Probability of *Globodera rostochiensis* Spread on Equipment and Potato Tubers[1]

B. B. Brodie[2]

Abstract: The probability of spreading cysts of *Globodera rostochiensis* on farming equipment and potato tubers was investigated in naturally infested field plots. The number of cysts recovered from soil that adhered to equipment differed significantly between different pieces of equipment. These differences were related to initial nematode density and, in most cases, to the volume of soil that adhered to the equipment. At an initial density of 0.04 egg/cm^3 of soil, significantly more cysts were recovered from a potato digger than from a potato hiller, cultivator, or plow. At an initial density of 0.90 egg/cm^3 of soil, significantly more cysts were recovered from the plow than from the other equipment. Although the population density was 22 times greater, only 10 times more cysts adhered to equipment used in soil with a density of 0.90 egg/cm^3 of soil than when used in soil infested at 0.04 egg/cm^3. The number of potato tuber samples (4.5 kg) that contained cysts with viable eggs was positively correlated with the initial densities of *G. rostochiensis* in soil in which they were produced. The percentage of tuber samples with cysts containing viable eggs was 10–12% for tubers harvested from soil with densities less than 1 egg/cm^3 and 30–76% for tubers harvested from soil with densities greater than 4 eggs/cm^3 of soil.

Key words: dispersal, distribution, golden nematode, potato cyst nematode, spread.

Although it is extremely limited in distribution, the golden nematode, *Globodera rostochiensis,* continues to be a threat to the potato industry of the United States (6). This threat is perpetuated by the relative ease with which *G. rostochiensis* can spread and establish new infestations, as illustrated by its spread throughout the world from its center of origin in the South American Andes (7,8,10). The objective of the program to control *G. rostochiensis* in the United States is to manage the nematode at densities low enough to eliminate the risk of its spread to noninfested areas.

Several factors contribute to successful survival and subsequent spread of *G. rostochiensis.* Chief among these factors are the ability of encysted eggs to withstand desiccation for long periods of time and the requirement for a host-mediated stimulant to induce hatch of encysted eggs (2,11). The ability of *G. rostochiensis* to go undetected at low population densities for 4–6 years contributes further to its successful spread (11). Any means by which soil can be moved is considered a potential means of spreading *G. rostochiensis* (9). Local spread (within and between fields) is attributed primarily to farming activities (5). Long-distance spread (within and between continents) is usually achieved by movement of used machinery, potato seed tubers, root crops, or their packaging materials (11,12).

Early in the golden nematode control program in the United States, the decision was made to keep nematode population densities low enough to prevent nematode spread (5). Consequently, *G. rostochiensis* population densities were kept extremely low by a control program based primarily on soil fumigation (5). Even under conditions of extremely low population densities and strict regulatory measures, however, some local spread occurred (2,5,7). It has been suggested that most of this spread was from infestations that reached population density levels from which spread occurred before they were detected by a soil survey (7). Previous studies have indicated that spread of *G. rostochiensis* is possible at extremely low population densities and that the probability of spread is influenced by soil moisture (1,10).

The dynamics of extremely low population densities of *G. rostochiensis* have received little attention and are poorly un-

Received for publication 15 June 1992.
[1] Cooperative investigations of the U.S. Department of Agriculture, Agricultural Research Service, and Cornell University Agricultural Experiment Station.
[2] Research Plant Pathologist, USDA ARS, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

derstood. Recent studies suggest that the probability of *G. rostochiensis* establishment is positively correlated with numbers of viable eggs in the soil but is unrelated to the age of the eggs (4). The objective of this study was to determine the probability of golden nematode spread from subdetectable population densities (not detectable by the current regulatory soil survey) on different farming equipment and on potato tubers.

Materials and Methods

Spread on equipment: The equipment used in these experiments included a two-row potato hiller, a two-row cultivator, a two-bottom (40 cm) moldboard plow, and a two-row potato digger. These pieces of equipment were used in the normal sequence of farming operations and were operated in six plots (10 m × 15 m) each at two different population densities of *Globodera rostochiensis*. The two density levels of *G. rostochiensis* studied, 0.04 and 0.90 egg/cm^3 of soil, are both below the level of detection by methods used in the golden nematode survey (5). The golden nematode survey employs a sampling procedure with an area per sample point of 1.13 m^2, whereas the area per sample point in this study was 0.37 m^2. The soil moisture during these experiments ranged from 10–15% by weight. Each piece of equipment was used once in six different plots infested at each of the two population densities. The soil in these plots was 9.1% sand, 46.9% silt, and 44.0% clay. Before use, each piece of equipment was cleaned free of soil with a high-pressure water system (Hotsy®) and allowed to dry. After use in a *G. rostochiensis*-infested plot, the equipment was placed on a sheet of polyethylene sufficiently large to accommodate the particular piece of equipment currently under study. The equipment was then cleaned free of soil with brushes and spatulas, and the resultant soil was collected on the polyethylene sheet. The volume of soil from each piece of equipment was measured and processed using a USDA cyst extractor (modified Fenwick can) (10,12). The number of cysts in each sample was recorded, and the number of viable eggs that they contained was determined by crushing individual cysts.

Spread on tubers: To determine the potential of potato tubers as a means of *G. rostochiensis* spread, tubers were harvested annually for 18 years from plots (10 m × 15 m) with known nematode population densities. Tuber samples were taken from the same plots each year, but the *G. rostochiensis* population densities in the plots differed each year because of the different cropping sequence imposed on them. The soil in these plots was the same as in previous experiments: 9.1% sand, 46.9% silt, and 44.0% clay. Soil moisture varied with year but was in the normal range for potato harvesting (12–20% by weight). The tubers were harvested mechanically, collected in metal baskets, and then dumped into burlap bags. From the burlap bags, the tubers were poured onto a potato grader and were re-collected in unused burlap bags. These processes removed most of the free soil adhering to the tubers. Afterwards, a 4.5-kg sample of tubers was selected at random from the tubers harvested from each plot. The tubers in these samples were then individually washed free of soil. The resultant wash water from each 4.5-kg sample of tubers was passed through a 841-μ sieve nested over a 250-μ sieve. Cysts that were collected on the 250-μ sieve were transferred to a filter sock and extracted with acetone as previously described (3). The number of cysts per sample was recorded and the cysts were crushed to determine the number of viable eggs they contained. The data on the recovery of cysts from tubers were grouped according to seven preselected soil population density ranges of *G. rostochiensis*. These density ranges were 0.01–0.04; 0.05–0.20; 0.21–0.99; 1–4; 4–15; 15–50; and >50 eggs/cm^3 of soil. A regression analysis was performed independently of years to determine the correlation of cyst recovery from potato tubers with *G. rostochiensis* population density.

Results

Spread on equipment: The volume of soil recovered from equipment differed significantly ($P = 0.05$) with different types of equipment (Table 1). Less soil was recovered from the hiller and cultivator than was recovered from the plow and digger. The volume of soil recovered from the hiller and cultivator in the two tests did not differ, nor did the volume of soil recovered from the plow and digger.

Significant differences in the number of cysts recovered from different pieces of equipment were related to both the amount of soil adhering to the equipment and, in most cases, to nematode population density (Table 1). At a density of 0.04 egg/cm^3 of soil, fewer cysts were recovered from the hiller, cultivator, and plow than were recovered from the digger. The fewest number of cysts was recovered from the hiller and the greatest number of cysts was recovered from the digger. The hiller and digger also contained the least and most amount of adhering soil, respectively.

Similar results were obtained when the equipment was used in plots infested with *G. rostochiensis* at a density of 0.90 egg/cm^3 of soil (Table 1). Again, fewer cysts were recovered from the hiller, which contained the least amount of adhering soil. Although considerably more cysts were recovered from the cultivator and digger in the second test than in the first test, their numbers did not differ from the number of cysts recovered from the hiller. At a density of 0.90 egg/cm^3 of soil, significantly more cysts were recovered from the plow than from the hiller. The plow also contained the largest volume of adhering soil in this experiment.

The number of viable eggs in cysts recovered from adhering soil differed with different types of equipment and was usually, but not always, related to the number of cysts recovered from the different types of equipment (Table 1). Cysts recovered from the plow and digger contained more viable eggs than did those recovered from the hiller and cultivator. The average number of eggs/cyst differed more for cysts recovered from equipment used in plots with a density of 0.04 egg/cm^3 than in plots with a density of 0.90 egg/cm^3 of soil.

Significantly ($P = 0.01$) more cysts and viable eggs were recovered from soil that adhered to equipment used in plots infested with *G. rostochiensis* at 0.90 egg/cm^3 of soil than from plots infested at 0.04 egg/cm^3 of soil. At an average density of 0.04 egg/cm^3 of soil, an average of 11 cysts containing 1,380 viable eggs adhered to the equipment, whereas an average of 112 cysts containing 5,419 viable eggs adhered to equipment used in plots at an average density of 0.90 egg/cm^3 of soil. The magnitude of these differences was not as great as the magnitude of differences in population densities that existed in the soil. The high population density of 0.90 egg/cm^3 of soil was 22 times greater than the low population density of 0.04 egg/cm^3 of soil. However, the numbers of cysts and eggs recovered from equipment used in plots infested at the high density were only 10

Table 1. Volume of soil and number of cysts and viable eggs recovered from different types of equipment used in soil infested with *Globodera rostochiensis* at average densities of 0.04 and 0.90 egg/cm^3 of soil.

Type of equipment	cm^3 of soil		No. of cysts		No. of eggs	
	0.04	0.90	0.04	0.90	0.04	0.90
Hiller	18.6 a†	19.7 a	0.3 a	2.0 a	3.3 a	68.3 a
Cultivator	248.3 a	330.5 a	9.8 a	72.0 ab	323.3 a	3,951.6 ab
Plow	1,154.5 b	1,451.5 b	7.0 a	237.0 b	285.0 a	7,531.6 b
Digger	1,855.3 b	988.3 b	27.0 b	136.6 ab	4,910.0 b	8,863.0 b

Means of six replications.
† Means in the same column followed by the same letters are not significantly different ($P = 0.05$) according to Fisher's PLSD test.

and 4 times greater, respectively, than the numbers of cysts and eggs recovered from equipment used in plots infested at the low density.

Spread on tubers: The experiment to measure the relation of *G. rostochiensis* population densities to adherence of cysts to potato tubers was repeated yearly for 18 years. Because these tests were done in plots with different cropping sequences, population densities varied each year. Consequently, the data were combined and grouped into seven preselected population densities that existed at harvest of each year. Accordingly, the number of tuber samples from plants grown at a particular nematode population density varied each year. The number of tuber samples assayed ranged from a low of 50 samples at population densities greater than 50 eggs/cm^3 of soil to a high of 208 samples at densities of 1.0–4.0 eggs/cm^3 of soil.

The number of tuber samples containing cysts with viable eggs increased with increase in nematode population density and was not influenced by the number of samples examined (Table 2). The percentage of cysts with viable eggs recovered from tubers was highly correlated with population density of *G. rostochiensis* at the time of harvest (Fig. 1). At a density of 0.01–0.04 egg/cm^3 of soil, 10% of the tuber samples contained at least one *G. rostochiensis* cyst with viable eggs. The percentage of tuber samples that contained cysts with viable eggs increased to 76% at population densities greater than 50 eggs/cm^3 of soil.

TABLE 2. Number of tuber samples examined over 18 years and number of samples with viable cysts at several densities of *Globodera rostochiensis*.

Nematode density (eggs/cm^3 of soil)	No. of tuber samples examined†	No. samples w/ viable cysts
0.01–0.04	112	12
0.05–0.20	140	14
0.21–0.99	188	24
1.00–4.00	208	35
4.01–15.00	172	53
15.01–50.00	134	49
50>	50	38

† Each sample consisted of 4.5 kg of tubers.

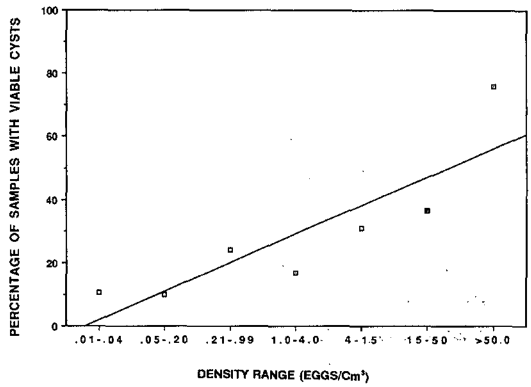

FIG. 1. The percentage of tuber samples with cysts that contained viable eggs in relation to *Globodera rostochiensis* population density at the time of tuber harvest. The data points represent the means of 112, 140, 188, 208, 172, 134, and 50 samples examined from lowest to the highest population densities, respectively. The regression model based on the means is $Y = 7.2571 + 9.1357X$, $R^2 = 0.75$.

DISCUSSION

These studies represent the first effort to quantify the potential for *G. rostochiensis* spread from different population densities. Other reports that address spread of *G. rostochiensis* deal with detectable population densities (7,8,11,12), and none of them attempt to quantify the potential for *G. rostochiensis* spread.

Three important events are necessary for successful spread of *G. rostochiensis:* acquisition of cysts with viable eggs, deposition of these cysts in the vicinity of a host plant, and successful population establishment. Population establishment is a weak link in the chain of events leading to successful spread of *G. rostochiensis*. Not only is population establishment influenced by inoculum density (4), it is severely limited by certain cropping sequences (5) and is essentially prevented by growing *G. rostochiensis*-resistant cultivars (2,7). Only the probability of acquiring cysts was considered in these experiments. Acquisition of cysts represents only a potential of spreading *G. rostochiensis* and does not address successful deposition of cysts or population establishment.

The equipment used in this study was operated in the infested plots in the normal sequence of farming operations, i.e., the plow in early spring to fit the land, the hiller in the early growing season, the cultivator in mid-season, and the digger during harvest. The number of viable eggs per cyst in the soil changes throughout this period, being reduced after plant emergence and increasing when new cysts are formed. The cyst density on the soil remains the same during the growing season and increases at harvest. The differences in cyst recovery from the plow and digger when used in plots at the low population density could be a result of difference in cyst density in the soil at the time of use. However, differences in cyst recovery from the plow and digger were not evident at the high population density, indicating the importance of amount of adhering soil in the spread of *G. rostochiensis*. Earlier studies in this laboratory indicated that spread of *G. rostochiensis* cysts on potato farming equipment was positively associated with the amount of soil adhering to equipment, which, in turn, is related to the type of equipment used (10). The current study confirmed these previous results and identified the relation between different types of equipment and the amount of soil they removed from a field. Contrary to previous studies (10), viable eggs per volume of adhering soil were concentrated by the equipment to levels higher than the egg density of the soil in which the equipment was used.

The potential for spread of *G. rostochiensis* was relatively less on potato tubers than on equipment. Although cysts adhered to tubers that were harvested from plots with low nematode population densities, the percentage of tuber samples that contained cysts was relatively low (10–12%) at subdetectable (<1 egg/cm^3 of soil) population densities. Because adherence of cysts to potato tubers potentially permits all three events of successful *G. rostochiensis* spread, seed potato production is banned in the United States in known *G. rostochiensis*-infested areas (5). This study supports the rationale for this ban.

In these studies, a potato digger represented the greatest potential risk for spreading *G. rostochiensis* cysts. The risk was greater not only because of the large amount of soil that adheres to the digger but also because population densities are highest at harvest time following production of a susceptible cultivar. Additionally, the new cysts present at harvest contain a large number of viable eggs that provide a greater chance of successful population establishment (4). Because the mold-board plow was used in the spring when population densities are lowest, the risk of spread of *G. rostochiensis* with the plow was associated primarily with the large volume of adhering soil. These experiments indicate clearly that spread of *G. rostochiensis* cysts on farming equipment and on potato tubers is possible from nematode population densities that are undetectable by the soil sampling techniques currently used to survey for the golden nematode (12).

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