The Effect of Six Different Soil Temperatures on Infection and Development of Nacobbus Batatiformis in Sugar Beets[1]

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Nacobbus batatiformis Thorne and Schuster is a serious nematode pest of sugar beets in western Nebraska (5,6)[3]. Knowledge of the basic problems on the effect of soil temperature and moisture on infection and development of this nematode has not been published in detail for the Nebraska root-galling nematode (4). This paper pertains to experimental work on some of these problems; and the authors believe that some of the results of this research may be of practical economic importance to commercial sugar beet growers.

Materials and Methods

The influence of soil temperature upon the nematode-induced disease was studied in the greenhouse with the aid of temperature tanks heated by electrical heating coils that were thermostatically controlled to provide various soil temperatures ranging from 15° to 35°C at 5-degree intervals. To obtain temperatures below 15°C, constant temperature, refrigerated, rapid set thermo-regulator water baths which provide closely controlled temperatures −10° to 65°C were used in the laboratory test. The greenhouse experiments were repeated 3 times and the laboratory test was conducted once. Each treatment was replicated 4 times in the greenhouse and 3 times in the laboratory experiments.

During the winter, most of the experiments were conducted in a greenhouse with a fairly constant air temperature of about 22°C; the air temperature in the laboratory was about 24°C. The effect of air temperature on soil temperature was minimized by keeping the culture cans covered until emergence of seedlings.

A soil mixture of 6 parts sand, 2 parts field soil, and 1 part compost was steam sterilized for 3 hours at 20 lb pressure to decrease variation due to other soil organisms; 9 kg of the sterilized soil mixture was added to each can; in the laboratory test, 900 grams of soil were added to each 600 ml pyrex beaker.

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[3] Numbers in parentheses refer to literature cited.

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An attempt was made to maintain the moisture content at 50 percent of the moisture-holding capacity of the soil by watering once or twice daily since our experimental evidence indicated that this moisture content favored infection and maintained plant growth to good advantage, and that 35 and 80 percent moisture levels at 25°C had little differential effect on pathogenesis of *N. batatiformis*. A soil temperature of 25° C was selected because this temperature proved optimum for sugar beet growth based on plant height in the noninoculated series of preliminary and subsequent experiments. To insure the least possible change of soil temperature during periodic waterings, water of the same temperature as the soil was added to the respective culture cans.

Although 10 sugar beet seeds of varieties Great Western 304R or Great Western monogerm were sown per culture can or beaker, respectively, the final number of plants ranged from 5 to 10 per replication. The plants in three or four of the six or eight containers were used as controls, and those in the remaining three or four containers were infested with infected sugar beet roots. Prior to seeding of the cans, inoculum was incorporated in the soil; this inoculum consisted of 10 grams per container of *N. batatiformis*-infected lateral roots chopped to facilitate dispersion in the soil. Prior to inoculation, examination of the inoculum revealed the presence of egg sacs in the galled roots.

The sugar beet plants were carefully dug 36 days after inoculation. The roots were then washed by submerging in a pan of water and then fixed in 5 percent formalin preparatory to staining. To facilitate examination of nematode development, the roots were dipped in hot lactophenol-acid fuchsin for one minute and cleared in lactophenol. The stages of the nematode and the degree of galling were recorded after this staining procedure.

Observational ratings were employed in classifying the symptoms. Each plant could be conveniently classified into one of four categories with respect to severity or absence of infection.

Infection categories described are as follows: 0 = no sign of infection, as evidenced by the absence of any galls on the main or secondary roots; 1 = slight infection, as evidenced by the presence of small and few galls on the secondary roots; 2 = moderate infection, as evidenced by the presence of larger and more galls on the secondary roots and occasional galls on the main root; 3 = abundant infection, as evidenced by numerous large galls on the main and secondary roots with coalescing of galls, a common occurence. The method was satisfactory and practical in the comparative determination of the amount of root galling in the experiments.

A disease index method appeared satisfactory in comparative determination of the amount of disease in the soil temperature experiments. It is as follows:

$$\text{Disease index} = \frac{(\Sigma \text{ Category numbers} \times \text{no. of plants}) \times 100}{\text{Total no. of plants} \times 3}$$

The 3 in the denominator represents maximum infection, and 100 is used to convert to percentage. The summarization of category numbers is obtained by multiplying the number of plants in each category by their respective category numbers from 0 to 3, and adding the products. The result is the comparative infection rating for the different treatments.

Results and Discussion

The information on the effect of soil temperatures on the incidence of the sugar beet root-galling disease caused by *N. batatiformis* is presented in Table 1. At the lower temperatures, 10° and 15°C, little or no infection was evident. The disease severity increased with increase in temperature with little difference at 25°, 30° or 35°C. These temperatures may be most favorable for expression of symptoms. Based on top growth measurements, 25°C appeared to be optimum; the average plant height 36 days after planting was 18, 24, 26, 22, and 19 cm at 15, 20, 25, 30, and 35°C, respectively. The height was measured from the crown to the top of the oldest leaf. Root growth measurements would be more meaningful from the standpoint of root infections, but they were not recorded. Godfrey (1) found that temperature affected top and root growth differently; top growth of tomatoes increases with increases in temperature up to 35°C, but root growth was much reduced at this temperature compared with the peak growth reached at 25° and 30°C.

Table 1.—Effects of six soil temperatures on the incidence of disease caused by *Nacobbus batatiformis* in sugar beet roots in the greenhouse (Experiments 1, 2, 3) and laboratory (Experiment 4), Lincoln, Nebraska.

Soil temperature °C	Disease index					
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Avg. for expts.	Avg. for controls
10	[a]	....	....	0	0	0
15	17	0	0	0	4	0
20	48	3	15	73	35	0
25	55	5	27	79	42	0
30	43	28	54	. .	45	0
35	38	27	65	. .	43	0

[a] No test made.

The optimal temperature for sugar beet root growth may be different from that of tomatoes as reported by Godfrey (1).

For four highest soil temperatures studied, the average plant height in the *N. batatiformis*-inoculated series was 10 percent less than that of the controls 36 days after inoculation; at 15°C the top growth for the inoculated and control sugar beets was the same. Apparently stunting due to nematode action was present for the four temperatures, 20°, 25°, 30° and 35°C. Under certain field conditions, *N. batatiformis* induces severe stunting and actual killing of sugar beet seedlings and young plants. In fact, under field conditions roots of surviving but severely infected sugar beets would commonly average about 1 inch or less in diameter at harvest time.

Table 2 includes the data on the effects of six soil temperatures on infection, development and reproduction of *N. batatiformis* in sugar beet roots. Infection was absent at 10°C; at the 15°C temperature larvae and females were present in the roots 36 days after inoculation, but galls were not developed to any extent (Table 1). In Experiment 1, the number of females at 15°C was greater where some root galling was present than Experiments 2 and 3 in which no galling was evident. Female development and gall development were associated to some degree. This was brought out at the higher temperatures for there is a positive correlation between rate of female development and disease index. A similar correlation seems to exist for reproduction rate and disease index. Although the number of females and egg-laying female nematodes was not high, there was an apparent relationship. In none of the control plants was any galling or nematodes evident in roots.

Based on the data on soil temperature effects on the root-galling disease, earlier planting of sugar beets should lessen the incidence of the malady because a definite relationship exists

Table 2.—Effects of six soil temperatures on development and reproduction of *Nacobbus batatiformis* in sugar beets in one laboratory and three greenhouse experiments, Lincoln, Nebraska.

Soil temperature °C	Avg. number per root		
	Females	Egg-laying females	Larvae
10	0.0	0.0	0.1
15	0.1	0.0	0.2
20	0.3	0.1	0.7
25	1.2	0.5	1.3
30	1.1	0.8	1.3
35	1.8	0.6	0.8

between soil temperature and the disease index. At the lower soil temperature the disease index is absent or low, but the disease severity increases with an increase in temperature up to 35°C, the maximum tried. Also, female development and reproductive capacity of *N. batatiformis* is more favorable above 10°C. The lower temperature may decrease the rate of nematode movement and thereby lessen infection rate and penetration. By permitting the sugar beet seedling to escape infection during its critical period of development, earlier planting may result in a good stand and yield of beets despite later infections of older sugar beet plants. Tyler (7) showed that at 16.5°C, the minimum time required for the life cycle of the root-knot nematode (*Meloidogyne sp.*) from larva to larva was 87 days, decreasing to 25 days at 27°C. A comparable situation exists with respect to temperature effects on the life cycle of *N. batatiformis*. Miss Tyler (7) also found that development from gall formation to egg-laying required 15 days at 27°C but 79 days at 14.3°C. A similar relationship appears to exist with *N. batatiformis,* however, the experiments were not continued beyond 36 days. Therefore, a more complete understanding on gall formation and egg-laying could not be determined at the lower temperatures. Tyler (7), Oteifa (2), and others reported that temperature is not the only factor influencing root-knot nematodes, but that differential rates of development could be inherent in the host plants or depend on the nutritional status of the host plant. Godfrey (1) found that temperature operated both directly on the nematode and indirectly through its effects on plant growth. We have observed in a plot cropped to sugar beets for the past 53 years, that fertilization in one-half the plot for the past 23 years had decreased the severity of *N. batatiformis*. Thus nutrition, as was shown by the effect of potassium on infection by *Meloidogyne incognita* (2), may affect the infection and development of *N. batatiformis*. *N. batatiformis* affects the physiology of the sugar beet by inducing starch formation and other effects (3). This carbohydrate might play an important role in the development and reproduction of this nematode. Nutreints and other factors may in turn affect the ability of the Nebraska root-galling nematode to induce starch formation, and thus affect its pathology.

Summary

The influence of six soil temperatures ranging from 10° to 35°C at 5-degree intervals was studied on infection and development of *Nacobbus batatiformis* in sugar beet roots. Few or no root galls were evident at 10° and 15°C. The disease index

increased with an increase in temperature with little difference between 25°, 30° and 35°C. Based on top growth measurements, 25°C appeared optimum for sugar beet growth. For four temperatures (20° - 35°C), the top growth in the noninoculated controls was 10 percent greater than the inoculated series, indicating that the nematode stunts the sugar beet plants. At 15°C, the top growth was the same for inoculated and controls. With an increase in temperature above 15°C, there was an increase in development and reproductive capacity of *N. batatiformis*. At the four higher temperatures, the disease index apparently was correlated with the rate of nematode development and reproductive capacity.

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