A Bucket Microplot Technique for Studying Phytonematodes

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ABSTRACT

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Inexpensive microplots were constructed from modified 19-L plastic buckets. Bucket microplots permitted experiments to be established with plants at field-planting density. Weekly whole-plant sampling was achieved with minimal disturbance to the adjacent plots. The entire soil profile in the rooting zone and the intact root system in each microplot was collected for nematode population density and distribution studies. Nematode densities increased 1,100-fold after 134 days. Eggs in the soil, eggs in egg masses on roots, and second-stage juveniles in the soil accounted for 49, 41, and 10%, respectively, of the final nematode population.

The study of soilborne plant pathogens has been hampered by the variability of edaphic factors and the contagious dispersal of soil biota under natural field conditions. These difficulties are overcome by using microplots that permit replicated experimental units and manipulation of population densities. Various types of field microplots have been used to study plant pathogens including phytonematodes (1).

Meloidogyne chitwoodi (Golden et al), the Columbia root-knot nematode, can cause severe potato (*Solanum tuberosum* L.) tuber deformation (6). Studies of *M. chitwoodi* population dynamics within the soil and plant were conducted to ascertain when tuber damage was initiated and how it progressed. The study required weekly destructive root and tuber sampling of replicated plants. Sampling plants under field conditions resulted in root damage, plant stress, and poor plant survival. A bucket microplot system was developed to allow whole-plant sampling without modifying the environment of the remaining plants.

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MATERIALS AND METHODS

Microplot units were constructed from 19-L plastic buckets 30 cm in diameter × 35 cm deep and equipped with a moveable handle. Eight circular holes were cut in the bottom of buckets with a 5-cm carpenter's hole saw attached to an electric drill (Fig. 1A). Areas selected for microplot studies were fumigated with metham sodium (Vapam) at 916 L/ha. Parallel trenches, 30 cm wide × 30 cm deep and 0.8 m apart, were excavated with a power trencher. Buckets were set in the bottom of the trench and spaced

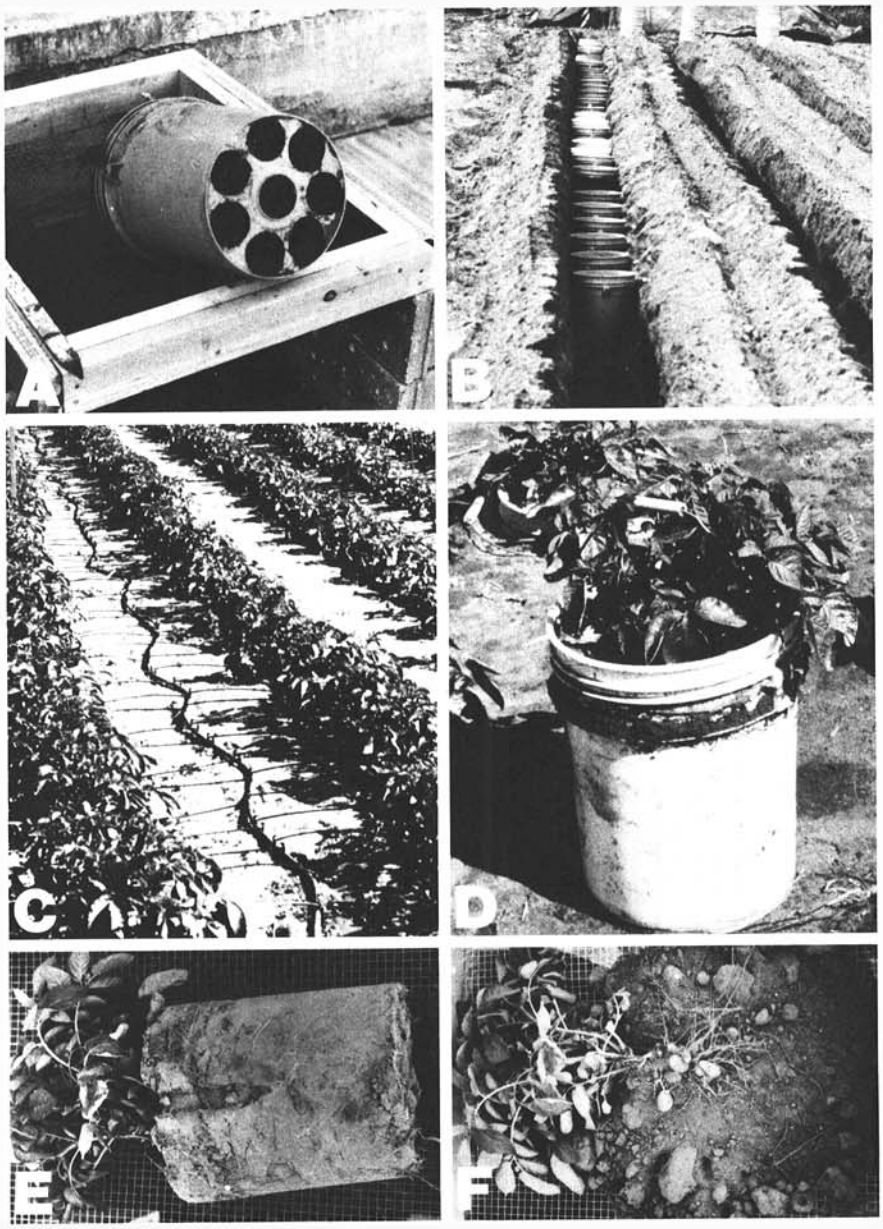

Fig. 1. (A) Bucket with drainage holes. **(B)** Buckets placed in trenches. **(C)** Established microplots showing drip irrigation system and interplants. **(D)** Bucket uplifted from ground. **(E)** Contents of bucket placed on a screen. **(F)** Plant showing early root and tuber growth.

Table 1. *Meloidogyne chitwoodi* egg and second-stage juvenile (J2) population dynamics on Russet Burbank potato grown in bucket microplots[a]

Days after planting	Number/250 cm³ of soil		Number/g fresh root		Total population microplot[b] (10^3)
	J2 (10^3)	Eggs (10^3)	Eggs (10^3)	Egg masses	
0	0.0	0.16	0.0	0	12
9	0.01	0.0	0.0	0	0.3
33	0.0	0.0	0.0	0	0
43	0.0	0.0	0.0	0	0
53	0.0	0.0	4.0	28	80
61	0.16	4.2	3.0	18	400
68	0.37	2.4	9.6	32	550
75	0.62	7.5	6.0	16	840
82	0.93	11.2	5.9	24	1,230
89	0.19	8.6	7.6	49	990
96	1.21	36.4	12.1	44	330
110	3.50	23.9	11.9	89	313
134	17.98	87.1	72.2	344	11,010

[a]Values are means of five replications.
[b]Total population = [(J2/L + eggs/L soil)(19 L)] + [(eggs/g root)(total g root)].

30 cm apart (Fig. 1B). Excavated soil was backfilled around each bucket to 5 cm of the top and the excess soil was stockpiled.

M. chitwoodi inoculum was prepared by NaOCl extraction of eggs from Columbian tomato (*Lycopersicon esculentum* Mill.) roots (5) and adjusted to 120 eggs/ml of water. Methyl bromide fumigated (0.3 kg/m³) soil was added to microplots in four increments and 25 ml of inoculum was thoroughly incorporated in each increment of soil. Soil was tamped to assure that microplot soil was in intimate contact with the subsoil.

Fertilizer was incorporated in the last 5 L of soil added. A single certified seed piece of Russet Burbank potato was planted at 15 cm in each microplot. Seed pieces were also planted between each plot and in border rows on either side of the microplot area. A drip irrigation system with a single emitter at each plant was used to irrigate microplots and interplants (Fig. 1C). Border rows were furrow-irrigated.

Five plants were sampled at regular intervals through the crop season by lifting the buckets from the ground by the handles (Fig. 1D). The holes were filled with stockpiled soil. The intact soil mass was slid out of buckets onto a large 1-cm mesh screen (Fig. 1E). The soil was carefully worked free of the roots and tubers (Fig. 1F). The screened soil was thoroughly mixed and samples were collected. Soil was processed by elutriation-centrifugation (2) and nematode population densities were estimated. Eggs were extracted from soil (3) and from root samples (5) after quantifying egg masses. Five tubers and the root system were collected from each plot, stained (4), and examined to determine nematode development stage, density, and distribution within the plant.

RESULTS AND DISCUSSION

M. chitwoodi populations developed well on Russet Burbank potato in the bucket microplot system (Table 1). No juveniles were recovered from the soil between 9 and 61 days, when second-generation eggs hatched. Second-stage juvenile (J2) numbers increased through the season and reached 17,980/250 cm³ of soil at harvest, a level comparable to densities recorded in field studies (7). Egg masses were first observed on the roots 53 days after inoculation. The number of eggs and eggs per gram of fresh root remained stable until about 89–96 days, when populations increased greatly. Numbers of free eggs in the soil samples were greater than the sum of all other population parameters measured. Population increase, as measured by J2 and total nematode life stages, was notably different by harvest with 37× and 1,100× increases, respectively. Whole-plot samples at each date allowed this partitioning and analysis of population

dynamics among J2, egg masses, and eggs.

Tuber set was similar to that observed in field plots with up to 15 tubers developing. Although many tubers were deformed due to their confinement in the bucket, they could be sectioned for nematode assay. J2, females, and females with egg masses were first detected in tubers at 75, 82, and 89 days after planting, respectively. Nematode numbers were too high in the tuber and root samples by the later part of the study to make accurate estimates. A lower inoculum level is needed to study *M. chitwoodi* population dynamics within potato plants.

The bucket microplot is an ideal experimental unit for *M. chitwoodi* population dynamics and host-nematode studies. The small plot unit allowed the installation of the large number of plots required for a weekly destructive sampling schedule. The handle-equipped buckets were easily lifted from the ground and removed from the experimental area without disrupting the plants remaining to be sampled. The sample unit allowed the partitioning of samples to study the spatial distribution of nematodes within the soil and plant.

Small bucket microplots permit the establishment of experiments that approximate field conditions and minimize interplot variation. Bucket microplots with interplants permit plants to be spaced at field density and they reduced plot border effects. The microclimate under the plant canopy should more closely represent commercial field conditions than would larger microplot units. Small plot volume permitted homogenous soil to be fumigated and used in all microplots. By amending inoculum while filling microplots, inoculum distribution in the soil profile was consistent in all plots at the start of the experiments.

Bucket microplots provided a low-cost alternative to other types of microplots. Those used in our studies were obtained free from local agricultural fertilizer distributors. Buckets are extremely durable, and, unlike fiberglass, clay, or ceramic tiles, do not break or crack with continuous use. The buckets have been steam-cleaned and used for three seasons. Specialized equipment is not necessary to establish bucket microplot experiments. Although a power trencher has been used, buckets were set in hand-dug holes in one commercial field.

Although microplots may not be suitable for all experimental needs, they provide flexibility that other microplot systems lack.

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