Project Number: 88-W2

Summary of Research pertaining to *Prunus* Rootstock Development: Screening for Resistance/Tolerance to Root Lesion nematode (*Pratylenchus vulnus*) and Ring nematode (*Criconemella xenoplax*) during 1988.

By Craig Ledbetter

Introduction

Developing nematode resistant rootstocks for stone fruits is a major goal of the USDA Horticultural Crops Research Laboratory in Fresno California. Prior work emphasized resistance to root-knot nematodes (*Meloidogyne* sp.). Cultivars 'Nemaguard' and 'Nemared' were released in 1959 and 1981, respectively to provide growers with root-knot resistant stocks. Before the release of these cultivars, root-knot nematodes were by far the most damaging nematode problem in the California stone fruit industry.

Since root-knot resistant cultivars are readily available, emphasis has shifted toward developing stocks resistant to root lesion (*Pratylenchus vulnus*) and ring (*Criconemella xenoplax*) nematodes. These nematodes are currently inflicting economic losses on the stone fruit industry. A recent survey of almond orchards in the San Joaquin Valley has indicated that over 25% are infested with *P. vulnus*. Ring nematode association with stone fruit roots has now been documented to predispose trees to bacterial canker complex.

Screening procedures are now available for root lesion, root-knot, and ring nematodes. The message from industry is clear that future rootstocks must be resistant/tolerant to all these nematodes. In 1988, the USDA began actively screening candidate rootstocks for resistance/tolerance to these pests.

I. Standardized procedures for lesion nematode screen.

Plant materials used in this screen are rooted softwood cuttings. Cuttings are obtained from active growth of field grown candidate rootstocks. Adequate quantities of wood are best obtained from mid-May through the end of June. Prior experience has shown that successful rooting declines if wood is collected between July and September, although cuttings taken during October will again root adequately.

Shoots removed from candidate rootstocks are cut into 6" segments. The basal end of the cuttings are dipped into a solution of 1000 ppm indole butyric acid to facilitate production of roots. Dipped cuttings are placed in a pre-moistened mixture of perlite and vermiculite (1:1). The cuttings are then held in a partially shaded greenhouse equipped with a mist system that operates at a frequency of 15 seconds every 30 minutes.

Rooting of the softwood cuttings usually takes from three to five weeks. Rooted cuttings are carefully removed from the misting bench, and transplanted into a 3:1 mix of sterile sand:sterile sandy loam which allows nematode establishment. Twenty rooted cuttings are usually planted in this soil mix to make certain that there are at least 10 actively growing plants which is the minimum number necessary to achieve satisfactory accuracy in this screen.

Inoculum for the screen is obtained by extracting the nematodes from roots of bitter almond. Previous work identified several bitter almond selections that were extremely susceptible to lesion nematode. These inoculated plants are held in the greenhouse and used as a 'mother block' of inoculum. Nematodes are extracted from the bitter almond roots with a misting system. The liquid extract is

collected, and sampled to determine the number of nematodes present per volume of extract (titer). In this manner, a known number of live nematodes can be inoculated with a high degree of accuracy.

Inoculated plants are carefully managed in the greenhouse to provide ample light, nutrients and water for maximum plant development. As stated earlier, a minimum of 10 actively growing cuttings are necessary for each candidate rootstock being run through the screen. Half of the cuttings are harvested after 90 days (short term), and the remaining plants are harvested after 250 days (long term). The short term screen yields data on the ability of the nematode to penetrate root tissue of candidate rootstocks. The nematode's ability to establish an active population on the root system is observed from root and soil samples of long term plants.

Plants are destructively sampled at harvest. Fresh and dry weights of both roots and shoots are obtained. Nematodes are extracted from both soil and roots to provide an indication of tolerance and/or resistance. Live nematodes per gram of root (fresh weight) has been demonstrated to be an accurate indication of lesion nematode tolerance or susceptibility.

II. Ring nematode screening procedures.

Screening procedures for ring nematode (*Criconemella xenoplax*) have been developed by USDA personnel in Byron, GA. Survivors from a field having a high incidence of peach tree short-life are currently being put through the screen at Byron. Genotypes appearing to have some degree of resistance/tolerance to ring nematode have been sent to Fresno for propagation and future testing in the lesion screen.

Currently there are several experiments set up to examine ring nematode screening procedures as they pertain to California conditions. Procedures for nematode extraction have been examined to identify a method that will maintain a high degree of live, active nematodes. There are three extraction procedures being tested. First, nematodes are simply washed out of infested soil with water. We believe that this is a very mild treatment, and produces low nematode mortality. The second extraction procedure, sugar flotation, is used by USDA personnel in Byron, GA. We believe that this is a harsh treatment for the nematodes. When sugar flotation is used, larger numbers of ring nematode are necessary to reduce significantly the amount of dry matter compared to other procedures, all other factors being equal. The third procedure used in this experiment is to simply use infested soil with a known inoculum level. A thoroughly mixed volume of infested soil is sub-sampled and tested for the number of ring nematodes present. With soil titer known, a volume of soil may be applied with confidence that a specific number of nematodes has been inoculated.

Inoculum levels for the ring nematode screen have also been examined. Screening procedures at Byron, GA call for 8,000 sugar extracted nematodes to be inoculated per plant. With different extraction procedures, we believe that the number of nematodes necessary to operate the screen may be reduced. Levels of 100, 1,000, and 10,000 nematodes are being used with each of the extraction procedures. Inoculum tests are being conducted on 'Nemaguard' peach (a susceptible host). We are attempting to identify the lowest level of nematodes necessary to produce a significant amount of dry matter reduction in a given period of time.

III. Current screening of rootstocks for resistance/ tolerance to lesion nematode.

Seven candidate rootstocks were inoculated with 150 lesion nematodes per cutting on 5 August 1988. The first harvest (short term) was accomplished on 3 November 1988. Data obtained from this harvest are presented below in Table 1.

Significant differences were noted between candidate rootstocks after 90 days. The rootstock accessions S60-1, PI442380, SL1410 and SL1665 had significantly fewer nematodes per gram of root than the candidate rootstock 14DR51. It should also be noted that PI442380 had significantly greater weight of tops than all other tested selections. No differences in root growth between the candidate rootstocks were evident after 90 days.

Cuttings from 11 other *Prunus* accessions were placed in the mist greenhouse for root development on 11 October 1988. Most cuttings had rooted by mid-November. However, vegetative buds did not immediately break and required treatment with gibberellic acid. It appears that cuttings should be ready to inoculate by mid-December. *Prunus* species and hybrids included in this set of cuttings are:

Accession	**Description**
P. mira	smooth pit peach
P. simonii	apricot plum
P85-203	*P. texana* hybrid
P. subhirtella	higan cherry
P. blirieana	*P. cerasifera* cv 'atropurpurea' X *P. mume*
P. spinosa	blackthorn, sloe
Winered	*P. saliciana* X *P. besseyi*
P. maritima	eastern beach plum
B 5-13	*P. besseyi* X *P. tomentosa*
P. mume	Japanese apricot
P60-100	B 5-13 open pollinated

Table 1. Averages of growth parameters and *Pratylenchus vulnus* counts for seven candidate rootstock and control ('Nemaguard') at 90 days after inoculation. Plants were inoculated with 150 live nematodes on 5 August 1988.

	Fresh weight of		Number of Nematodes		
Selection	Tops (g)[x]	Roots (g)[y]	Soil	Roots	per gram root
S60-1	1.4 b[z]	5.5	8	251 bc	51 b
PI442380	5.5 a	5.6	70	421 abc	75 b
TN R2	0.6 b	3.6	176	316 abc	143 ab
Nemaguard	1.9 b	5.9	67	497 ab	157 ab
Lovell #2	1.5 b	4.8	94	367 abc	89 ab
14DR51	1.4 b	3.4	70	571 a	185 a
SL 1410	1.9 b	4.7	15	204 c	48 b
SL 1665	2.3 b	4.2	12	293 abc	75 b

[x] Variable consisted of only active growth produced by the cuttings after inoculation.

[y] Variable consisted of all below ground portions of the cutting.

[z] Means followed by the same letter within a column are not significantly different at the 0.05 level according to the Duncan's Multiple Range test.

IV. Status of Candidate Rootstock Materials.

During 1988, candidate rootstock accessions from the USDA Byron, GA station have been successfully established at the USDA station in Fresno. It appears that many of these trees will have ample wood by June 1989 for testing in the lesion nematode screen. A listing of these accessions, as

well as other accessions to be tested during 1989, is presented below (Table 2).

Table 2. Candidate rootstock accessions established at USDA Fresno, CA to be screened for lesion nematode resistance/tolerance in 1989.

Accession	Description
FL 4-1-11	from Fla 14-9 PE open pollinated
FL 2-1-11	"
FL 4-3-16	from Fla 14-18 PE open pollinated
FL 2-3-16	"
SL 2760	Blue Goose PL open pollinated
SL 1089	520-9 PE open pollinated
SL 1090	"
SL 1990	Edible Sloe PL open pollinated
SL 1171	7446-14 PL open pollinated
Baladi #1	PI82413
Transvasal Yellows	PI134151
Bokhara	seedling PE
Yunnan 55885	"
Macedonian Wild	*P. cerasifera*
Etter's Best	*P. subcordata* X *P. domestica*
Bruce	*P. salicina* X *P. angustifolia*
G2S2544-1-2	Korean PE
P2037	*P. besseyi* X Myrobalan

V. Lathhouse test of candidate rootstock for resistance/ tolerance to ring nematode.

A long term lathhouse test of three candidate rootstocks was established at Kearny Field Station in March 1988. The

experiment was designed to evaluate inoculation procedures of ring nematode and examine growth differences between inoculated and uninoculated plants. One of the rootstocks, *Prunus tomentosa*, was determined to be highly resistant to lesion nematode in a previous screen. The lathhouse test will give an indication of *P. tomentosa*'s tolerance to ring nematode.

The experiment was set up with two inoculation procedures; water extracted nematodes, and infested soil. A third treatment was an untreated check. Five plants from each treatment were to be harvested at each of three dates;4, 8 and 12 months after inoculation. Root and shoot weights were obtained, and soil was sampled from each container to examine the populations of ring nematode. 'Nemaguard' peach, 'Citation' hybrid, and *P. tomentosa* were used in the study. The four month evaluation occurred on 18 July 1988. Remaining test plants were pruned back to 18.0cm and allowed to continue growth. On 17 November 1988, plants were sampled for the eight month evaluation. Data from these harvests are presented in Table 3.

There were no significant differences noted between treated and untreated plants with regard to root or shoot weights. This was true on both a fresh and dry weight basis. These data demonstrate some of the complexity involved in screening for ring nematode resistance and why methods must be developed for screening procedures specific to California conditions. Nycezepir et al. (1988) observed significant reductions in dry stem weight of 'Nemaguard' peach six months after inoculation under Georgia conditions. We must first be capable of documenting differences in growth between inoculated and non-inoculated plants before we begin an active screening program.

Table 3. Shoot and root fresh weights (g) from ring nematode treatments in lathhouse test at Kearny Agricultural Center.

Cultivar	Average ± Standard Deviation Water Extract	Infested Soil	Untreated
Four month evaluation			
Nemaguard			
(shoot)	363.8±127.9	328.8±71.5	435.9±114.6
(root)	101.7±31.1	88.7±35.0	104.5±25.5
Citation			
(shoot)	47.5±10.0	--	50.5±6.9
(root)	43.3±17.2	--	49.7±10.7
P. tomentosa			
(shoot)	61.4±29.5	--	94.7±44.5
(root)	41.8±17.5	--	64.4±22.5
Eight month evaluation			
Nemaguard			
(shoot)	113.7±17.9	128.1±44.0	131.3±59.3
(root)	281.3±50.0	255.8±82.8	248.3±90.5
Citation			
(shoot)	88.2±10.2	--	87.7±8.8
(root)	110.4±18.0	--	124.7±45.5
P. tomentosa			
(shoot)	109.8±19.8	--	97.6±16.2
(root)	162.7±40.4	--	130.2±45.0

VI. Summary

A feasible greenhouse screening procedure for the lesion nematode (*Pratylenchus vulnus*) has been developed. Candidate

rootstocks are currently being screened at both 90 and 250 days after inoculation with 150 live adult nematodes. We believe that 40 candidate rootstocks can be screened in 1989. Procedures are still being developed for screening *Prunus* accessions for resistance/tolerance to the ring nematode (*Criconemella xenoplax*). Future releases of rootstock materials should possess resistance/tolerance to root-knot, lesion and ring nematodes.

Peggy & Paul —

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To: Paul LaVine (Almond Board)
Gary Obenauf (Prune Board)
Jon Fields (California Tree Fruit Agreement)
Dave Williams (Cling Peach Board)

From: Craig Ledbetter

Re: Summary of 1988 Research Results

Enclosed are results of research performed during 1988 on project # 88-W2 'Genetic Improvement of Stone Fruit Rootstocks for Resistance to Nematodes'. Currently, the project is going quite well and we are gearing up for some larger scale nematode screening in 1989.

If there are any questions regarding the report, please feel free to contact me at (209) 453-3064.

Sincerely,

Craig Ledbetter
Research Geneticist

Enclosure
cc: Warren Micke
Steve Southwick
Scott Johnson
Ted Dejoung