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Physiological Response of Resistant and Susceptible *Vitis vinifera* Cultivars to *Meloidogyne incognita*

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Abstract: The effect of *Meloidogyne incognita* on growth, general physiological response, and the concentration of reducing and nonreducing sugars at the nematode feeding sites of French Colombard (susceptible) and Thompson Seedless (moderately resistant) *Vitis vinifera* cultivars was studied up to 2,100 degree-days (DD-base 10 C). Nematode stress dosage, measured as the product of cumulative number of juveniles and females and their total energy (calories) demand, accounted for up to 15 and 10% of the energy assimilated by French Colombard and Thompson Seedless plants, respectively. Total leaf area, total carbon dioxide fixed, transpiration rate, stomatal conductance, and internal leaf CO_2 concentration were not affected, but energy assimilated into plant tissue and respiration were decreased by nematode infection in both cultivars. Energy consumed by nematodes accounted for most of the difference in total energy assimilated between infected and uninfected plants on French Colombard but not on Thompson Seedless, suggesting that the resistant cultivar may be using more energy to curtail the nematode's activity. Nematodes did not affect the concentration of reducing sugars, but the concentration of nonreducing sugars increased in French Colombard and decreased in Thompson Seedless. This indicates that there was more translocation of photosynthate to the feeding sites of the susceptible than to those of the resistant cultivar, and may explain why *M. incognita* causes more damage to French Colombard than to Thompson Seedless.

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

Our ability to understand plant–nematode interaction depends on investigation of the changes that take place at the feeding sites and the host's physiological processes that are affected. Energy demand for *Meloidogyne incognita* (Kofoid & White) Chitwood growth and reproduction in grape (*Vitis vinifera* L.) results in decreased productivity of nematode-infected grape vines (7,8). Fewer *M. incognita* became established and produced fewer eggs in roots of a moderately resistant than in a susceptible cultivar, but the adult females grew to comparable sizes (8). This has led to speculation that resistant and susceptible cultivars may partition their energy reserves differently either to defend themselves against nematode infection and reproduction or to repair damage caused by nematode infection. One way a host may respond to nematode infection is by changing the concentration of reducing and nonreducing carbohydrates. This may cause the host to synthesize chemicals which may require intermediates of carbohydrate catabolism as precursors (1). Such mobilization of energy reserves may result in less biosynthesis of storage carbohydrates and energy production, placing further stress on the host's ability to maintain energy homeostasis through carbohydrate synthesis alone (1).

The objectives of this study were 1) to compare the growth and physiological response of susceptible and moderately resistant *V. vinifera* cultivars to *M. incognita* infection and 2) to determine how the concentration of reducing and nonreducing sugars correspond with other changes in host physiology.

Materials and Methods

Two sets of experiments were conducted on French Colombard (susceptible) and Thompson Seedless (moderately resistant) cultivars that were rooted from two-bud dormant cuttings in a mist chamber (7).

In the first experiment, 4-week-old cuttings were inoculated with 0 (control), 3,000, 6,000, 12,000, or 24,000 *M. incognita* second-stage juveniles (J2) pipetted into the soil (7). Treatments were replicated

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four times and plants were randomly placed in a growth chamber at 30 C for a 12-hour light (400 μE m^{-2} s^{-1}; 700–400 nm) and at 25 C for a 12-hour dark period for over 2,100 degree-days (DD base 10 C). Plants were watered to saturation twice a week with normal strength Hoagland solution (3), otherwise daily with de-ionized water.

Leaf area, photosynthetic rate, stomatal conductance, internal leaf CO_2 concentration, and transpiration rate (physiological parameters) measurements were taken at approximately biweekly intervals. Procedural details for plant growth and for physiological measurements were as described by Melakeberhan and Ferris (7), with the exception that there were no primary leaves in the present study. The dry weight of 20 plants (four replications times five treatments) were measured at monthly intervals after inoculation.

The rates of leaf area and plant dry weight increase and other physiological parameters were determined for each treatment over 2,100 DD and treatment totals were compared (7). The formulae for the calculations of calorific equivalents of total CO_2 fixed, respiration, energy partitioning within the plant, and energy budget balance sheets were as described in Melakeberhan and Ferris (7).

At each harvest date, nematode population and developmental stages were determined from 1-g samples of randomly selected roots (5) and total energy demand (nematode stress dosage) was calculated (7, 8). Total energy consumed by the nematodes was subtracted from the total energy assimilated by the plants at the end of the study. 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.

In the second experiment, the effect of 0, 10,000 or 20,000 *M. incognita* J2 on reducing and nonreducing sugars of similar age plants and experimental conditions as in the previous experiment was studied for 1,260 DD. Twenty plants (four replications times five treatments) were harvested at approximately biweekly intervals. Nematode populations were estimated from 1-g root samples and processed as in the first experiment. The concentrations of reducing and nonreducing sugars were measured from randomly selected 100-mg samples from galled (nematode-infected) and ungalled (control) roots. The 100-mg samples were cut into ca. 5-mm pieces, freeze dried in liquid nitrogen, lyophilized over night, and ground to powder using a pestle and mortar. Subsamples of 10-mg each were boiled for 2 hours in test tubes containing 10 ml distilled water and centrifuged at 500 g for 15 minutes (1). Supernatant from each sample was collected, divided into two equal volumes, and analyzed for the concentration of reducing sugars by the Anthrone colorimetric method and for nonreducing sugars by the Somogyi microcopper method (1,4). Sugar concentrations were determined from glucose (reducing) and sucrose (nonreducing) standard curves. The change in sugar concentration for each treatment over the duration of the experiment was calculated separately and treatments were compared.

Data for all the parameters were subjected to regression analysis against nematode stress dosage (7, 8).

Results

In the first experiment, there was no increase in nematode stress dosage above the inoculum level of 4,000 *M. incognita* J2 per plant in French Colombard, whereas the increase was linear ($r^2 = 0.95$) in Thompson Seedless (Fig. 1). Moreover, it took four times the level of inoculum on French Colombard to produce the same level of nematode stress dosage on Thompson Seedless (Fig. 1).

Total leaf area (not shown here) and the energy equivalent of total CO_2 fixed per plant (Fig. 2) were not significantly affected by nematode treatment on either cultivar; however, Thompson Seedless grew ca. 15% larger than French Colombard at the same age.

The total energy equivalents assimilated into plant tissue, plant tissue and respira-

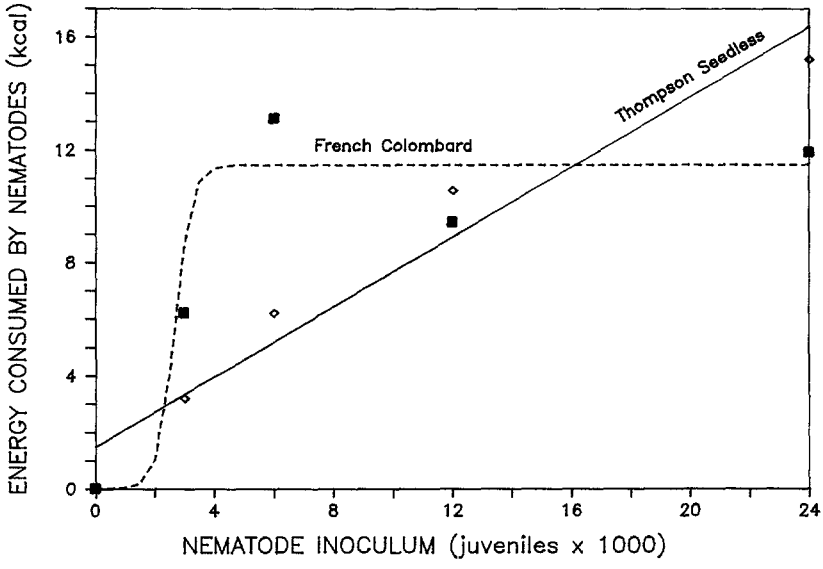

FIG. 1. Response of *Meloidogyne incognita* reproduction and energy (kcal) demand of same age Thompson Seedless and French Colombard grapes over 2,100 DD. The product, nematode stress dosage, was used for expressing the relationship with the different parameters. Thompson Seedless: $y = 1.4933 + 0.00062X$, $r^2 = 0.95^{**}$. French Colombard: $y = A/(1 + Ce^{BX})$. A = 11.4801, C = 10,000.00, B = −0.00345 and residual mean square = 3.51.

tion, including energy consumed by nematodes, declined ($P = 0.05$) by 31, 34.3, and 27.3%, respectively, compared with the controls in Thompson Seedless (Fig. 3). The equivalent figures for French Colombard were 32.9, 33.5, and 21.6%, but the total energy assimilated, including that consumed by nematodes, was not significantly affected (Fig. 3). The proportion of energy unaccounted for increased with nematode

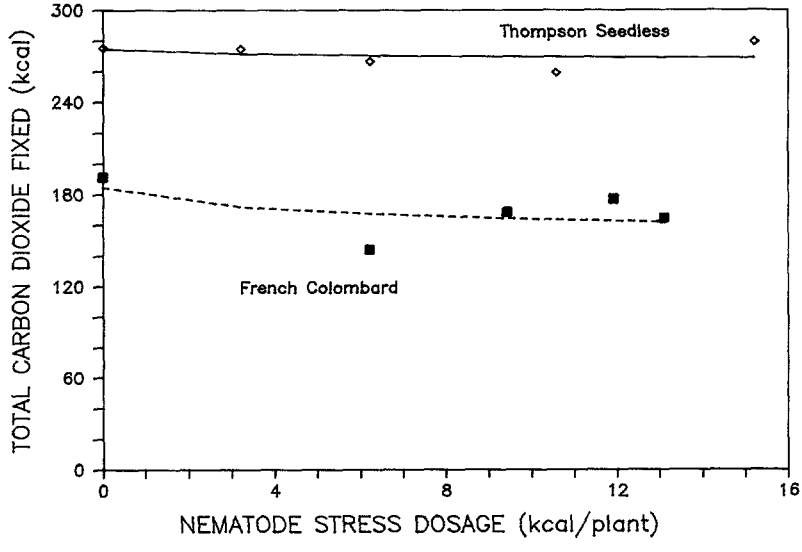

Fig. 2. Energy equivalent (kcal) of total carbon dioxide fixed by *Meloidogyne incognita*-infected same age Thompson Seedless and French Colombard grapes over 2,100 DD. Thompson Seedless: $y = 274.7058 - 0.10715 \log(X + 1)$, $r^2 = 0.01$. French Colombard: $y = 184.7124 - 1.1546 \log(X + 1)$, $r^2 = 0.12$.

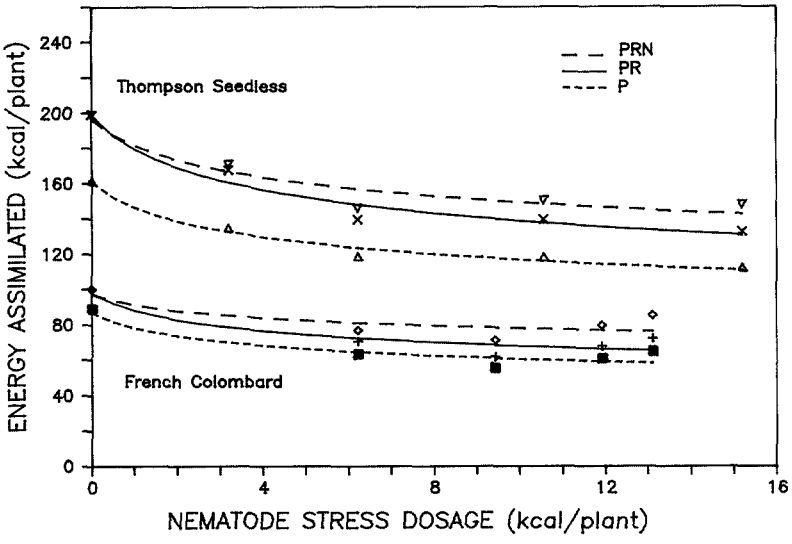

Fig. 3. Effect of *Meloidogyne incognita* on energy (kcal) incorporated into plant tissue (P), P and respiration (R) (PR) and including that consumed by nematodes (N) (PRN) of same age Thompson Seedless (upper three lines) and French Colombard (lower three lines) grapes over 2,100 DD. Thompson Seedless: P = 161.2894 − 0.13315 log(X + 1), r^2 = 0.97**; PR = 199.8766 − 0.15065 log(X + 1), r^2 = 0.95**; PRN = 197.1766 − 0.11467 log(X + 1), r^2 = 0.90*. French Colombard: P = 87.2956 − 0.15061 log(X + 1); r^2 = 0.83*; PR = 98.5141 − 0.15401 log(X + 1), r^2 = 0.84*; PRN = 97.8172 − 0.09213 log(X + 1), r^2 = 0.60.

stress dosage in both cultivars, but more so in French Colombard than in Thompson Seedless (Fig. 4).

There was no significant change in transpiration rate, stomatal conductance, internal leaf CO_2 concentration, and specific leaf area with nematode treatment over the duration of the experiment (data not shown here).

In the second experiment, the nematode

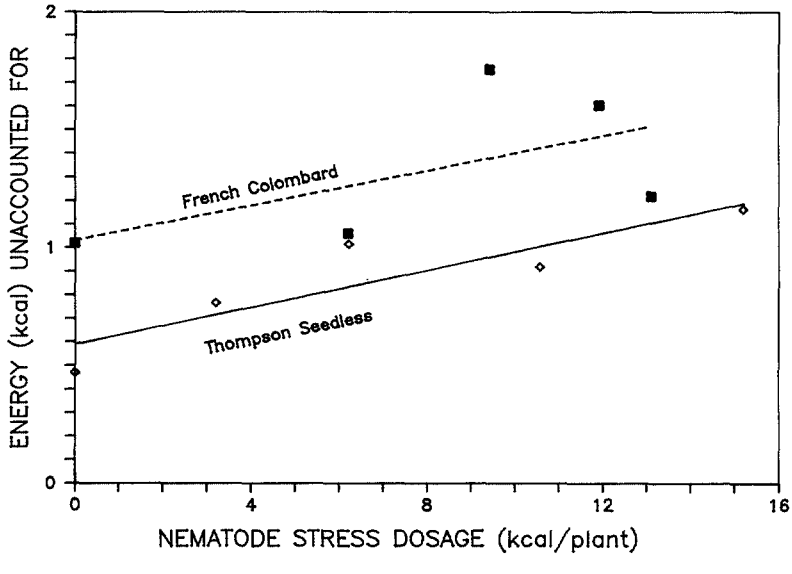

Fig. 4. Effect of *Meloidogyne incognita* stress dosage on the total energy unaccounted for proportional to plant weight of same age Thompson Seedless and French Colombard grapes over 2,100 DD. French Colombard: y = 1.0324 + 0.0369X, r^2 = 0.34. Thompson Seedless: y = 0.5904 + 0.03941X, r^2 = 0.79*.

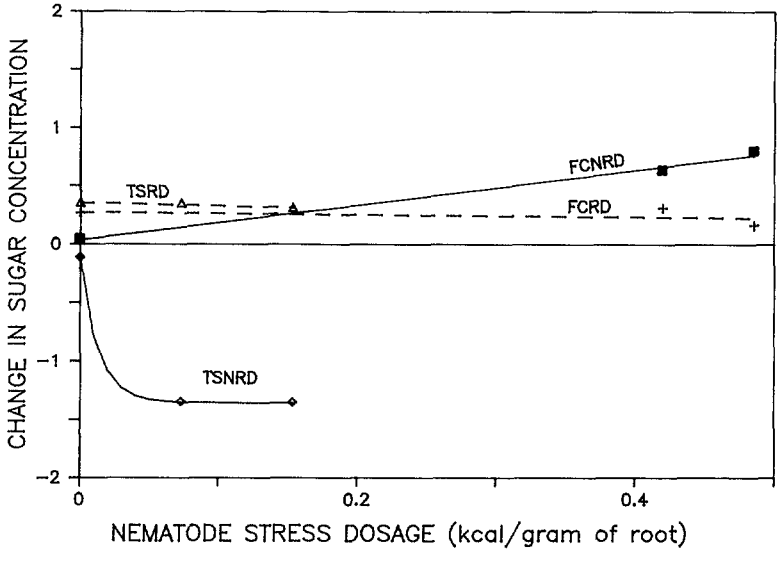

Fig. 5. Changes in the concentration (μg/DD) of total reducing (RD) and nonreducing (NRD) sugars of *Meloidogyne incognita*–infected galls of same age French Colombard (FC) and Thompson Seedless grapes (TS) over 1,260 DD. Data points represent slopes of changes of each treatment up to the end of the experiment. French Colombard: FCRD = 0.27706 − 0.09295X, r^2 = 0.11; FCNRD = 0.04178 + 1.509X, r^2 = 0.99. Thompson Seedless: TSRD = 0.36254 − 0.28312X, r^2 = 0.87; TSNRD = a + be^{cx}. a = −1.35, b = 1.2388, c = −76.22 and residual mean square = 0.0204.

stress dosage per gram of galled root tissue in Thompson Seedless was about one-third of that in French Colombard, but there was little change in the concentration of reducing sugars in either cultivar (Fig. 5). The concentration of nonreducing sugars increased in French Colombard, whereas it decreased in Thompson Seedless with increasing nematode stress dosage (Fig. 5).

Discussion

Our earlier study on French Colombard, conducted over a shorter period of time, indicated that the energy demand for growth and reproduction of *M. incognita* accounted for about 15% of the total energy entering the plant system (7). As a result, less energy was partitioned into leaf area expansion which, in turn, affected the energy entering the system and further decreased biomass production in grapes. In the present study, comparing slightly older French Colombard and Thompson Seedless plants and 2–3 times higher nematode stress dosage than in the previous work, we found that the nematode energy demand accounted for up to 15% and 10% of the total energy assimilated by the two cultivars, respectively. Although the energy consumed by nematodes was slightly higher in Thompson Seedless than in French Colombard at the end of the study (Fig. 1), Thompson Seedless grew larger than French Colombard and suffered less from nematode stress. Moreover, the difference in the trend of nematode stress dosage between the two cultivars indicates that the nematode reproduction is faster in French Colombard than in Thompson Seedless until a threshold was reached (2). Nematode reproduction may also be limited by root size in French Colombard.

The plant growth and physiological response parameters had a similar trend in both cultivars, but with higher magnitude on French Colombard than on Thompson Seedless. Leaf area per plant and the energy equivalent of total CO_2 fixed were not affected by nematode treatment in the two cultivars. This was possibly because plants were large enough at the time of inoculation to be able to sustain this degree of

nematode stress without suffering significant decrease in leaf area and (or) total photosynthesis over the duration of the experiment. However, the decrease in total energy assimilated into plant tissue and respiration with increasing infection suggests an increase in energy demand from the nematode and inefficient biosynthetic processes. Moreover, the higher increase in the proportion of energy unaccounted for (comprising leakage, photorespiration, and other processes), with higher nematode stress on French Colombard than on Thompson Seedless, indicates additional effect on host physiological processes other than nematode energy demand. The decrease in total energy assimilated, including energy consumed by nematodes, in Thompson Seedless but not in French Colombard suggests that nematode energy demand accounts for most of the difference in dry weight between infected and uninfected plants in French Colombard but not in Thompson Seedless. Although Thompson Seedless may suffer less physiological damage by investing more energy for defense against the nematode invasion and reproduction and (or) repair of damage than French Colombard, we speculate that the moderate level of resistance of Thompson Seedless may have a high energy price.

In our earlier report (7) we hypothesized that greater nematode stress and longer infection periods may be necessary to show an effect on transpiration rate, stomatal conductance, and internal leaf CO_2 concentration, but we found no effect on these parameters in this study. Because the effect of a root-parasitic nematode on these host physiological processes is usually indirect, the nematode may not have affected the processes that influence the physiological parameters measured.

More than 90% of the material translocated in the phloem consists of carbohydrates, with nonreducing sugars accounting for 10–25% (10,11). Studies on the effect of *Meloidogyne* infection on the concentrations of reducing and nonreducing sugars vary with the type of host–parasite interaction. For example, Nasr et al. (9) found a decrease in both forms of sugars in *M. javanica*-susceptible almond and peach plants, whereas that was not the case in resistant peaches. Our results support the notion that the host may be responding to the nematode infection by changing the concentration of these sugars (1). The cultivars did not differ in their response of changes in the concentration of reducing sugars, indicating little effect on storage carbohydrates. Although the mechanisms are unknown, the decrease in the concentration of nonreducing sugars in the moderately resistant cultivar suggests less photosynthate translocation to the nematode's feeding site and may explain why the nematode produces fewer eggs than in the susceptible cultivar (8). However, the increase in the concentration of nonreducing sugars with duration of infection and level of inoculum in the susceptible cultivar suggests a higher rate of photosynthate translocation to the feeding site (6) and may explain the greater physiological inefficiency. Moreover, as the nematode's activity increases, the effect on energy partitioning and associated physiological processes will diminish productivity.

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