HortScience 14(1):27–28. 1979.

Methods for Screening Snap Beans for Resistance to Root-knot Nematodes[1]

J. E. Wyatt and George Fassuliotis[2]
U. S. Vegetable Laboratory, Charleston, SC 29407

Additional index words. Phaseolus vulgaris, Meloidogyne spp., vegetable breeding

Abstract. **A method was developed to evaluate snap beans, *Phaseolus vulgaris* L., for resistance to root-knot nematodes, *Meloidogyne* spp., which permits selected plants to survive, making either hybridizations or seed increase possible in the same generation. Nematode inoculum are added to commercial potting medium in greenhouse benches and snap beans are grown either in peat or clay pots filled with uninoculated soil and buried in the inoculated media. Roots protruding through holes in the bottom of the pots are evaluated for root gall and reproduction indices.**

A difficulty encountered in breeding snap beans for resistance to root-knot nematodes has been the propagation of resistant plants following their evaluation for root galling and nematode reproduction. Bean plants must be at least 30 days old before roots are examined for galls in order to eliminate the susceptible segregates (1). Plants of this age are sensitive to uprooting and will seldom survive the shock of transplanting. Consequently, plant breeders must resort to using remnant seed of resistant lines, a procedure which has hampered breeding progress.

One alternative to using remnant seed is to uproot the plants and examine the roots about 50 days after seeding under greenhouse conditions. At this age, pods are partially mature and seeds will germinate, although vigor is greatly reduced. The period of anthesis is over, consequently, the opportunity for backcrossing or crossing the resistant selection with another parent has passed. Also, in older plants, root senescence and root rotting organisms often cause sloughing of the cortex, resulting in difficulties in evaluating the root knot response.

The objective of this study was to develop a technique for evaluating individual snap bean plants for resistance to root-knot nematodes and to preserve alive those resistant individuals selected. This enables the breeder to use the selected plants for crossing or selfing.

We used 3 snap bean lines that had different levels of resistance to the southern root-knot nematode, *M. incognita* (Kofoid & White) Chitwood: resistant Plant Introduction (PI) 165426 (1), susceptible 'White Seeded Provider,' and B4151-9N, a breeding line segregating for resistance. Growing medium was 3 parts commercial potting mixture, composed of shredded peat moss and horticultural vermiculite, and 1 part builders' sand. The medium was steam sterilized.

Nematodes were reared on tomato, *Lycopersicon esculentum* Mill. cv. Homestead 24, and eggs used for inoculum were extracted with 1.0% NaOCl solution (2).

Peat pot experiment. Holes were made 10 cm deep and 8 cm in diameter at 20 cm intervals within rows 30 cm apart in the potting medium in greenhouse benches. About 12,000 eggs of *M. incognita* were placed in the bottom of each hole with an automatic pipette.

Holes 2.2 cm in diameter were cut in the bottom of 7.6 cm diameter peat pots. The peat pots were placed in the holes in the benches and filled with the uninoculated growth medium. Three seeds of snap bean were planted in each pot and 4 pots comprised a plot. Seedlings were thinned to 1 plant per pot. The experimental design was a factorial with 3 snap bean lines and 3 evaluation dates with 3 replications.

Plants were dug with the peat pot intact at 35, 45, and 55 days after seeding. The roots that emerged through the hole in the bottom of the peat pot were removed and evaluated for root knot response. Because some susceptible bean plants exhibit a non-galling response but show female nematode development and egg mass formation, 2 responses were recorded. Galling was scored on a 1 to 5 scale with 1=no galling and 5=severe galling. Nematode reproduction was scored on a 1 to 5 scale with 1=no enlarged females or egg masses and 5=many enlarged females or egg masses. The remainder of the root system in the peat pot was not disturbed. Plants were replaced in the benches and watered as needed until seed matured. Pods were harvested as they matured.

This technique was satisfactory for evaluating snap beans for root-knot nematode response, but several problems were encountered. If the seedling tap root failed to emerge through the hole in the bottom of the pot, root samples were small and consisted of secondary roots only. However, there were enough roots from all plants to rate the root knot response. Roots that grew through the sides of the pot were seldom infected with nematodes or had not had time to form knots. Another problem was the deterioration of the peat pots, especially those in the 45 and 55 day evaluation periods. This made handling more difficult and the root systems remaining within the pots were often damaged. Plants wilted severely after digging but all, except those evaluated at 55 days, recovered after 1 or 2 days.

Thirty-five days after seeding, most plants were in full bloom with small pods 3 to 5 cm long. At 45 days, most pods were 10 to 12 cm long, the optimum size for green pod harvest. Pods remained green but were almost mature 55 days after seeding and seeds were near their maximum size. Plants dug 55 days after seeding never fully recovered and remained permanently wilted until maturity.

No differences occurred in galling or nematode reproduction among evaluation times (Table 1). Root galls and nematode egg masses were prominent, and female nematodes were well developed at all evaluation dates. Plant-to-plant and varietal differences were distinct at all evaluation times.

Seed yield did not differ among evaluation dates (Table 1). Plants uprooted 35 or 45 days after seeding recovered and seed yields were comparable to plants dug at 55 days when seeds were nearing maturity. Plants retained most of their root system which continued to grow into the medium outside the pots.

As expected, the 3 bean lines were significantly different in their galling and nematode reproduction responses. PI 165426 was highly resistant to infection by *M. incognita*, although small galls were observed occasionally. 'White Seeded Provider' was moderately to severely infected by the root-knot nematodes. Many galls and numerous large egg masses were produced by the large, well-developed female nematodes. B4151-9N, a segregating breeding line, exhibited a variety of responses; some plants were completely resistant to both root galling and nematode reproduction, some were free of root galls but had numerous enlarged female nematodes and egg masses, while some had both root galls and egg masses.

Differences in yield of seed among bean lines cannot be totally ascribed to nematode resistance. PI 165426 has

[1]Received for publication May 30, 1978. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

[2]Research Geneticist and Research Zoologist, respectively, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture.

Table 1. Root gall and nematode reproduction indices and seed yield of 3 bean lines grown in peat pots 35, 45, and 55 days.

Variable	Root gall index[z]	Reproduction index[y]	Seed yield (g/plot)
Days after planting			
35	2.91 a[x]	2.86 a	12.8 a
45	2.36 a	3.06 a	10.8 a
55	2.61 a	3.18 a	13.2 a
Bean line			
White Seeded Provider	4.44 a	4.62 a	5.0 a
B4151-9N	2.41 b	3.46 b	12.8 b
PI 165426	1.03 c	1.03 c	19.0 c

[z]1 = no galling, 5 = severe galling.

[y]1 = no egg masses or enlarged females, 5 = many egg masses or enlarged females.

[x]Mean separation within columns within variable by Duncan's multiple range test, 5% level.

an indeterminate growth habit and sets pods over a long period of time. B4151-9N is a vigorous, determinate bush that sets more pods than most other cultivars under greenhouse conditions. 'White Seeded Provider' is a determinate variety that produces an average yield in the greenhouse.

Clay pot experiment. Seeds of each line were germinated and grown in paper towels until radicles were 6 to 8 cm long. The seedlings were then placed in 10 cm clay pots with the root tips protruding 1 cm through the drain hole in the pot. Enough potting medium was added to cover the seedling. Holes 1 cm deep were made in the growth medium in benches. Spacing of holes was the same as in the peat pot experiment. About 6500 *M. incognita* eggs were added to each hole in the growth medium and the clay pots were placed over the holes. Plants were removed at 25, 35, and 45 days after transplanting and roots were evaluated as described in the peat pot test. Plants were returned to the greenhouse bench and grown to maturity for seed harvest.

Clay pots were better than peat pots for evaluating root knot symptoms. Root systems which grew into the growth medium through the drain holes of the clay pots (Fig. 1) were large, vigorous, and more amenable to evaluation for root knot response than those grown in peat pots. Root systems developed uniformly after the tap root grew through the drain hole in the clay pots. In contrast, in the peat pot experiment, only small secondary roots grew through the hole in some instances. Numbers of root galls and nematode reproduction rates were more uniform in clay pots since the roots were similar in size and development when infected by the nematode larvae.

Plant survival after root removal was good. Plants evaluated 25 days after transplanting recovered within 1 day, but plants evaluated at 35 and 45 days after transplanting required longer recovery periods. However, no plants evaluated 45 days after transplanting remained permanently wilted.

There were no differences in root gall indices among the 3 evaluation dates (Table 2). Nematode reproduction indices were significantly lower 25 days after transplanting due to prevalence of immature female nematodes. As in the peat pot experiment, differences were significant among bean lines for nematode resistance using either root gall or reproduction indices (Table 2).

Yield of seed increased significantly as evaluation time was delayed. Plants in the clay pots had little opportunity to develop additional roots extending through the drain hole. Roots were confined to the small volume of the pot and earlier removal of the roots growing through the drain hole of the pot resulted in reduced yield.

The optimum time for evaluation will depend on the purpose of the test. If selections are to be used in making crosses, evaluation should be made 25 to 30 days after transplanting to allow recovery before anthesis. However, if selections are for generation advance, evaluation should be delayed to 45 days or longer after transplanting to maximize yield of seed per plant.

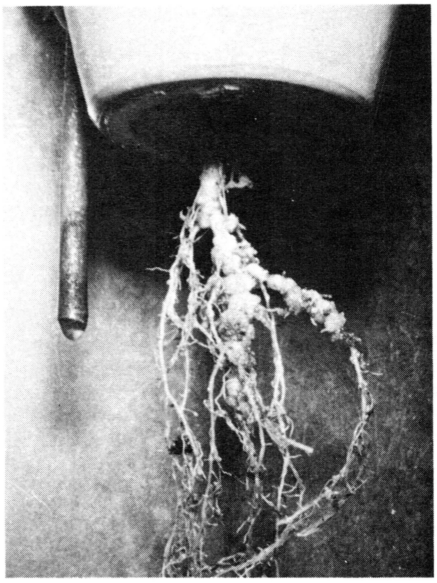

Fig 1. Bean root system infected with southern root-knot nematode, *Meloidogyne incognita*. The roots outside the clay pot will be removed for evaluation and the plant grown to maturity.

Table 2. Root gall and reproduction indices and seed yield of 3 bean lines grown in clay pots 25, 35, and 45 days.

Variable	Root gall index[z]	Reproduction index[y]	Seed yield (g/plot)
Days after transplanting			
25	2.67 a[x]	2.84 a	35.1 a
35	3.12 a	3.46 b	42.8 b
45	2.68 a	3.32 b	52.7 c
Bean line			
White Seeded Provider	4.91 a	5.00 a	28.1 a
B4151-9N	2.52 b	3.61 b	45.1 b
PI 165426	1.05 c	1.02 c	57.5 c

[z]1 = no galls, 5 = severe galls

[y]1 = no egg masses or enlarged females, 5 = many egg masses or enlarged females.

[x]Mean separation within columns within variables by Duncan's multiple range test, 5% level.

Literature Cited

1. Fassuliotis, G., J. R. Deakin, and J. C. Hoffman. 1970. Root-knot nematode resistance in snap beans: breeding and nature of resistance. *J. Amer. Soc. Hort. Sci.*. 95:640-645.
2. Hussey, R. S. and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rptr.* 57: 1025-1028.