). Dry weight equivalents and A_1 were converted into calories using 4.7 kcal per gram dry weight (9).

Energy conversion efficiencies were calculated as

$$GPE = P/TCF \times 100,$$
$$NPE = P/A_1 \times 100,$$
$$TAE = A_2/TCF \times 100$$

where GPE = gross production efficiency, NPE = net production efficiency, and TAE = total assimilation efficiency. Total energy balance and assimilation efficiencies were compared by treatments.

The mean rates of transpiration, stomatal conductance, internal CO_2 concentration, and change in specific leaf area over the duration of the experiment were compared for each treatment.

Data of all the parameters were subjected to regression analysis against nematode stress dosage.

Results

The number of nematodes in the roots increased with nematode inoculum to a maximum of about 250 mg dry weight at the end of the study (Fig. 1A). Sampling error in determining the number of nematodes in the roots was about 16%. Nematode stress dosage ranged from about 0.8 kcal at the lowest to 3.4 kcal at the highest nematode inoculum level (Fig. 1B).

Increasing nematode stress dosage resulted in a decline of total (15%) and secondary (32%) leaf area ($P = 0.05$) but had no effect on the primary leaves (Fig. 2A). Leaf, stem, shoot, root excluding nematodes, and total plant dry weight decreased significantly ($P = 0.05$) with nematode infection (Fig. 2B), whereas root growth including nematodes was not affected.

Photosynthetic rate was not significantly affected by nematode infection, however, the amount of CO_2 fixed by the total and secondary leaves decreased by 12 and 55%, respectively, with increasing nematode stress dosage (Fig. 2C). Calculated maintenance respiration was not affected but growth and total respiration ($P = 0.05$) decreased with increasing nematode stress dosage (Fig. 2C). The CO_2 dry weight equivalent in the leaves, stems, shoots, roots, and plant total followed the same trend as that of the plant dry weight.

Calculation of energy partitioning showed a significant ($P = 0.05$) decrease in energy assimilated into plant tissue (A_1, bottom line) but not when energy consumed by nematodes (A_2, middle line) was included (Fig. 3A). Treatments did not differ in the amount of energy unaccounted for, the difference between the top and middle lines (Fig. 3A). Proportional to plant weight ($[TCF - A_2]/A_2$), however, energy unaccounted for increased with increasing stress dosage. Nematode stress dosage, represented by the difference between the bottom (A_1) and middle (A_2) lines of Figure 3A accounted for up to 15% (Fig. 3B) of the energy assimilated into plant tissue plus energy consumed by nematodes (A_2 in Fig. 3A).

Gross production and total assimilation (nematode growth included) efficiencies decreased by 14.3 and 2.7%, respectively, whereas net production efficiency was not affected by nematode infection (Fig. 3C).

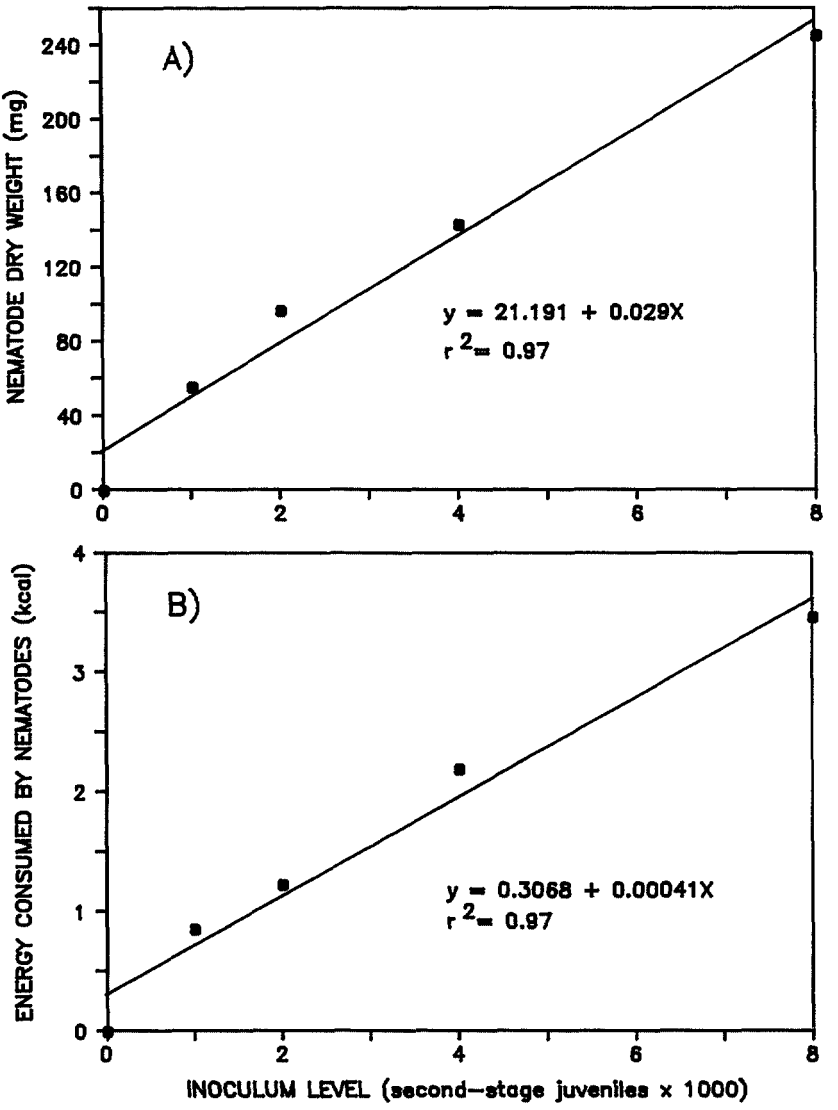

FIG. 1. Relationship of *Meloidogyne incognita* inoculum level with A) total dry weight of juveniles and female recovered from roots and B) nematode stress dosage (kcal) at 59 days after nematode inoculation.

The slopes of transpiration rate, stomatal conductance, internal CO_2 concentration, and specific leaf area did not significantly differ with nematode treatment over the duration of the experiment.

DISCUSSION

This study establishes the influence of root-knot nematode parasitism on the total energy entering the grape plant system and the partitioning of the energy. Moreover, calculating nematode dry weight allows us to separate nematode dry weight from root weight and assess the significance of nematode biomass. Calculated respiration of the uninfected whole plants accounted for about 20% of the total CO_2 fixed. This is within the range of other woody plants (21); however, total plant respiration calculated with nematodes excluded, was decreased by nematode infection, whereas total respiration including nematode weights was not affected. The nematode stress dosage level achieved in this study decreased total plant photosynthesis but did not affect the rate of photosynthesis per unit of leaf area.

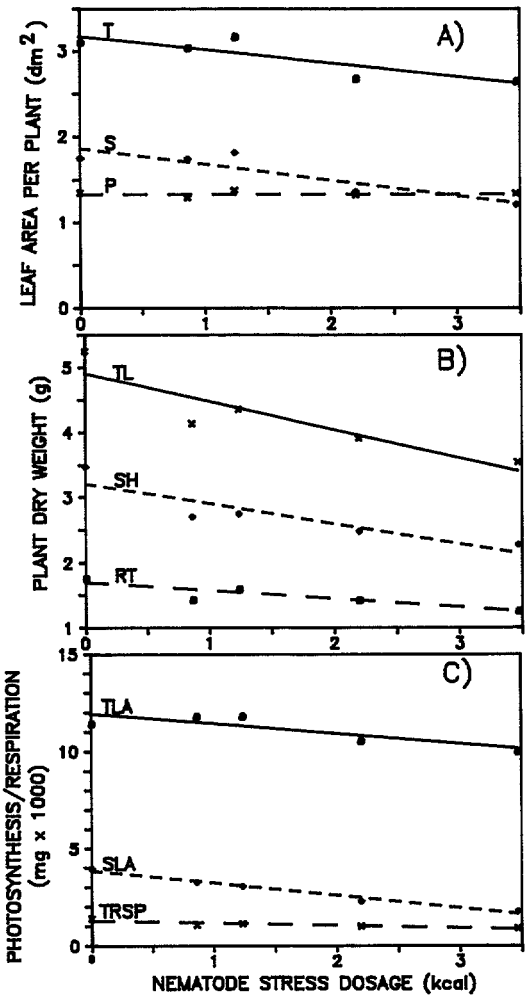

FIG. 2. Effect of *Meloidogyne incognita* stress dosage on A) expansion of primary (P), secondary (S), and total (T) leaf area (dm^2) of French Colombard grapes, over 59 days. P = 1.336 + 0.00006X, r^2 = 0.01; S = 1.866 − 0.186X, r^2 = 0.81*; T = 3.174 − 0.159X, r^2 = 0.73. B) Shoot (SH), root (RT) excluding nematodes and total (TL) plant dry weight (g) of French Colombard grapes over 59 days. SH = 3.212 − 0.306X, r^2 = 0.81*; RT = 1.699 − 0.128X, r^2 = 0.78*; TL = 4.912 − 0.435X, r^2 = 0.82*. Root plus nematode growth (not shown here) was not affected, but leaf and stem were significantly (P = 0.05) affected by increasing nematode stress dosage. C) Total plant photosynthesis (mg CO_2) on secondary (SLA) and total (TLA) leaf area basis and total calculated respiration (TRSP) (mg CO_2) of French Colombard grapes over 59 days. TLA = 11943.008 − 516.147X, r^2 = 0.72; SLA = 3851.048 − 641.601X, r^2 = 0.98**; TRSP = 3211.595 − 314.18, r^2 = 0.80*. Slopes significantly different from zero at P = 0.05 (*) and P = 0.01 (**).

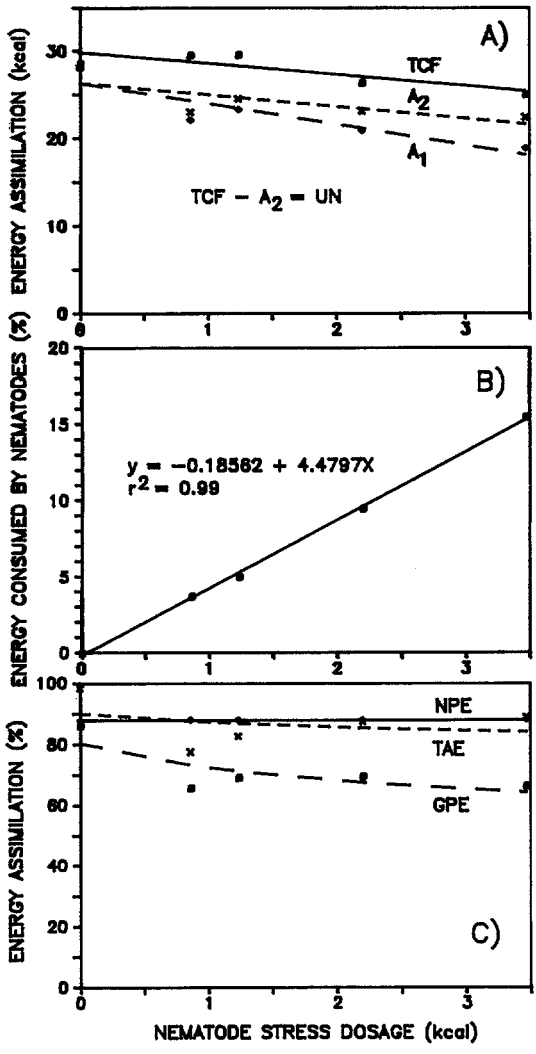

FIG. 3. Effect of *Meloidogyne incognita* stress dosage on A) calorific equivalents (kcal) of total CO_2 fixed (TCF), total energy assimilated into plant tissue plus nematodes (A_2), energy assimilated into plant tissue (A_1) excluding that consumed by nematodes. The difference between TCF and A_2 represents the energy unaccounted for (UN) which, proportional to plant weight ([TCF − A_2]/A_2), increased with stress dosage. TFC = 29.8575 − 1.2904X, r^2 = 0.72; A_2 = 26.2967 − 1.3566X, r^2 = 0.60; A_1 = 26.2967 − 2.3566X, r^2 = 0.82*. B) Energy consumed by nematodes (A_2 − A_1) as a percentage of A_2 of French Colombard grapes over 59 days. C) Gross (GPE) and net (NPE) production efficiency and total (TAE) assimilation efficiency of French Colombard grapes over 59 days. GPE = 80.42 − 0.152 (log X + 1), r^2 = 0.61; NPE = 87.79 + 0.1293X, r^2 = 0.73; TAE = 90.05 − 0.046 (log X + 1), r^2 = 0.09. *Slopes significantly different from zero at P = 0.05.

The decrease in total plant photosynthesis was associated with decreased expansion of the secondary leaves. The rates of photosynthesis of these leaves are low until they are fully expanded (8). In annual crops such as beans, leaf area expansion was significantly slowed after being subjected to similar nematode inoculum in a time period shorter than in this experiment (15). This indicates that the nematode might be affecting the same physiological processes, but at different rates. The reduced rate of leaf area increase, however, seems to be due to the slowed development of the secondary leaves, leading to a significant decline in total leaf area (12). The low rate of expansion of secondary and total leaf area, significant decrease in the overall plant dry weight, and lack of effect on root growth (including nematodes) and primary leaf area with increasing nematode stress dosage, suggest that the nematode is altering the growth pattern of the host. Anwar (1) reported a decrease in root : shoot ratio of *M. incognita*-infected French Colombard plants over longer periods of infection and suggested a greater effect on root than shoot growth. In this study, shoot and root weights, excluding nematode weights, were equally affected, whereas when nematode weights were included the effect of the nematode is greater on the shoot than on the root. Thus, both the present study and that of Anwar (1) suggest that the nematode might be altering the partitioning of the photosynthate.

In plants infected with *Meloidogyne* spp., a significant amount of photosynthate is diverted to the feeding site (11) and the nematode that otherwise would have been partitioned for plant growth. Such diversion of energy results in lower gross production efficiency. However, net production efficiency, a measure of partitioning of assimilated energy, did not change because respiration was proportional to dry matter accumulation. Although there is a 16% sampling error associated with our estimate of the number of nematodes used for calculating the energy consumption, our calculations show that nematodes consumed up to 15% of the total energy assimilated (A_2). The treatments did not differ in total assimilation, including nematode consumption, indicating that energy consumption of *M. incognita* is an important component of the host–nematode interaction and probably accounts for most of the decrease in plant growth.

Poskuta et al. (18) reported that *Heterodera glycines* does not affect the in vivo kinetics of ribulose biphosphate carboxylase-oxygenase and suggested that the effect of the nematode is more on partitioning of photosynthate and root physiology than on the photosynthetic process and apparatus. Studies on transpiration rate of nematode-infected plants showed no clear trend (16). At this stage of our study, we see no evidence that the nematode affects transpiration rate, stomatal conductance, internal CO_2 concentration, and specific leaf area, which are key indicators of the CO_2 uptake and fixation process and the physical status of the photosynthetic apparatus. Given higher nematode stress and longer infection period, however, it is possible that there may be an effect on these parameters. The relationship between nematode densities and these parameters provides a mechanistic understanding of the physiological processes that are affected by the nematode. Our ability to estimate the energy consumed by the nematode allows us to separate the demand aspect from the impact on host physiological processes and furthers our understanding of host–nematode interaction. Accordingly, the data indicate that the energy consumed by *M. incognita* accounts for a significant portion of the total energy assimilated (A_2) by the plant and is a major damage component. This alters the partitioning of the photosynthate in such a way that leaf area expansion is slowed which, in turn, affects the total energy entering the system. Moreover, *M. incognita* infection and (or) energy demand increase the energy unaccounted for. This is presumed to include leakage, photorespiration, or repairing the cost of

nematode damage, etc., and decreases the physiological efficiency of nematode-infected grapes. Further studies are needed to address associated damage components and to extend our understanding of the host–nematode system.

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