Journal of Nematology 25(2):286–290. 1993.

Effects of Inoculum Density and Egg Age on Establishment of *Globodera rostochiensis* Populations[1]

B. B. Brodie and M. L. Brucato[2]

Abstract: The establishment of *Globodera rostochiensis* Ro1 populations was examined under greenhouse conditions. The probability of *G. rostochiensis* population establishment was calculated from the number of plants that produced new cysts with viable eggs following inoculation with various numbers of eggs of different ages. Probability of population establishment was positively correlated with inoculum density but was not affected by the age of eggs used in these experiments. The probability of *G. rostochiensis* establishment ranged from 5% at densities of 2 eggs/pot to 100% at densities of 25 eggs/pot or greater. At densities of 3 eggs/pot and beyond, there was no correlation between inoculum density and the number of viable eggs/new cyst. Also, the number of plants that produced new cysts was a function of inoculum density and not age of eggs. Juveniles from eggs 1 year old or older were equally as infective as were those from eggs in newly developed cysts (4 months old).

Key words: Globodera rostochiensis, golden nematode, nematode spread, population establishment, potato, *Solanum tuberosum.*

Globodera rostochiensis Ro1 (golden nematode) continues to be a serious threat to the potato industry of the United States some 40 years after its discovery in this country (2). Control of this quarantined pest in the United States is aimed at preventing the establishment of new infestations by managing the nematode at population densities low enough to eliminate its spread. The dynamics of extremely low population densities of the golden nematode have received little attention and are poorly understood (4,5).

Several factors influence the reproduction and subsequent establishment of golden nematode populations. The most important of these is initial inoculum level or initial population density (6). Reproduction rate of the golden nematode is known to be low at low population densities, increasing to a maximum, and then slowly decreasing as the density further increases (5). It has been suggested that at very low densities, difficulties in mate finding may reduce the chances of golden nematode population establishment (5). However, sex attractants, promiscuity, and the grouping of eggs in cysts tend to overcome some of the difficulties in mate finding and favor establishment of new populations (3,4).

Infectivity of *G. rostochiensis* juveniles is reportedly associated with amount of their lipid reserves at the time of hatching (8). Lipid reserves in juveniles decline with prolonged storage (9,11), and later hatched juveniles exhibit impaired infectivity and delayed development (8). Low lipid reserves are related to the age of cysts and cause reduced juvenile mobility, which may influence the subsequent infectivity of the juveniles (9).

The cysts are the dispersal units of the golden nematode. Successful dispersal not only depends on a cyst being close enough to a host root for hatch stimulation, but also on a cyst containing enough infective progeny to initiate a new population. This study examined the relation of egg density and age of eggs to establishment of golden nematode populations. A preliminary report has been given (1).

MATERIALS AND METHODS

In the first experiment, cysts of *Globodera rostochiensis* Ro1 were extracted from naturally infested soil. These cysts were individually crushed and categorized according to the numbers of viable eggs that they contained. Cyst categories were established as follows: Category I = 2–9 viable eggs/cyst, Category II = 10–49, Category III =

Received for publication 12 June 1992.

[1] Cooperative investigation of the U.S. Department of Agriculture, Agricultural Research Service, and Cornell University Agricultural Experiment Station.

[2] Research Plant Pathologist and Research Support Specialist, USDA ARS, and Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

50–99, and Category IV = 100 or more viable eggs/cyst. Potato plants (12 cm tall) growing singly in 7.6-cm clay pots filled with a 1:1 soil:sand mixture were individually inoculated with the eggs from cysts of each cyst category. Each plant was inoculated by placing the viable eggs from a single cyst into a small depression 2.5 cm deep in the soil of each pot. Each inoculum density was replicated 20 times. The same number of plants was similarly inoculated with a comparable number of viable eggs from 4-month-old cysts produced under controlled conditions and termed vintage cysts (7). The plants were grown in a greenhouse at 23–25 C until they senesced (ca. 12 weeks). Then the plant tops were excised and the soil in the pots was air dried for 3 weeks. Afterwards, new cysts were extracted by flotation and individually crushed to reveal their contents. The number of plants with cysts containing viable eggs (viable cysts), number of viable cysts per plant, number of cysts with viable eggs, and the number of viable eggs per cyst were recorded for each pot. The above experiment was repeated using the same procedures. The data from these experiments were analyzed using Fisher's PLSD test. Additionally, a basic logistic regression analysis was performed with the data. Significant differences detected by these two methods of analysis were identical. The results from Fisher's PLSD test are given.

In the third experiment, inoculum consisted only of eggs from 4-month-old vintage cysts. The inoculum was applied at sequential densities of 2 to 15 viable eggs/pot to potato plants (12 cm tall) growing in 7.6-cm clay pots. Each inoculum density was replicated 20 times. The plants were grown in the greenhouse at 23–25 C until they senesced. After senescence, the plant tops were removed and the soil in each pot was air dried for 3 weeks. New cysts were extracted from the soil and individually crushed to reveal their contents. The number of plants with viable cysts, number of viable cysts per plant, and number of viable eggs per cyst were recorded from each pot. These measurements were used to calculate the probability of golden nematode population establishment at different inoculum densities.

RESULTS

The probability of golden nematode population establishment was positively correlated with inoculum density but was not influenced by age of eggs (Fig. 1). There were no differences in the percentage of plants that produced cysts with viable eggs (viable cysts) following inoculations with eggs from newly formed (vintage) cysts or with eggs from older (field) cysts. Results of the first two experiments were essentially the same, and only data from the first experiment are given. Inoculations with eggs from Category I (2–9 viable eggs/cyst) field cysts averaged 40% probability of population establishment as calculated from the average number of plants out of 20 that produced viable cysts. From the same measurements, inoculations with a like number of eggs from vintage cysts averaged 30% probability of population establishment. Inoculations with eggs from Category II (10–49 viable eggs/cyst) field cysts averaged 70% probability of population establishment, and the

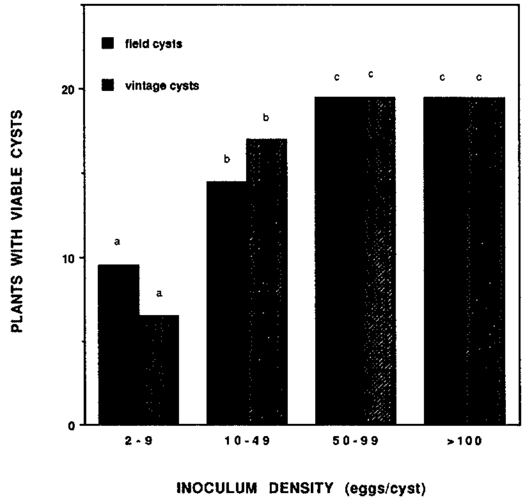

FIG. 1. The relation of inoculum density and age of *Globodera rostochiensis* to the numbers of plants out of 20 bearing cysts with viable contents. Bars with the same letter are not significantly different ($P = 0.05$).

same number of eggs from vintage cysts averaged 80% probability of establishment. Inoculations with eggs from field cysts of Categories III (50–99 viable eggs/cyst) and IV (>100 eggs/cyst) and inoculations with a comparable number of eggs from vintage cysts resulted in up to 100% probability of golden nematode population establishment.

In the first two experiments, based on analysis of 20 individual replicates inoculated with eggs from Categories I and II field cysts or a like number of eggs from vintage cysts, inoculum densities of 3 eggs/pot or fewer resulted in no plants with viable *G. rostochiensis* cysts. Percentage of plants with viable cysts increased sharply at 4 eggs/pot and varied between 25% and 60% up to 12 eggs/pot. At 13 eggs/pot, percentage of plants with viable cysts was 88%, and at 14–24 eggs/pot, the percentage of plants with viable cysts varied between 75% and 100%. At 25 eggs/pot or greater, the percentage of plants with viable cysts was 100%.

In the third experiment, eggs from only 4-month-old vintage cysts composed the inoculum, and the highest density used was 15 eggs/pot. Percentage of plants with viable cysts varied somewhat, but population establishment was positively correlated with inoculum density (Fig. 2). At an inoculum density of 2 eggs/pot, only 5% of the plants had cysts containing viable eggs. Percentage of plants with cysts that contained viable eggs increased with increasing inoculum density to a high of 85% at 13 eggs/pot, but was only 55% at an inoculum density of 15 eggs/pot.

The number of viable eggs per cyst in the new cyst generation varied widely and was not related to inoculum density or age of eggs. Numbers of viable eggs per cyst were very low following inoculation with eggs from vintage cysts at 2 eggs/pot but increased dramatically to 72 eggs/cyst at an inoculum density of 3 eggs/pot (Table 1). At inoculum densities greater than 3 eggs/pot, the number of viable eggs per new cyst varied considerably, reaching an average of 149 eggs/cyst at an inoculum density of 15 eggs/pot. Similar, but more variable, results were obtained following inoculation with eggs from field cysts.

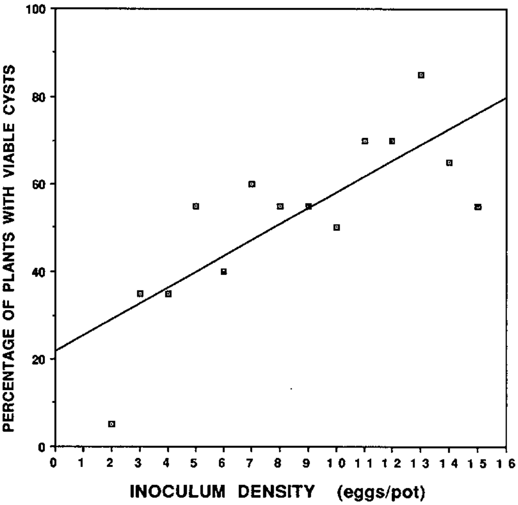

FIG. 2. The relationship between inoculum density of *Globodera rostochiensis* from vintage cysts and the percentage of plants bearing cysts with viable contents. The values represent the means of 20 replications, and the regression model based on the means is $Y = 21.582 + 3.6374X$, $R^2 = 0.61$.

Inoculum density was positively correlated with the number of new cysts that contained viable eggs (Fig. 3). At an inoculum density of 2 eggs/pot, only 50% of the cysts in the new generation contained viable eggs, whereas an average of 92% of

TABLE 1. Influence of inoculum density of *Globodera rostochiensis* on the number of viable eggs/cyst in the new generation.

Inoculum density (eggs/pot)	Average no. viable eggs/cyst†
2	3
3	72
4	128
5	78
6	80
7	60
8	48
9	47
10	57
11	78
12	77
13	67
14	74
15	149

† Based on analysis of 20 replications.

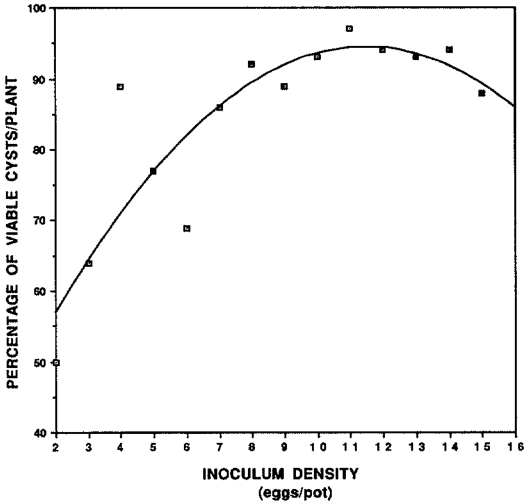

FIG. 3. The relationship between inoculum density of *Globodera rostochiensis* and the percentage of new cysts with viable contents. The values represent the means of 20 replications, and the regression model based on the means is $Y = 39.265 + 9.6095X - 0.41827X^2$, $R^2 = 0.77$.

the cysts in the new generation contained viable eggs at inoculum densities of 8 to 15 eggs/pot.

DISCUSSION

The amount of lipid reserves in juveniles of *Globodera rostochiensis* at the time of hatching has been implicated in their subsequent infectivity (9,11). Furthermore, lipid reserves decline during storage, and juveniles in older cysts tend to have less lipid reserve (11). The exact age of juveniles was unknown for the field cysts used in our experiments. However, previous studies in our laboratory demonstrated that the number of viable eggs per cyst was related to cyst age and that older cysts contained fewer viable eggs (6). For the purposes of the present study, we presumed that cysts containing only 2–9 viable eggs were at least 5 years old because we had previously shown experimentally that most cysts of *G. rostochiensis* contained 2–9 viable eggs after exposure to five cycles of a potato cultivar resistant to the golden nematode (6). It was, therefore, surprising in our present study that juveniles from cysts containing only 2–9 viable eggs were equally as infective as were juveniles from new cysts. Low lipid reserves of juveniles hatched from older cysts are reported to be associated with decreased juvenile mobility, which could influence host finding and subsequent infection (11). However, the conditions of our experiments favored nematode infection as we reduced energy loss in host finding by placing eggs in the close vicinity of roots, which could account for the high infectivity of juveniles from older cysts.

An earlier report indicated that the number of viable eggs per cyst in a new generation of *G. rostochiensis* is negatively correlated with egg density when the initial density is at least 30 eggs/g of soil (10). In our experiments, initial egg density was not related to the number of viable eggs in the new cyst generation. The highest inoculum density used in our experiments was 0.07 egg/g of soil, which is far too low to meet the criteria set forth in earlier studies (10). Therefore, the differences in our results and those reported earlier (10) can be attributed to differences in initial egg densities. However, our results do support the suggestion that because potato cyst nematodes have their eggs in packets (cysts) in the field, the local density is rarely so small that mating fails (4). Also, the fact that inoculum density in our tests was unrelated to the number of viable eggs in new cysts supports an earlier finding that fecundity of *G. rostochiensis* females is not dependent on multiple matings with different males (3).

Our study clearly indicates that when the average number of viable golden nematode eggs in a cyst is fewer than 50, the probability is significantly reduced that this cyst will result in the establishment of a new population. However, we did not identify what density between 15 and 50 eggs/cyst assures 100% probability of establishing a new population. Because of the many factors that influence population establishment of *G. rostochiensis*, the number of eggs/cyst that ensures successful population establishment undoubtedly differs under different circumstances. However, it was clear that a cyst of *G. rostochiensis* with as few as 2 viable eggs can, albeit with an extremely low probability, establish a new infestation.

LITERATURE CITED

1. Brodie, B. B., and M. L. Brucato. 1989. Relation of cyst age and egg density to establishment of *Globodera rostochiensis* populations. Journal of Nematology 21:552–553 (Abstr.).
2. Brodie, B. B., and W. F. Mai. 1989. Control of the golden nematode in the United States. Annual Review of Phytopathology 27:443–461.
3. Green, C. D., D. N. Greet, and F. G. W. Jones. 1970. The influence of multiple mating on the reproduction and genetics of *Heterodera rostochiensis* and *H. schachtii.* Nematologica 16:309—326.
4. Jones, F. G. W. 1965. The population dynamics and population genetics of the potato cyst-nematode *Heterodera rostochiensis* Woll. on susceptible and resistant potatoes. Pp. 301–316 *in* Report of the Rothamsted Experiment Station for 1965.
5. Kort, J. 1962. Effect of population density on cyst production in *Heterodera rostochiensis.* Nematologica 7:305–308.
6. LaMondia, J. A., and B. B. Brodie. 1986. Effects of initial nematode density on population dynamics of *Globodera rostochiensis* on resistant and susceptible potatoes. Journal of Nematology 18:159–165.
7. LaMondia, J. A., D. Rawsthorne, and B. B. Brodie. 1986. Methods of reducing experimental variation in *Globodera rostochiensis.* Journal of Nematology 18:415–418.
8. Robinson, M. P., H. J. Atkinson, and R. N. Perry. 1985. The effects of delayed emergency on infectivity of juveniles of the potato cyst nematode. Nematologica 31:171–178.
9. Robinson, M. P., H. J. Atkinson, and R. N. Perry. 1987. The influence of soil moisture and storage time on the mobility, infectivity, and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida.* Revue de Nématologie 10:343–348.
10. Seinhorst, J. W. 1984. Relation between population density of potato cyst nematodes and measured degrees of susceptibility (resistance) of resistant potato cultivars and between this density and cyst content in the new generation. Nematologica 30:66–76.
11. Storey, R. M. J. 1984. The relationship between neutral lipid reserves and infectivity for hatched and dormant juveniles of *Globodera* spp. Annals of Applied Biology 104:511–520.