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Standardization of Host Suitability Studies and Reporting of Resistance to Root-Knot Nematodes

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Introduction

Research on *Meloidogyne* species has taken place at an accelerated rate in recent years. This increased research has been due partially to the organized effort to study root-knot nematodes brought about by the International *Meloidogyne* Project (IMP). The IMP was funded from 1975 through 1984 by the United States Agency for International Development and headquartered at North Carolina State University.* It comprised a network of scientists from approximately 75 developing nations who collaborated on an extensive and detailed study of root-knot nematode biology and control.

A worldwide survey conducted by the IMP showed that approximately 95% of all root-knot nematode damage to crop plants is caused by one or more of the four major *Meloidogyne* species, namely *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. However, only one or two of these species are likely to be present in any one area. Since this is the case, the planting of crop varieties resistant to even one of the four predominant species can be a practical and appropriate management strategy for use by small-scale farmers against root-knot nematodes. Screening for resistance and development of resistant cultivars is, at the present, an area highly emphasized in the CNRCP program of research.* This type of research has been carried out for decades, but there is no recognized standard procedure for conducting this work and reporting the results.

Designations of host suitability, i.e. degree of susceptibility or resistance, have been primarily subjective in the past. Due to differing criteria for separation of resistant cultivars from susceptible ones, a cultivar categorized as moderately resistant (MR) in one study could be rated differently in another study. Accordingly, development of a standardized quantitative method based on actual nematode reproduction in the host and damage to the plant is necessary to promote increased uniformity of host-resistance designations and thereby allow broader interpretation and comparison of results.

Literature Review

Many parameters have been devised to estimate nematode reproduction and, in some cases, also to indicate host resistance. In 1966, Oostenbrink introduced the reproduction factor (R) as a measure of the reproductive capabilities of nematodes: $R = P_f/P_i$ (11). The variable P_f represents the final population of nematode juveniles and eggs recovered from the soil and roots of infected plants; P_i the initial population of eggs and/or juveniles with which the plants were inoculated. The R factor has been used in research on both migratory and sedentary endoparasitic nematodes as an indicator of host suitability (10, 12). However, no specific degree of resistance (DR) designations, e.g. highly resistant, moderately resistant or susceptible, have been based on this parameter.

Taylor developed a host "resistance" classification system in which he compared nematode reproduction on tested plants with that on a known susceptible cultivar of the same species (13). On the basis of this comparison, host suitability designations were defined as follows: nematode reproduction that is 25 to 50% of that on the susceptible host designates a slightly resistant plant (SR); 10 to 25%, a moderately resistant plant (MR); 1 to 10%, a very resistant plant (VR); less than 1%, a highly resistant plant (HR); and 0%, an immune plant. According to this system, nematode reproduction is represented by the total number of eggs and juveniles extracted from the soil and roots of infected plants. This scale was somewhat arbitrarily designed, and the degree of resistance designation assigned to any one cultivar could vary greatly depending on the susceptible cultivar chosen for comparison.

The egg-mass index (EI), which was introduced in the literature by Taylor and Sasser (14), was originally developed by members of the Southern Regional Technical Committee on Nematodes (Table 1). It gives an approximate measurement of root-knot nematode reproduction based on numbers of egg masses/plant instead of actual numbers of eggs and juveniles/plant. Since the number of eggs per egg mass varies, the EI is not a quantitative measurement of reproduction; still, it has been used as a basis for estimates of host efficiency. Likewise, the gall index (GI), which is based on the same scale as the EI, has been used as an indicator of plant damage even though little work has been done to correlate GI with crop yield. In the differential host test an average EI and an average GI of 2 or less are interpreted as in-

* The IMP has now been superseded by the USAID-funded Crop Nematode Research and Control Project (CNRCP).

Table 1. Taylor & Sasser's rating scale for the presence of root-knot nematode galls or egg masses on roots.

Number of galls or egg masses	Gall index (GI) or egg mass index (EI)
0	0
1-2	1
3-10	2
11-30	3
31-100	4
100+	5

dicating host resistance and those greater than 2 as indicating host susceptibility.

In their work on host suitability of tomato, Hadisoeganda and Sasser (6) proposed the following more detailed relationship between EI values and degree of resistance (DR) designations: EI range of 0.0-1.0 = highly resistant (HR); 1.1-3.0 = very resistant (VR); 3.1-3.5 = moderately resistant (MR); 3.6-4.0 = slightly resistant (SR); and 4.1-5.0 = susceptible (S) (6). It was hoped that DR designations could be assigned by means of this scale without the lengthy and tedious process of counting eggs and calculating the index of reproduction (IR): (number of eggs produced on test host/the number of eggs produced on a known susceptible host)x100. Unfortunately, the scale could not be applied to other crops with any degree of accuracy because the relationship between EI and IR varies too greatly among crops (unpublished data). This variation is due to the wide differences in nematode reproduction on the various standard susceptible cultivars chosen for comparison.

The host suitability designations used by the IMP over the past several years have been based on the index of reproduction (14). However, several problems have been associated with this method. First of all, standard susceptible cultivars have to be designated for each host crop to be tested. This necessity presents no problem in the case of tomato since the cultivar 'Rutgers' is widely available and clearly susceptible to all of the major species and races of *Meloidogyne*.

For other crops, selection of standard susceptible cultivars becomes complicated. For example, a standard may not be susceptible to all major species and races of *Meloidogyne* (often this information is not known when evaluations begin) hence several standard susceptible cultivars would have to be designated depending on the nematode species and race. In such a situation, meaningful comparison of a cultivar's resistance to various *Meloidogyne* species and races could not be made. Additionally, cultivars selected for standards might not be available on a worldwide basis.

Secondly, criteria for selection of standard susceptible cultivars must be very stringent because host resistance designations vary greatly depending on the degree of susceptiblity of the standard cultivar chosen for comparison. Thirdly, since each crop screened is compared to its own standard susceptible cultivar, comparison of DR designations among different crops is also meaningless. For example, nematode reproduction on a highly resistant (HR) tomato cultivar might be approximately the same as that on a moderately resistant (MR) or slightly resistant (SR) corn cultivar. Last of all, the IR is not a true indicator of host resistance, but only one of comparative and relative host efficiency.

Canto-Saenz (3) recently proposed a series of host suitability designations based jointly on host efficiency and damage to the plant. According to this system, which is a variation of one proposed earlier by Dropkin and Nelson (4), plants are categorized as resistant, tolerant, hypersusceptible or susceptible (Table 2). Although a quantitative basis for assessment of host efficiency and plant damage was not proposed by Canto-Saenz, the development of such a system, in which extent of nematode reproduction is used to quantify host efficiency and extent of galling or yield loss to quantify plant damage, may make it possible for plants to be categorized so that meaningful comparisons can be made.

Table 2. Host suitability designations proposed by Canto-Saenz, 1983.

Nematode reproduction on host	Damage to plant	
	Statistically significant	Insignificant
Efficient	Susceptible	Tolerant
Inefficient	Hypersusceptible	Resistant

Proposed Methodology for the Screening of Crop Germ Plasm for Resistance to Root-Knot Nematodes

Rationale. With so many methods being used in the determination of nematode reproduction and host suitability, the need for standardization is apparent. Within the International *Meloidogyne* Project (IMP), hundreds of cooperators throughout the world have screened cultivars for resistance. In fact, over the past four years, more than one thousand lines and/or cultivars of crop plants have been screened against the major species and races of *Meloidogyne* at the Project Center alone. Several International Agriculture Research Centers have provided the germ plasm for this effort: tomato and Chinese cabbage from the Asian Vegetable Research & Development Center (AVRDC), pigeon pea and chickpea from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), bean and the forage crop *Desmodium* from the International Center for Tropical Agriculture (CIAT), potato from the International Potato Center (CIP), and corn, wheat, barley and triticale from the International Center for Maize and Wheat Improvement (CIMMYT). The methods that have evolved for conducting this research and for reporting the results are outlined in detail. It is hoped that this methodology will be adopted by scientists throughout the world and thereby give continuity to programs that screen crop germ plasm for resistance to root-knot nematodes.

Increasing inoculum. It is extremely important that all *Meloidogyne* populations used for inoculum be accurately identified to species *and* race. Performance of a host differential test and examination of perineal pattern morphology before a population is used will ensure against use of mixed species populations or those of unknown physiological races (8, 14).

Large quantities of eggs of the four major *Meloidogyne* species for use as inoculum can be produced on susceptible tomato cultivars such as 'Marglobe' and 'Rutgers.' Over one million eggs can be collected from a single, heavily infected tomato plant (5). The fact that all of the major species and races of *Meloidogyne* reproduce heavily on these susceptible tomato cultivars makes them a good choice for inoculum production. However, some less common *Meloidogyne* species, especially those that primarily infect woody plants, reproduce poorly on or will not infect tomato. In such cases, other plants must be used for increasing inoculum. The procedure for increasing inoculum is outlined in the following paragraphs.

Tomato seedlings of a cultivar highly susceptible to root-knot nematodes are grown in steam-sterilized soil and then inoculated with a known species and race of *Meloidogyne*. Plants are ready to be harvested for inoculum approximately 60 days after inoculation, under greenhouse conditions (22-30°C). Once the soil is washed from the roots, the roots are cut into pieces about 2-cm long and placed in a 1000-ml container with 200 ml of 0.5% sodium hypochlorite (NaOCl) solution (10% solution of household bleach) (9). The tightly capped container is shaken vigorously for three minutes. Shaking partially dissolves the gelatinous matrix, thus freeing eggs from the egg mass. Exposure to the NaOCl for longer than four to five minutes adversely affects the viability of the eggs. The liquid suspension of eggs is poured through a 200-mesh sieve nested upon a 500-mesh sieve. Eggs suspended in the agitated solution pass through the 200-mesh sieve, which removes root debris, and are collected on the 500-mesh sieve. Eggs are washed free of residual NaOCl solution under a slow stream of cool tap water.

The inoculum concentration is calibrated as follows. The egg suspension is adjusted to a known volume, usually 100 ml, and then poured into a flask. Since one milliliter of solution generally contains too many eggs to be easily counted, a dilution series is necessary. When an aliquot of the egg suspension is transferred, the suspension should be well agitated so that the eggs are dispersed evenly throughout the known volume. Swirling the flask, blowing air into the suspension through a pipette, and then quickly transferring the eggs before they settle accomplishes this purpose. A 0.5-ml sample is transferred by pipette to a flask containing 4.5 ml of tap water. A 0.5-ml aliquot of the diluted suspension is then transferred to a clear counting dish, and the eggs are immediately dispersed with tap water. The counting dish should be etched with lines that divide the bottom surface into equal fractions. Counting the number of eggs covering $\frac{1}{4}$ of the counting surface and then multiplying that number by 80 gives an estimate of the number of eggs/ml in the original suspension. The estimate is more reliable and accurate when the dilution series is repeated and the results of the two replicates averaged. The egg suspension is then diluted so that the appropriate number of eggs can be dispensed in a convenient amount of water (usually 5 ml) (8).

Growth and Inoculation of Plants to be Screened. The lines or crop cultivars to be screened are planted in disinfested soil. Cultivars may be seeded into germination flats and transplanted later to individual pots or may be directly planted in pots, depending on the growth habits of the plant type being evaluated. A fine sandy loam soil mixed with an equal amount of coarse sand has proven satisfactory. Fertilizer is applied as necessary. Once the plants are

Table 3. Quantitative scheme for assignment of Canto-Saenz's host suitability (resistance) designations.

Plant damage (gall index)	Host efficiency (R factor)	Degree of resistance (DR) designation
≤ 2	≤ 1	resistant
≤ 2	> 1	tolerant
> 2	≤ 1	hypersusceptible
> 2	> 1	susceptible

well established (usually in the 2- to 4-true-leaf stage), an Oxford pipet (Fisher Scientific Co., Pittsburgh, PA 15219) is used to dispense 5000 *Meloidogyne* eggs (P_i) near the base of each plant stem. Three to ten replications of each treatment are advisable. Several 'Rutgers' tomato plants are also inoculated to serve as controls.

Plants are grown in greenhouse conditions for approximately 60 days at a temperature of 22 to 30° C. At the higher end of the temperature range, 45-50 days of growth may be sufficient. If the tomato has more than 100 galls and egg masses, sufficient time has elapsed and the test can be terminated. It should be remembered, however, that temperatures approaching 32.5° C or more tend to decrease cultivar resistance (1).

Rating Plants and Determination of Host Suitability. After the allotted time period, tops of the test plants are cut off and the soil gently washed from the roots. Roots are soaked in a solution of 15 mg phloxine B/liter of water for 15-20 minutes and the egg masses become stained pink (7). The roots are then relatively easy to rate according to the system of Taylor and Sasser (Table 1) (14).

Generally, the gall index (GI) and the egg mass index (EI) for any given plant are approximately the same when plants are rated after 60 days. Ideally, one gall and one egg mass develop for each female present in the roots, and the egg masses, stained by the phloxine B, are readily visible. However, variation does occur. For example, *M. incognita* infects and reproduces in corn roots without causing much noticeable galling or subsequent yield loss. In the rating system that we propose here, the GI is used to indicate nematode damage to the plant.

An estimate of nematode reproduction based on the number of eggs extracted per plant is also necessary if cultivars are to be separated into host suitability designations (Table 3). Eggs are extracted as for inoculum except that the root system is finely chopped in a blender and a 1% NaOCl solution is used since egg viability is not of concern. Extracted eggs are stained red with a few drops of acid fuchsin-acetic acid solution (3.5 g acid fuchsin, 250 ml acetic acid, 750 ml distilled water). The egg suspension with stain must be brought briefly to a boil (2).

The egg number/plant is counted and averaged with the other replicates. If 5 to 10 replicates are used, 3 to 5 randomly selected replicates are sufficient for estimation of egg number since this process tends to be time consuming. The reproduction factor, $R = P_f/P_i$, is then calculated, with P_f being the average final egg count among replicates and P_i, in this case, being the 5000 eggs used as original inoculum. An R factor of less than or equal to one indicates no apparent increase in the nematode population.

In order to utilize Canto-Saenz's host suitability designations, we are proposing a new rating system based on the gall index (GI) as an indicator of plant damage and on the reproduction factor (R) as an indicator of nematode reproduction or host efficiency (Table 3). Cultivars with GI's greater than 2 are designated as either susceptible [efficient host ($R>1$); significant damage ($GI>2$)] or hypersusceptible [poor host ($R\leq1$); significant damage ($GI>2$)]. Since such cultivars are damaged whether or not significant nematode reproduction occurs, they usually can be eliminated from a screening program. The remaining categories are assigned as follows: resistant [poor host ($R\leq1$), minimal damage ($GI\leq2$)], and tolerant [efficient host ($R>1$), minimal damage ($GI\leq2$)]. Although plants with no nematode reproduction ($R=0$) and no damage ($GI=0$) could be categorized as immune, such a label is not assigned during a preliminary screening because the procedure is not sensitive enough. Very low numbers of galls or egg masses may go undetected in even the most rigorous screening.

Some Results from the IMP Screening Program. Lines of crop plants received from International Agriculture Research Centers have been screened against the major species and races of *Meloidogyne* according to the method just described. Degree of resistance designations as shown in Table 3 have been assigned to the following crops: 25 lines of tomato (AVRDC), 5 lines of Chinese cabbage (AVRDC), 95 lines of chickpea (ICRISAT), 27 lines of pigeon pea

(ICRISAT), 109 lines of bean (CIAT), 73 lines of *Desmodium* (CIAT), 24 lines of corn (CIMMYT), 30 lines of durum wheat (CIMMYT), 272 lines of triticale (CIMMYT), and 213 lines of barley (CIMMYT). To illustrate the application of the proposed system in the classification of host suitability, we have presented representative data from tests completed (Table 4). Each of these lines or cultivars has been subjected to the screening procedure only once.

Summary

The method for assigning degree of resistance designations that is outlined here is uniformly applicable to all crops and eliminates the need for standard susceptible cultivars. Also, if the decision is made to choose only resistant and tolerant selections for use in subsequent breeding programs, then other categories (GI>2) can be quickly separated thus eliminating tedious and time-consuming egg counting. Although the relationship of the GI to yield has not been examined for all crops, it is the best preliminary indicator of damage currently available. Widespread use of this method will allow researchers to compare easily the results of their root-knot nematode resistance studies. A brief summary of the approach is as follows:

1. Fill appropriately sized pots with disinfested soil, preferably a sandy loam soil mixed with an equal amount of coarse sand.

2. Plant cultivars to be screened, replicating each three to ten times.

3. Once plants are well-established, inoculate each pot with 5000 eggs of a known species and race of *Meloidogyne*.

4. After approximately 60 days, depending on the temperature, terminate the test by harvesting plants and staining roots with phloxine B (7).

5. Assess plant damage by rating for galls (14).

6. Extract eggs from roots of plants, especially those with gall indices of 2 or less, using a 1% NaOCl solution (9).

7. Assess nematode reproduction and host efficiency by determining the average final population among replicates and calculate Oostenbrink's (11) reproduction factor: $R = P_f/P_i$, where P_i = the initial inoculum level, 5000 eggs.

8. Assign Canto-Saenz's host suitability designations on the basis of the scale proposed in Table 3. For examples of the application of this system, see Table 4.

Table 4. Host suitability of selected cultivars to various *Meloidogyne* species.

Breeding line or cultivar	Nematode species	Damage to host: Mean gall index*	Damage to host: Standard deviation	Host efficiency (Mean R factor)**	Suitability designation (resistance)
		— Tomato — (5 replications)			
CL 106-5-1-0	*M. incognita* (R-1)	0.8	0.84	0.03	Resistant
L672	*M. arenaria* (R-1)	4.0	0.71	1.78	Susceptible
L274	*M. hapla*	5.0	0.00	5.23	Susceptible
L4129	*M. arenaria* (R-2)	5.0	0.00	177.34	Susceptible
		— Bean — (3 replications)			
Alabama 1 (G03736)	*M. incognita* (R-1)	0.0	0.00	0.05	Resistant
Saginaw (G03941)	*M. incognita* (R-1)	4.6	0.58	1.57	Susceptible
Calima P642	*M. incognita* (R-1)	4.6	0.58	7.58	Susceptible
Tender Pod (G06731)	*M. javanica*	5.0	0.00	52.72	Susceptible
		— Pigeon pea — (5 replications)			
ICP-8868	*M. hapla*	0.0	0.00	0.00	Resistant
ICP-11297	*M. javanica*	1.2	1.30	0.14	Resistant
ICP-11293	*M. incognita* (R-3)	0.6	1.34	1.42	Tolerant
ICP-8860	*M. arenaria* (R-2)	2.2	0.84	0.70	Hypersusceptible
ICP-11295	*M. incognita* (R-1)	3.6	0.55	2.62	Susceptible
ICP-8863	*M. arenaria* (R-2)	4.0	0.00	6.69	Susceptible
ICP-11292	*M. incognita* (R-2)	5.0	0.00	27.38	Susceptible

* Gall index scale: 0 = 0 galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = 100+ galls.
** The R factor is calculated as the average final egg count divided by 5000 eggs (number of eggs with which each pot was inoculated).

Table 4 Cont.

Breeding line or cultivar	Nematode species	Damage to host: Mean gall index*	Damage to host: Standard deviation	Host efficiency (Mean R factor)**	Suitability designation (resistance)
		— *Desmodium ovalifolium* — (3 replications)			
CIAT #3674	*M. incognita* (R-1)	0.0	0.00	0.01	Resistant
CIAT #3788	*M. incognita* (R-3)	2.0	0.00	1.79	Tolerant
CIAT #3652	*M. hapla*	3.3	1.53	0.81	Hypersusceptible
CIAT #3666	*M. arenaria* (R-1)	3.0	0.00	1.56	Susceptible
CIAT #3793	*M. incognita* (R-2)	4.0	2.31	6.92	Susceptible
CIAT #3652	*M. arenaria* (R-2)	5.0	0.00	61.33	Susceptible
		— Corn — (5 replications)			
Sids 7929	*M. hapla*	0.0	0.00	0.00	Resistant
Savedra 7926	*M. arenaria* (R-1)	0.6	0.89	0.72	Resistant
Cotaxtla 7921	*M. arenaria* (R-2)	2.4	0.55	0.69	Hypersusceptible
Across 7936	*M. javanica*	4.8	0.45	24.61	Susceptible
Suwan 8035	*M. incognita* (R-1)	5.0	0.00	85.50	Susceptible
		— Durum Wheat — (5 replications)			
EDM	*M. hapla*	0.0	0.00	0.00	Resistant
SHWA "S"-YAV"S" CD 20626-6M-2Y-1M-0Y	*M. arenaria* (R-1)	2.4	1.24	0.50	Hypersusceptible
CNDO ROK"S" CD-1895-12Y-0Y-2E-3B-0Y	*M. arenaria* (R-1)	3.4	0.55	3.38	Susceptible
CHUR"S" CD-20124-11M-3Y-2M-1Y-1Y-0M	*M. arenaria* (R-2)	4.6	0.55	8.84	Susceptible
	M. arenaria (R-2)	5.0	0.00	15.62	Susceptible

* Gall index scale: 0 = 0 galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = 100+ galls.
** The R-factor is calculated as the average final egg count divided by 5000 eggs (number of eggs with which each pot was inoculated).

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