Electrical Stimulation Applied to Second-Stage Larvae of Heterodera rostochiensis to Determine Viabilty

IRVING GRANEK[1]

Viability of cysts of the golden nematode, *Heterodera rostochiensis* Wollenweber, recovered from treated fields on Long Island, New York, is based on appearance and movement of second-stage larvae. If internal structures of one or more larvae appear to be normal in appearance, observations are made at random intervals for 1-2 days to detect movement. This is time-consuming and questionable as a viability test because golden nematode larvae are relatively immobile. In an earlier unpublished investigation, I found that electrical stimulation of viable golden nematode larvae caused movement and stylet projection.

The objective of this test was to determine if electrical stimulation could be used to determine viability of second-stage larvae of *H. rostochiensis*. Soil containing cysts was collected and stored for about 3 weeks in the laboratory. Cysts were then extracted from the soil and kept in water from 1-3 h before extracting the larvae. Three groups of larvae were tested as follows: 100 larvae whose viability was determined by visible movement (Group A); 100 nonviable larvae—50 killed by hot water treatment and 50 killed chemically by fumigation [25 by 1,3-dichloropropene, 1,2-dichloropropane mixture (D-D®) and 25 by Vorlex® (D-D + methyl isothiocyanate)] (Group B); and 100 larvae of questionable viability whose internal structures were intact but in which no detectable movement was observed (Group C).

Electrical equipment consisted of a variable transformer, which had a range of 0-140 volts with a maximum of 10 amps, and a fixed pair of stainless steel electrodes embedded in plastic, the tips of which were exposed and ground down to tapered needle points. The distance between the points was 1 mm. Each larva was placed in a drop of tap water on a glass slide and positioned on the stage of a stereomicroscope. The electrodes straddled the larva. Voltage was increased, from a 5-volt starting point, by increments of 5 up to 70. The electrodes were kept in place for a period of 5 sec at each interval.

Group A showed stylet projection with 82% doing so at 15 and 20 volts (Table 1). Stylets were not projected in Group B up to 70 volts. Stylets of 77% of the larvae in Group C were projected, but only 35% were projected at 15 and 20 volts. The stylet remained projected in most instances; some were retracted after a short time; others were retracted almost immediately.

This method seems to provide a quick and accurate means of determining viability of golden nematode larvae and should be especially useful in regulatory work. The technique may also be helpful in taxonomic studies because accurate measurements of the stylet can be made when the tip is projected outside of the head. In addition, ac-

TABLE 1. Projection of stylets after electrostimulation of second-stage larvae *Heterodera rostochiensis*.

Potential V (ac)	Viability groups[a] A	B	C
5	0	0	0
10	7	0	0
15	45	0	10
20	37	0	25
25	8	0	16
30	2	0	12
35	0	0	3
40	1	0	5
45	NA	0	3
50	NA	0	3
55+	NA	0	0
Totals[b]	100/0	0/100	77/23

[a]Group A—all mobile, Group B—all killed by heat or by chemicals, and Group C—all structurally intact but of undetermined viability (all groups had 100 larvae each).

[b]Total number of larvae showing projecting stylets/viability group.

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[1] Nematologist, Animal and Plant Health Inspection Service, U. S. Department of Agriculture, Golden Nematode Laboratory, Hicksville, New York. The author thanks Mr. John L. McDonough, Plant Protection Inspector, Animal and Plant Health Inspection Service, U. S. Department of Agriculture, for fabricating the electrodes and for his valuable technical assistance.

curate measurements can be made between the base of the stylet knobs and the dorsal esophageal gland orifice since projection of the stylet straightens the procorpus which is often coiled.

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