Root-knot nematodes in California and the development of resistant sugarbeet varieties

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Introduction

In California one of the major obstacles to sugarbeet production is the damage caused by plant parasitic nematodes, such as the root-knot group, *Meloidogyne* spp. Historically, root-knot nematodes occurred in more than twenty counties of the state (Caveness, 1959). Their extensive host ranges, and involvement with fungi, bacteria and virus in disease complexes, limit sugarbeet growers' nonhost crop rotation options. In places where root-knot nematodes occur, not only are root yields greatly affected but quality is also reduced. In severe cases whole crops may be destroyed. This is probably one of the elements why in recent years California sugarbeet planting acreage has decreased to less than one third of 1969's 360,000 acres (Goodwin, 1975; Nilsson, 1998). The best way to overcome such limitation would be planting sugarbeet varieties resistant to nematodes. To develop sugarbeet resistant to root-knot nematode, an understanding of current existing *Meloidogyne* species and their distribution in California sugarbeet growing regions is important. With a defined target, breeding sugarbeet resistance to root-knot nematode can be more meaningfully planned and pursued.

Materials and Methods

The root-galled sugarbeet and infested soil from various major root-knot nematode infested fields were acquired. Many of these samples were collected with the help of beet growers and sugar company personnel (Table 1), and sent to the Salinas ARS research station for evaluation.

Table 1. Main sources of galled root and infested soil samples from sugarbeet fields and involved personnel.

Sample	*Farm location*	*Person collected*
Roots:	Stockton Island Delta; Colusa farm	G. L. Fisk
	Sacramento-Yuba, Oji Bros; Woodland Ranch	J. D. Schulke
	Dos Palos-North; D.P.N. Palm-Roxbury	J. Martin
	Salinas, Hitchcock and Highway 68	S. T. Koike
	Tulare, Cartmill Road and Highway 99	J. L. Kimmell

	San Ardo Beet Farm	A. L. Pilgeram
	Merced County – Lewis Maiorino; Christina Santa Rita; Mengles Family Farming	J. L. Kimmell
	Mendota, sugabeet fields	J. Watson
	Riverside County	M. S. Munson
	Mantica, San Joaquin; Stockton area	W. Bassi
Soil:	Merced County – Nickel Entpr. DF/Sec 36, L/Sec 31	J. Sagaser
	Nickel Entpr. TL #2 - 5/Sec. 33	J. D. Schulke
	Del Testa 1A,B/Sec 11, 2A/Sec 12, 3A,B/Sec 1	J. Sagaser
	Dos Palos farm	J. Martin
	Monterey County, Gonzales	G. Fellows
	Fresno County – Nickel Entpr. S/Sec 34, F/Sec 36	J. H. Griffin
	Bred Coburn Shop, Sec 33	J. Sagaser
	Davis Drier and Elevator Farm, Firebaugh	C. H. Hu
	Brawley, New Side Farms	J. P. Singh

Nematode females and egg masses were extracted from root galls using forceps under a microscope. Twenty females per infected sugarbeet source were separately inoculated to 20 tomato (Tropic) seedlings that were growing in the 3 X 17-cm polyethylene cone-tainers, filled with an autoclaved mix sandy loam soil and coarse sand. For nematodes from infested soil, tomato seedlings were germinated directly in field soil containers. These inoculated tomato plants were examined for a probable root gall induction in 60 days. Isolates recovered from these procedures were increased for 100-120 days on tomato plants in several 15-cm pots to produce sufficient amounts of inoculum for the differential test plants.

The following plant varieties were used in the differential host assay:

Tomato, *Lycopersicon esculentum* Mill., cv Tropic or Rutgers
Cotton, *Gossypium hirsutum* L., cv Delta-Pine 16
Tobacco, *Nicotiana tabacum* L., cv NC 95
Pepper, *Capsicum frutescens* L., cv California Wonder
Watermelon, *Citrullus vulgaris* Schard, cv Charleston Grey
Peanut, *Arachis hypogaea* L., cv Florunner
Corn, *Zea mays* L., cv field corn

The differential host assay was conducted following a procedure developed by Sasser (1980) and Myers (1990), with minor modifications. Seeds of the designated hosts were planted in 10- or 12.7-cm pulp pots filled with the sterilized soil and sand mix according to a planting schedule chart (Table 2). Tobacco seed was first germinated in a pot of fine vermiculite, and then seedlings were transplanted to pots containing the soil mix. Cotton seed should be scarified with a file before planting.

Table 2. Days before start of *Meloidogyne* species and race identification by differential host assay.

120	90	42	24	0
Start *Meloidogyne* inoculum culture on tomato (Tropic or Rutgers)	Germinate tobacco in vermiculite; start cotton in pots	Plant seed of pepper, peanuts, watermelon; transplant tobacco to soil mix	Plant corn	Harvest nematodes from roots of inoculum culture plants; inoculate test plants in groups.

The root-knot inoculum that has been produced on tomato plants was obtained by removing tomato roots from the pots in a container of water. Root galls were carefully washed free of soil with slowly running tap water. The second-stage juveniles (J2) were recovered with the use of a mist extraction apparatus, a cabinet in which Baermann funnels were set up containing the galled-roots under an intermittent mist (Ayoub, 1980). Freshly hatched juveniles were collected daily for inoculations, 1000 juveniles per plant. Five or more replicates were used for each host. Plants were grown in greenhouses maintaining 25-30 C for 50 to 60 days.

Roots of all test plants were examined for the degree of root galling and the presence of egg masses. Gall counts were classed as a previous study (Yu, 1995; modified from Taylor and Sasser, 1978), by a scale consisting of 0 = zero, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >101galls/plant observed. The scores of galls (and egg masses) for each replicate were recorded separately. The average root-knot gall and egg mass score for each host differential was then calculated to determine susceptibility. A score of 0, 1 or 2 was designated negative (-) and scores of 3, 4 and 5 were designated positive (+). Compared to the results with Sasser's (1980) differential host test reactions as in Table 3, a *Meloidogyne* species or race was tentatively identified. Isozyme analysis to further confirm the *Meloidogyne* species (not races) of selected nematode samples was conducted in a nematology laboratory at the University of California, Davis.

Breeding sugarbeet resistance to root-knot nematode started in the early 1990s. Due to a lack of resistance materials, the first step taken in this project was to identify a resistant germplasm. A wide variety of cultivated and noncultivated *Beta* lines and accessions were screened to search for resistance to *Meloidogyne* spp. The identified resistance was isolated and introgressed to sugarbeet through hybrid crosses in the greenhouse. The strength and spectrum of such host-plant resistance was investigated via inoculation against the four most commonly occurring root-knot nematode species, i.e., *M incognita, M. javanica, M. arenaria,* and *M. hapla*. Field trials of the resistant derivative families were conducted to evaluate sugarbeet performance under infested soil conditions.

Table 3. Positive (+) and negative (-) reactions of the differential host test.

Meloidogyne species and race	Tobacco NC 95	Cotton Delta-Pine 16	Pepper California Wonder	Watermelon Charleston Grey	Peanut Flor-runner	Corn field
M. incognita						
Race 1	-	-	+	+	-	+
Race 2	+	-	+	+	-	+
Race 3	-	+	+	+	-	+
Race 4	+	+	+	+	-	+
M. javanica	+	-	-	+	-	+
M. arenaria						
Race 1	+	-	+	+	+	+
Race 2	+	-	-	+	-	+
M. hapla	+	-	+	-	+	-
M. chitwoodi	-	-	-	-	-	+

Results and discussion

In most cases, nematode females and egg masses were detected and extracted from root galls of the infected sugarbeet samples (Table 1). In samples derived from Colusa County, however, no females were found. About 50 days after inoculating one female (and egg mass) each to a tomato seedling, a range of 10 to 70% of the inoculated plants formed root galls. The majority of nematode populations used in this study was developed through this process. On the other hand, only a few isolates were recovered from infested soil by induction of root galls on susceptible hosts. Following the planting schedule (Table 2), sufficient amounts of inoculum were later produced for inoculations to test plants. Scores of positive (+) and negative (-) reactions to specific nematode species and races were obtained from plants of each differential host groups. In comparison to the chart listed in Table 3, there were four *Meloidogyne* species, five races, existing in California sugarbeet growing areas. They were, respectively, *M. incognita* Race 2 and Race 4, *M. javanica, M arenaria* Race 2, and *M. hapla* (Table 4).

Even though the available sample sizes were small, the generated information is important. Root-knot nematodes were existent in at least 11 sugarbeet growing counties in California, in 1990s (Table 4). Among the four nematode species, *M. incognita* was the most widely spread. Incidence of root-knot nematode occurrence also was reported in

several other counties, especially in Kern and Kings County; unfortunately, we were unable to obtain samples from these areas at the time of this investigation.

Table 4. *Meloidogyne* species identified in California sugarbeet production counties – a preliminary survey.*

M. incognita	M. javanica	M. arenaria	M. hapla
Merced	Fresno	Yolo	Monterey
Monterey	Imperial	Yuba	
Orange	San Joaquin		
Riverside	Merced		
Sacramento			
San Joaquin			
Tulare			

*Samples from some other counties, e.g., Kern and Kings, were not available for test.

Progress has been made on breeding sugarbeet resistant to root-knot nematode. Through inoculation and examination of wide varieties of sugarbeet genotypes and wild *Beta* materials, host-plant resistance to root-knot nematode was identified from the noncultivated sea beets, *B. vulgaris* ssp. *maritima* (L.) Arcang accessions (Yu, 1995). The resistance was determined to be effective against multiple species and races of *Meloidogyne* spp., including *M. incognita, M. javanica, M arenaria,* and *M. hapla* (Table 5), that have undergone inoculation study. These were exactly the same four species of root-knot nematodes currently identified in California sugarbeet fields (Table 4).

Table 5. Formation of root galls in *Meloidogyne* spp. resistant *Beta* lines inoculated with *M. incognita, M. arenaria, M. javanica* and *M. hapla* J2 combined.

Genotypes of *Beta*	No. of Plants	Susceptibility levels*					
		0	1	2	3	4	5
660-2	39	3	2	9	8	10	7
83A5	48		3	25	14	1	5
1614	45	2	4	15	10	6	8
2013	50		6	16	13	8	7
5802	40	6	2	14	14		4
58A2	41		1	11	6	3	20

*Based on gall counts: 0 = zero; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = >101 galls/plant.

Hybrid crosses between resistant wild beets and cultivar sugarbeet have been successfully conducted in the laboratory and greenhouse. Promising derivatives with stable root-knot nematode resistance transmission were generated and selected for further development. Among progeny groups of later generations, intensity of certain undesirable characteristics of sea beet, e.g., the annual bolting habits and sprangled root systems, decreased as the number of backcross generations increased. Positive results were demonstrated by the improved shape and size of tap root formation and root yield enhancement.

The infestation of root-knot nematodes in the United States was known to occurred in at least twelve sugarbeet producing states (Caveness, 1959). When root-knot nematode resistant varieties are developed, growers should be able to plant sugarbeet crops in the fields of their choice. Planting resistant sugarbeet varieties not only suppresses nematode populations in the soil, but it also simplifies crop rotation practices. It will relieve the limit of nonhost rotation farming options in California.

Summary

The status of root-knot nematode distribution in California sugarbeet fields was investigated. Samples of the galled plants and infested soil were collected from various major growing areas. To identify the specificity of *Meloidogyne* spp., nematodes were initially recovered with the use of susceptible hosts. Matured females and egg masses were extracted from infected plants and inoculated to individual tomato seedlings that were growing in cone-tainers; for nematodes from infested soil, seedlings were germinated directly in pots containing the field soil to induce galling. Isolates recovered from these procedures were increased to build populations. They were then inoculated to groups of test plants for differential host assay. The results indicated that the four most common species of root-knot nematode, i.e., *M. incognita, M. javanica, M. arenaria,* and *M. hapla*, were currently existent in California sugarbeet growing areas, occurring in eleven or more counties. Genetic sources of resistance to root-knot nematode is now available. Due to its multi-species resistance capability, sugarbeet production may be protected from serious root-knot nematode damages when the resistance is eventually incorporated into a commercial variety.

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