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GENETIC IMPROVEMENT OF STONE FRUIT ROOTSTOCKS FOR RESISTANCE TO NEMATODES

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ABSTRACT

Since 1988, this project has committed to screening 88 *Prunus* candidate rootstocks for resistance/tolerance or susceptibility to root lesion nematode (*Pratylenchus vulnus*). Currently 82 candidate rootstocks have been inoculated with this soil-borne pest. Accessions have been observed in plums, *Prunus* species, apricots and interspecific hybrids that are poor hosts to the lesion nematode. Data collected during 1990 have indicated that the long term harvest period should be shortened from 250 days to 150 days. We propose that this change in procedure become standard for future screening. A ring nematode (*Criconemella xenoplax*) test was also harvested during 1990. Significant differences in root or shoot growth were observed in three of the four seed propagated candidate rootstocks at harvest (144 days after inoculation). We were successful in obtaining seed from various hybridizations performed during the 1990 bloom. Thirty-six specific crosses were made between commercial rootstocks or root-knot resistant selections and accessions that demonstrated resistance or tolerance to root lesion nematode.

OBJECTIVES

(1) Screen 40 candidate rootstocks for resistance/tolerance to root lesion nematode. (2) Develop standardized procedures for greenhouse screening of candidate rootstocks for resistance/tolerance to ring nematode. (3) Identify lesion nematode resistant germplasm and hybridize with existing root knot resistant rootstocks.

PROCEDURES

Lesion Nematode Screen

Plant materials used in this standardized screening procedure were collected during the summers of 1989 and 1990. Specific names of accessions or cultivars are categorized in Table 1. Softwood cuttings used in the screen were rooted in a 8:1 mixture of perlite:peat. Seedlots to be screened were kept in a sterile environment at 4^{o}C until the emergence of root tips. All plants entering the lesion nematode screen were potted in a 3:1 mix of sterilized sand:sterile sandy loam soil. This non-organic soil mix is necessary to ensure growth and development of both the *Prunus* candidate rootstock and root lesion nematode.

For optimal results, 32 uniform explants of a candidate rootstock are necessary in the lesion nematode screen. Half the plants are used as uninoculated controls while each of the remaining plants are inoculated with 150 live lesion nematodes. Control and inoculated groups are further sub-divided into a short term (90 day) or long term (250 day) harvest period. Thus, a total of eight plants are sampled for both controls and inoculated at each harvest period.

At the designated harvest period total root and shoot fresh weights are measured. Root systems are mist extracted for five days to obtain lesion nematodes infesting the roots. The total number of nematodes extracted is divided by the root weight to standardize nematode numbers. Control and inoculated groups are compared for root weight, shoot weight and nematodes per unit weight of root by standard ANOVA procedures.

Improving efficiency of lesion nematode screen

A greenhouse experiment was conducted during 1990 to address the question of whether or not the long term harvest period could be shortened from 250 days to 150 days. The test was initiated with hopes of reducing overhead costs per accession and thereby improving the efficiency of the screen. Three seed propagated candidate peach rootstocks were used with harvests conducted at 90, 150 and 250 days. In addition, both large and small pot sizes were used for the 150 day treatments. Shoot and root fresh weights, as well as nematode populations infesting the roots, were sampled at each harvest period.

Development of ring nematode screening procedures

A greenhouse test accomplished during 1990 examined the effects of high levels of ring nematode on four seed propagated candidate rootstocks. Ring nematodes were extracted from infested sand using the sugar flotation - centrifugation technique. Each treated plant was inoculated with 10,000 ring nematodes. Harvest occurred 144 days after inoculation. Data collected from this test were root and shoot fresh weights as well as nematode levels per 250 cc of soil.

One other small test examined how soil type affected ring nematode populations on *P. tomentosa* roots. Specifically, the test compared sterile sand (standard ring nematode 'soil') with the standard 3:1 mix (sterile sand:sterile sandy loam) used with lesion nematode. Population levels of ring nematode were compared 120 days after inoculation.

RESULTS

Lesion nematode screen

Tables 2 and 3 document root lesion nematode population increases or decreases on a variety of candidate rootstocks at 250 and 90 days, respectively. Population decreases (below initial number of nematodes applied at inoculation) at the short term harvest have been detected in five of the six major categories. Root lesion nematode populations have dramatically increased on most peach germplasm inoculated to date. For peach germplasm specifically, accessions of 'Pillar' have demonstrated to be the least favorable hosts for root lesion nematode.

Improving efficiency of lesion nematode screen

Table 4 provides numerical data obtained from the time trial experiment. This experiment was set-up to indicate the possibility of whether or not the long term harvest period could be shortened from 250 days to 150 days. The main objections to the 250 day harvest were plant senescence and a confined root space. Studying the growth parameters in Table 4, there are a few interesting points brought out by this data. Roots of both inoculated and control plants continued to grow during the 150 to 250 day period. The effect of 'Pot' was insignificant for roots at 150 days while shoot growth was significantly affected by pot size at this same harvest period (Table 5). In general, we found it much easier to sample plants at the 150 day period than at the end of 250 days. Roots were not nearly as tangled or bound at the earlier harvest date. Plants grown for 250 days also are typically senescent by harvest. Leaf drop is quite common, and most of the plants have not actively grown (in shoots) for the last several months. Given these factors, we feel that the standardized procedures for the lesion screen should be changed. The short term (90 day) harvest period will remain in effect. The long term period should be shortened to 150 days. Since pot size was an insignificant factor in both root growth and NPGR, we would like to use the smaller pot. Reduced pot volume will save the limited greenhouse space and allow more accessions to be screened in the same given area. Changing these procedures will benefit both plant and nematode health as well as reduce total costs of the screening operation.

Further development of ring nematode screening procedures

Experiments conducted in 1989 indicated that sugar flotation - centrifugation was the most effective extraction technique for ring nematodes to be used infesting 'Nemaguard' roots. Using 10,000 ring nematodes per plant, we were able to demonstrate differences in root or shoot growth in three of the four seed propagated candidate rootstocks (Table 6). The interspecific hybrid 'R9.5' was unaffected significantly by the nematode treatment with regard to root or shoot fresh weights. Final nematode populations were quite variable within each accession. Mean population values between these accessions were not different significantly. The population values were a disappointment with this test. It was desired that populations would increase at least three-fold during the 144 day treatment period (as was found on 'Nemaguard' peach in 1989). Final population values declined by more than 90 % for each of the seed propagated accessions.

In all tests to date, we have used sterile sand exclusively for experiments involving ring nematode. This media makes growing *Prunus* even more difficult than the standard 3:1 mix of sterile sand:sterile sandy loam used for root lesion experiments. A small experiment was conducted during 1990 to examine the effects of different soil types on ring nematode. We conducted this test because of the low population levels encountered in the above mentioned seed propagated rootstock test.

Fourteen plants of *P. tomentosa* obtained from softwood cuttings were planted in both 3:1 mix and sterile sand. Both sets were inoculated with 800 ring nematodes per plant. All plants were harvested 120 days after inoculation. Root and shoot fresh weights did not differ significantly for the two treatments. Nematode populations infesting the soil were highly significant with regard to soil type. Nematodes infesting the sterile sand averaged 45±44 per 250 cc while those infesting the 3:1 mix averaged 865±519 per 250 cc. These data were very consistent and we will use the 3:1 mix for ring nematode (as well as root knot and root lesion) in future experiments.

Hybridization to incorporate root knot and root lesion resistance

During the 1990 bloom, only a limited number of screened candidate rootstocks possessed any degree of root lesion resistance/tolerance. Both *P. tomentosa* and *P. japonica* appear to be highly resistant to root lesion nematode. *P. tomentosa* is unsuitable as a female parent for root knot resistant peach pollen. From other rootstock studies, there also appears to be a great deal of graft incompatibility with this stock. Alleged 'hybrid' seed from root knot resistant peach X *P. tomentosa* crosses performed during the 1989 bloom turned out to be self pollinations. We did not utilize *P. tomentosa* during the 1990 bloom.

Recent field studies have demonstrated that certain accessions of *P. japonica* are compatible with a peach scion. This candidate stock has produced viable seed from crosses with root knot resistant peaches. At this time, 11 hybrids involving crosses on *P. japonica* with 'Nemared', 'PI442380' and '7788-147' have been reared to the seedling stage.

Besides the above mentioned *Prunus* species, 'PI442380' (an evergreen peach) was the most root lesion tolerant peach germplasm at the 1990 bloom. Our pollen supply was limited due to the small size of trees, but we were able to incorporate this accession in 7 of the 36 crosses involving root knot and root lesion resistant germplasm.

Looking ahead to the upcoming 1991 bloom, there are more candidate rootstocks available that appear to be unfavorable hosts for root lesion nematode. In particular are a number of promising plum and apricot accessions where root lesion nematode reproduction appeared to be very low or non-existent. Selections of 'Pillar Peach' and two accessions from Florida appear to be the best peach candidates with regard to root lesion resistance/tolerance.

CONCLUSIONS

A total of 82 candidate rootstocks have been inoculated with root lesion nematode. Poor hosts for this soil-borne pest have been identified in plums, *Prunus* species, apricots and interspecific hybrids. Experiments conducted in 1990 indicate that the long term harvest of the lesion screen should be shortened from 250 to 150 days. With regard to screening for ring nematode resistance/tolerance, significant differences in growth of shoots and/or roots were observed in three of the four candidate rootstocks harvested 144 days after inoculation. We have determined that the standard sterile 3:1 soil mix of sand:sandy loam used in the lesion nematode screen is superior to sterile sand with regard to ring nematode establishment. Thirty-six specific crosses were performed between root knot resistant accessions and germplasm that demonstrated resistance or tolerance to root lesion nematode.

Table 1. Candidate rootstocks to be screened for lesion nematode resistance/tolerance during 1989 and 1990.

Prunus sp.	Peach germplasm		Plum germplasm	
*P. blirieana**	95-17*	K62-68*	Red Glow*	3-4*
*P. minutiflora**	Fla. 2-1-11*	K62-67*	Abundance*	Bruce*
*P. orthosepala**	Fla. 2-3-16*	Boone Co. Sdlng.*	Deep Purple	Convoy*
*P. spinosa**	Fla 4-3-16*	9-21*	Lantz	Manor
*P. dasycarpa**	PI442378*	9-26*	Etters Best	K41-10*
*P. texana**	Pillar #67*	F #1	St. Anthony	PI304928
*P. tomentosa**	Pillar #70*	F #4	Toka	
P. fremontii	Pillar # 71*			
P. umbellata				
P. angustifolia				
P. munsoniana				

Standard rootstocks		Almond germplasm	Interspecific hybrids	
GF8.1*	S60-1*	A15.5-36*	P85-202*	Alf 44-18*
Harrow Blood*	Tenn. Nat.*	K3-90*	R9.5*	Alf 46-13*
M2624*	Okinawa*		P114-92*	Alf 46-19*
Pisa #2*	Higama*		S3400*	K187-1*
Myrabi	Montclar		P67-116	68-971*
St. Julian 53.7*	Nemaguard*		P67-154	69-1637P*
Lovell	Nemared		P67-158	7788-147*
GF 43*				

Apricot germplasm	Peach Tree Short Life materials
GF58.7*	SL1089*
PI418541*	SL1090*
PI418552*	SL2009*
PI506389*	SL2760*
PI506392*	SL1410*
Perfection*	SL1665*

* Indicates that the accession has been inoculated with lesion nematode

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Table 2. Average nematodes per gram of root (fresh weight) and population change of *Pratylenchus vulnus* 250 days after inoculation for 27 accessions of *Prunus*. Initial inoculation consisted of 150 live nematodes per plant.

Accession	NPGR[1]	% increase (decrease) from initial inoculum	Accession	NPGR	% increase (decrease) from initial inoculum
		Plums			
4	44	3281	8	33	1707
11	14	59	12	78	8544
15	151	8098	16 Bruce	3	(62)
17	12	155	18	31	418
21	42	838			
		***Prunus* species**			
5	323	23996	7	63	1076
13	32	3695	19 *P. tomentosa*	2	(76)
20 *P. tomentosa*	11	8			
		Apricots			
24	80	4071			
		Interspecific hybrids			
2	81	344	9	34	3707
		Peaches			
26	46	2421	1	96	4471
6	302	12681	22 Fla 4-3-16	26	311
23	33	320	25 Harrow Blood	52	2020
27	48	3568	28Tenn. Nat.	68	1809
29	34	326	3 Pisa #2	74	1105
30 Pillar	11	31			

[1] Nematodes per gram root, fresh weight

Table 3. Average nematodes per gram of root (fresh weight) and population change of *Pratylenchus vulnus* 90 days after inoculation for 67 accessions of *Prunus*. Initial inoculation consisted of 150 live nematodes per plant.

Accession	NPGR[1]	% increase (decrease) from initial inoculum	Accession	NPGR	% increase (decrease) from initial inoculum
		Plums			
58 M2624	64	281	59	129	270
60	124	420	61	1	(91)
62	70	280	63	108	433
64	80	297	65	45	127
66	68	223	67	7	(59)
68	29	31	4	45	644
8	17	34	11	126	68
12	163	519	15 GF43 cutting	157	1093
16	6	(69)	17	13	(46)
18	69	563	21	29	135
32 GF43 seed	316	1604	33	116	116
41	77	204	42 St. Julian 53.7	243	547
49	18	(36)	52	24	(16)
		***Prunus* species**			
5	123	1113	7	80	239
13	240	588	19 *P. tomentosa*	1	(89)
20 *P. tomentosa*	2	(89)	43	57	198
		Apricots			
24	24	112	45	7	(76)
46	105	117	47	126	41
48	21	(59)	53	2	(94)
		Interspecific hybrids			
2	113	404	9 GF8.1	163	737
36	73	267	37	154	1027
38	119	589	39	29	52
40	17	11	44	26	(18)
54	5	(42)	55	15	2
		Almonds			
57*	66	599	69	121	329
		Peaches			
26	29	138	30 Pillar seed	44	145
31	155	677	50 Pillar cutting	28	(38)
1	72	221	6	207	1404
22	37	46	23	27	38
25 Harrow Blood	58	164	27	37	172
28 Tenn. Nat.	37	228	29	87	237
34	81	528	35 Okinawa	138	913
3 Pisa #2	171	766	51	81	29
56	68	454			

[1] Nematodes per gram root, fresh weight

* Accession 57 was harvested after 150 days.

Table 4. Harvest parameters from 90, 150 and 250 day treatments of three seed propagated candidate rootstocks inoculated with root lesion nematode. Values indicate treatment means ± standard deviations.

	90 Days small pots		150 Days small pots		150 Days large pots		250 Days large pots	
Accession	inoc.	cont.	inoc.	cont.	inoc.	cont.	inoc.	cont.
Fresh Weight of Shoots								
Tenn. Nat.	10.7±1.6	10.4±0.6	5.4±0.5	5.7±1.1	9.5±1.7	10.4±2.3	10.1±1.9	9.3±1.4
95-17	7.1±2.1	6.7±0.7	6.0±0.8	6.4±0.7	9.3±1.0	12.2±2.0	7.9±1.0	11.2±2.5
P70-67	5.6±0.8	6.1±1.4	4.8±1.0	7.3±0.8	7.2±0.8	11.0±2.5	5.1±0.7	7.9±1.7
Fresh Weight of Roots								
Tenn. Nat.	13.4±1.3	11.5±1.2	11.1±1.1	14.5±2.0	21.7±3.8	27.7±5.1	42.3±5.2	37.1±11.5
95-17	5.5±1.7	6.4±0.7	7.9±0.5	10.4±1.4	18.8±2.3	22.5±4.8	18.8±4.1	23.2±2.8
P70-67	8.1±0.8	8.3±1.3	9.9±1.5	14.0±0.9	21.1±1.3	26.3±5.5	17.9±2.1	24.9±3.7
Nematodes per gram Root								
Tenn. Nat.	49±20	- -	176±39	- -	238±37	- -	68±32	- -
95-17	112±78	- -	114±54	- -	195±66	- -	34±35	- -
P70-67	67±48	- -	51±32	- -	145±38	- -	11±13	- -

Table 5. Probability values for various factor/dependent variable combinations obtained from lesion screen time trial experiment.

	Dependent Variable		
Factor	Shoot FW	Root FW	NPGR
150 day harvest - All pots			
Accession	.71	.30	***
Treatment	**	.86	–
Pot	*	.58	.43
AXT	**	.98	–
AXP	.10	.90	.34
TXP	*	.25	–
150 vs 250 days - Large pots only			
Accession	.79	*	*
Treatment	**	.14	–
Date	*	***	.13
AXT	**	.08	–
AXD	*	***	.24
TXD	.41	.27	–
250 days - All large pots			
Accession	***	***	**
Treatment	*	*	–
AXT	*	*	–

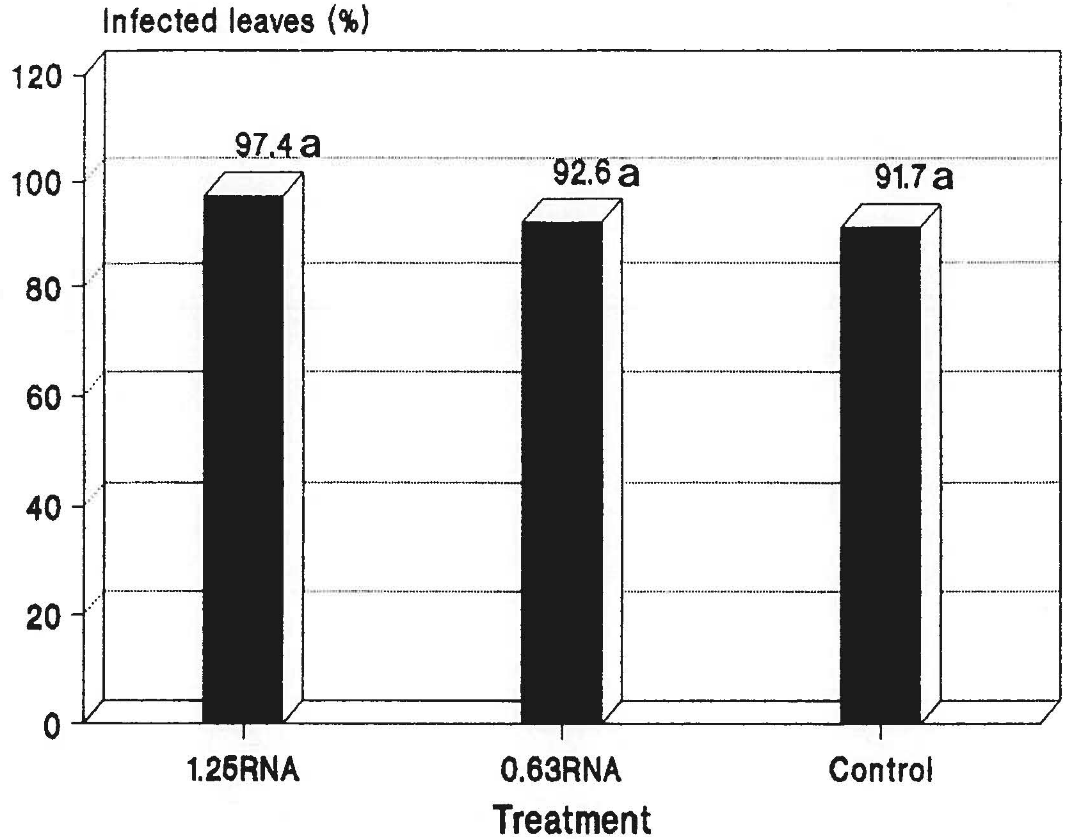
*, **, *** indicate significance at the 0.05, 0.01, and 0.001 levels, respectively

Table 6. Harvest data for four seed propagated *Prunus* accessions inoculated with 10,000 ring nematode (Cx) per plant. Harvest occurred 144 days after inoculation. Tabular values indicate treatment means ± standard deviations.

Accession	Treatment	FW shoots	FW roots	Cx/250cc soil
R 9.5				
	inoculated	15.2±4.6	18.1±6.2	463±513
	control	14.1±6.2	18.5±10.0	--
Harrow Blood				
	inoculated	14.7±1.8	24.4±1.8	379±262
	control	17.1±1.8**	29.6±3.1***	--
95-17				
	inoculated	17.0±2.3	22.0±2.8	821±658
	control	20.8±2.5**	29.0±4.4***	--
P70-67				
	inoculated	14.3±1.9	28.0±2.8	587±508
	control	15.9±2.3	36.4±3.8***	--

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 levels, respectively

Effect of RNA-TRIAD* ON SHOT-HOLE DISEASE (*Stigmina carpophila*)



*Inoculated and then treated

Figure 2B