Supplement to Journal of Nematology 24(4S):707–711. 1992.

Effects of Root Decay on the Relationship between *Meloidogyne* spp. Gall Index and Egg Mass Number in Cucumber and Horned Cucumber[1]

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Abstract: A greenhouse study was conducted to determine if root necrosis had an effect on the relationship between root-knot nematode gall index and egg mass number. Thirty-four cultigens of *Cucumis* (14 accessions, 12 cultivars, and six breeding lines of *C. sativus*, and two accessions of *C. metuliferus*) were evaluated against four root-knot species (*Meloidogyne arenaria* race 2, *M. incognita* race 1, *M. incognita* race 3, and *M. javanica*) measuring gall index, root necrosis, and egg mass number. Root necrosis affected the gall index–egg mass relationship. At lower root necrosis values, a stronger relationship existed between gall index and egg mass number than at higher root necrosis values. Root tissue was destroyed by root necrosis, and normal root-knot nematode reproduction would not occur, even though root galling was still observed. The races of *M. incognita* tested had a greater effect in predisposing *C. sativus* and *C. metuliferus* to root necrosis than did *M. arenaria* race 2 or *M. javanica*. This study showed that root necrosis had an adverse affect on the relationship between gall index and egg mass number in cucumber.

Key words: African horned cucumber, cucumber, *Cucumis sativus, Cucumis metuliferus, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica,* nematode, resistance, root-knot nematode.

Root-knot nematodes (*Meloidogyne* spp.) are highly destructive as primary partners in root-rot complexes. Those nematodes can predispose plants to severe damage from root necrosis. Root necrosis is thought to be part of the root-knot syndrome (6). Root necrosis has long been associated with the root-knot disease, and much yield loss attributed to root-knot nematodes may result from root necrosis (1). There is little information on the effect of root necrosis on root-knot nematode reproduction. Root necrosis may have detrimental effects on nematode populations. When root necrosis occurs, root tissue is destroyed, which may cause a decline in the nematode population. The objective of this study was to determine the effect of root decay on the relationship between gall index and egg mass number.

Received for publication 8 January 1992.
[1] The research reported in this publication was funded in part by the North Carolina Agricultural Research Service. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.
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MATERIALS AND METHODS

A greenhouse study was conducted to evaluate 34 cultigens (Table 1) of *Cucumis* (14 accessions, 12 cultivars, and six breeding lines of *C. sativus* L., and two accessions of *C. metuliferus* Naud.) for resistance to four root-knot nematodes (*Meloidogyne arenaria* (Neal) Chitwood race 2, *M. incognita* (Kofoid & White) Chitwood races 1 and 3, and *M. javanica* (Treub) Chitwood) by measuring gall index, root necrosis, and egg mass number. During harvest, we noticed that severely galled roots were usually highly necrotic and had only a few egg masses. Therefore, we reanalyzed the data to determine the relationship between gall index and egg mass number as affected by root necrosis.

All plants were grown from seed in 150-mm diameter (1.8 liters volume) clay pots on greenhouse benches. Five seeds were sown in each pot, which had been filled with sterile sand:soil in a 1:1 ratio, giving a mixture of 85% sand, 10% silt, and 5% clay. Plants were thinned to two per pot at the two-leaf stage and to one plant at the three-leaf stage. Irrigation with fertilizer (200 ppm N) was supplied twice daily using drip tubes.

Populations of *M. incognita* race 1, *M.*

TABLE 1. Cultigens of *Cucumis metuliferus* and *C. sativus* tested in root necrosis study.

C. metuliferus	*C. sativus*		
PI 482443	Chinese Long Green	Nepal Local 7	PI 344350
PI 482452	Delcrow	Ohio MR 17	PI 357865
	Dharampur-1	PI 169351	PI 432864
	Double Yield	PI 176953	PI 478364
	Early Russian	PI 206425	PI 483342
	Green Thumb	PI 211975	Poinsett
	Gy 4	PI 220169	Producer
	LJ 90430	PI 261608	Southern Pickler
	M 41 (NCSU)	PI 308915	Sumter
	Mincu	PI 319216	Wisconsin SMR 18
	National Pickling	PI 326594	

incognita race 3, *M. arenaria* race 2, and *M. javanica,* maintained in the greenhouse on tomato (*Lycopersicon esculentum* L. 'Rutgers'), were used as inoculum sources. Eggs for inoculum were obtained from tomato plants using the standard NaOCl procedure (5). Each *Cucumis* plant was inoculated with 25 ml of suspension containing 5,000 eggs, placed on the soil around the base of each plant. After all plants had been inoculated, 13 cm^3 of a 1:1 moist, sterile sand:soil mixture was placed on top of the soil in each pot to protect eggs from desiccation.

Roots were evaluated 11 weeks after planting (9 weeks after inoculation) using a gall index system on a scale of 0 to 100% injury (2), with 0 = immune, 1–10 = highly resistant, 11–20 = moderately resistant, 21–40 = slightly resistant and >40 = susceptible. Egg masses on roots were stained red with Phloxine B (4) and counted using the method of Hadisoeganda and Sasser (3), with 0 = resistant, 1–10 = moderately resistant, 11–50 = slightly resistant and >50 = susceptible. Root necrosis was determined by rating the percentage (0–100%) of the roots that were necrotic on each plant.

The experiment was a split-plot treatment arrangement in a randomized complete block design with five replications. Whole plots were the four nematodes and subplots were the 34 cultigens. Gall index, root necrosis, and egg mass number were analyzed using the GLM, REG, and CORR procedures of SAS (7). To linearize the relationship between gall index and egg mass number, data were transformed using arcsin (0.01 × percent galls) and $\log_{10}$ (number of egg masses + 1), respectively.

RESULTS

Averaged across the four root-knot nematodes evaluated, the mean necrosis values for *C. metuliferus* (2 cultigens) and *C. sativus* (32 cultigens) were 4.1 and 32.9, respectively (Table 2). Furthermore, the

TABLE 2. Mean gall index and root necrosis values for each *Cucumis* species tested over each nematode evaluated.

	Cucumis metuliferus		*Cucumis sativus*		Both *Cucumis* species	
Nematode	Gall index	Necrosis	Gall index	Necrosis	Gall index	Necrosis
M. arenaria race 2	3.6	5.1	50.8	23.1	48.1	22.0
M. incognita race 1	6.2	5.2	72.2	39.2	68.3	37.2
M. incognita race 3	5.2	3.2	69.7	35.3	65.9	33.4
M. javanica	5.8	2.8	63.4	34.0	60.0	32.1
Overall means	5.2	4.1	64.0	32.9	60.6	31.2
LSD ($P \leq 0.05$)	4.8	5.7	15.4	19.6	10.4	17.4

Data presented are means of five replications of either 2 cultigens (*C. metuliferus*), 32 cultigens (*C. sativus*), or 34 cultigens (both *Cucumis* species).

cultigens of *C. sativus* evaluated were more susceptible (mean gall index of 64.0 over all nematodes) compared to an overall gall index of 5.2 on the cultigens of *C. metuliferus*. Root necrosis and gall index indicated that the cultigens of *C. metuliferus* were highly resistant, whereas the cultigens of *C. sativus* were susceptible.

Over all treatment combinations, untransformed gall index and egg mass number were not highly correlated (r = 0.24). Several linear models were tested using transformed data. The best was $\log_{10}$ (egg mass number + 1) = 0.53 + 2.57 (percentage galls), with r^2 = 0.443 and P = 0.0001 (Fig. 1). Use of arcsin (0.01 × percentage galls) did not improve the fit of the model (r^2 = 0.417 and P = 0.0001). When regression was run on the original model using data from plants having root decay of less than 20%, r^2 was improved to 0.632 with P = 0.0001 (Table 3). When regression was run using data from plants having root decay >80%, there was no significant fit (r^2 = 0.038, P = 0.4545). The r^2 value became progressively smaller when analyzing root necrosis data <10% to <100% and >0% to >90%. Thus, at lower root decay values, gall index and egg mass number were better correlated. Also, when necrosis data above 40, 50, 60, 70, 80, and 90% were analyzed, the best linear models were not significant (Table 3). Therefore, the relationship between gall index and egg mass number was nonsignificant at high levels of root necrosis. When necrosis data above 40, 50, 60, 70, 80, and 90% were analyzed, this deletion of lower root necrosis values resulted in models with no significance.

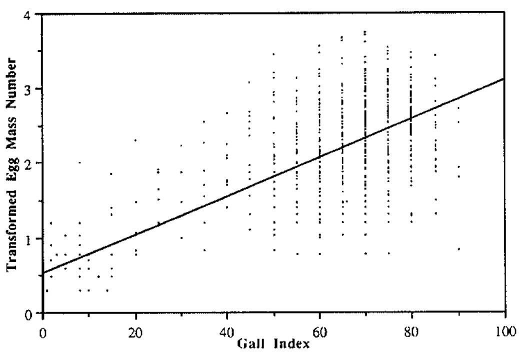

FIG. 1. Transformed egg mass number [$\log_{10}$ (egg mass number + 1)] versus gall index (percentage galls). Relationship between the two variables was linear (y = 0.53 + 2.57x; r^2 = 0.4431; n = 663; P = 0.0001).

Plants infected with various *Meloidogyne* species or races have been assumed to differ in their response to root necrosis. Races of *M. incognita* tested had a greater effect in predisposing *C. sativus* and *C. metuliferus* to root necrosis than did *M. arenaria* race 2 or *M. javanica*. Less severe root necrosis occurred with *M. arenaria* race 2 as compared to the races of *M. incognita* tested; no observations for *M. arenaria* race 2 were obtained when data above 80% root necrosis were analyzed (Table 4). The best linear model (r^2 value of 0.999, P = 0.0209) was obtained when necrosis data above 80% were analyzed for plants inoculated with *M. javanica*. This correlation indicated that *M. javanica* did not predispose the species of *Cucumis* tested to root necrosis. In contrast, both *M. incognita* race 1 and *M. incognita* race 3 provided poor fit to the best linear models when data above a root necrosis rating of 80% were analyzed (r^2 = 0.080, P = 0.4964 and r^2 = 0.035, P = 0.7235, respectively), indicating that the races of *M. incognita* tested were associated with much root necrosis (Table 4). The races of *M. incognita* evaluated predisposed the *Cucumis* cultigens to a greater amount of necrosis than did *M. arenaria* race 2 or *M. javanica*, which was directly related to the fact that the cultigens of *Cucumis* evaluated were more susceptible to *M. incognita* than the other nematodes tested (Table 2). Overall gall indices for *M. incognita* race 1 and *M. incognita* race 3 were 68.3 and 65.9, respectively; whereas gall indices for *M. javanica* and *M. arenaria* race 2 averaged 60.0 and 48.1, respectively (Table 2). However, only *M. arenaria* race 2 was significantly different ($P \leq 0.05$) from the races of *M. incognita* tested with respect to gall index. Some of the yield loss thought to be caused directly by *M. incognita* in cucumbers may actually be caused by root necrosis.

TABLE 3. Specific necrosis data percentages over all root-knot nematode species and races evaluated for the best linear model using data for gall index and egg mass number.

Necrosis data used	Best linear model†	r^2 value	*n*	*P* value
All	Tegg mass = 0.53 + 2.57 (gi)	0.443	663	0.0001
= 0%	egg mass = −1.98 + 209.87 (Tgi)	0.081	18	0.2382
= 100%	Tegg mass = 2.83 − 1.23 (Tgi)	0.304	5	0.2570
<10%	Tegg mass = 0.22 + 3.26 (Tgi)	0.674	69	0.0001
<20%	Tegg mass = 0.26 + 3.24 (gi)	0.632	206	0.0001
<30%	Tegg mass = 0.35 + 3.02 (gi)	0.556	399	0.0001
<40%	Tegg mass = 0.44 + 2.79 (gi)	0.492	581	0.0001
<50%	Tegg mass = 0.46 + 2.72 (gi)	0.478	614	0.0001
<60%	Tegg mass = 0.49 + 2.67 (gi)	0.468	639	0.0001
<70%	Tegg mass = 0.49 + 2.65 (gi)	0.467	644	0.0001
<80%	Tegg mass = 0.51 + 2.62 (gi)	0.458	648	0.0001
<90%	Tegg mass = 0.52 + 2.59 (gi)	0.450	655	0.0001
<100%	Tegg mass = 0.52 + 2.58 (gi)	0.446	657	0.0001
>0%	Tegg mass = 0.60 + 2.46 (gi)	0.371	644	0.0001
>10%	Tegg mass = 0.76 + 2.23 (gi)	0.259	619	0.0001
>20%	Tegg mass = 1.23 + 1.53 (gi)	0.092	542	0.0001
>30%	Tegg mass = 1.72 + 0.83 (gi)	0.020	388	0.0051
>40%	Tegg mass = 2.03 + 0.29 (gi)	0.004	146	0.4717
>50%	egg mass = −61.40 + 359.67 (gi)	0.032	62	0.1616
>60%	egg mass = −86.27 + 326.31 (Tgi)	0.064	32	0.1546
>70%	Tegg mass = 2.22 − 0.32 (Tgi)	0.014	20	0.6157
>80%	egg mass = 10.60 + 143.45 (Tgi)	0.038	16	0.4545
>90%	egg mass = 18.67 + 150.70 (Tgi)	0.042	12	0.5006

† Tegg mass is transformed egg mass number using $\log_{10}$ (egg mass number + 1), and Tgi is transformed gall index (gi) using arcsin (0.01 × gall index).

DISCUSSION

Root tissue was destroyed by root necrosis, resulting in lower root-knot nematode reproduction, although some root galling was still observed. It was assumed that populations of sedentary forms of nematodes such as *Meloidogyne* spp. were generally depressed under conditions of high root necrosis. We found that root decay had an adverse effect on the relationship between gall index and egg mass number, resulting in fewer egg masses on severely galled and decayed roots. When only data

TABLE 4. Two necrosis levels and all data analyzed for each root-knot nematode tested with the r^2 value for the best linear model using both gall index and egg mass number.

Root-knot nematode and necrosis data used	Best linear model†	r^2 value	*n*	*P* value
M. arenaria race 2 <20%	Tegg mass = 0.31 + 2.92 (gi)	0.567	95	0.0001
M. arenaria race 2 >80%	No observations	—	—	—
M. arenaria all data	Tegg mass = 0.53 + 2.29 (gi)	0.403	167	0.0001
M. incognita race 1 <20%	Tegg mass = 0.08 + 3.41 (Tgi)	0.732	28	0.0001
M. incognita race 1 >80%	egg mass = −30.42 + 257.19 (Tgi)	0.080	7	0.4964
M. incognita race 1 all data	Tegg mass = 0.36 + 2.80 (gi)	0.450	163	0.0001
M. incognita race 3 <20%	Tegg mass = 0.39 + 3.03 (gi)	0.628	41	0.0001
M. incognita race 3 >80%	egg mass = 182.92 − 105.86 (Tgi)	0.035	5	0.7235
M. incognita race 3 all data	Tegg mass = 0.76 + 2.14 (gi)	0.300	168	0.0001
M. javanica <20%	Tegg mass = 0.32 + 3.56 (gi)	0.673	39	0.0001
M. javanica >80%	Tegg mass = 2.43 − 1.00 (Tgi)	0.999	2	0.0209
M. javanica all data	Tegg mass = 0.60 + 2.77 (gi)	0.461	162	0.0001

† Tegg mass is transformed egg mass number using $\log_{10}$ (egg mass number + 1), and Tgi is transformed gall index (gi) using arcsin (0.01 × gall index).

above root necrosis ratings of 40, 50, 60, 70, 80, and 90% were analyzed, the best linear models indicated that the relationship between gall index and egg mass numbers was not significant. The best linear models obtained through analyses of data below a root necrosis rating of 20% for each nematode tested were highly significant ($P = 0.0001$). Thus, with less root necrosis, a positive relationship existed between gall index and egg mass number.

Some plant breeders and nematologists use only numbers of eggs, numbers of egg masses, or an egg mass index in determining resistance to root-knot nematodes. Those measurements do not always reflect accurately the resistance of a plant, because it could be highly susceptible to root-knot nematodes and have few egg masses. This study showed that roots can be severely galled and have a high amount of root necrosis and an associated reduction in the number of egg masses found on the roots. In determining resistance to root-knot nematodes, gall index (percentage of roots galled) and root necrosis rating may be a preferable measure, because a susceptible plant will usually have a large percentage of its roots galled and a large amount of necrosis on these roots. There is usually a positive relationship between egg mass number and gall index. However, if roots become necrotic, root tissue will be destroyed and there will be no correlation in that relationship.

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