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University of California
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# Project Plan/Research Grant Proposal

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roject Title Genetic Improvement of Stone Fruit Rootstock for Resistance to Nematodes

eywords lesion nematode, root knot nematode, ring nematode, screening, hybridization

ommodity(s) almond, apricot, peach, plum prune Relevant AES/CE Project No. N/A

roblem and Its Significance:

A 1986 survey by Dr. Mike McKenry of almond orchards in the San Joaquin Valley has indicated that over 25% have economically damaging levels of lesion nematode (*Pratylenchus vulnus*). This nematode is currently inflicting economic losses on the California stone fruit industry. Currently, there are no available rootstocks compatible with scion varieties and resistant/tolerant to lesion nematode. Of the sandy orchard soils in San Joaquin, Stanislaus and Merced counties, more than half are infested with high levels of ring nematode (*Criconemella xenoplax*). The ring nematode can occur simultaneously with lesion nematode which necessitates resistance/tolerance to both pests in the same rootstock. Future widespread chemical controls of these pests is unlikely due to public concerns, and Environmental Protection Agency guidelines restricting contamination of groundwater with nematicides. The most efficient and environmentally sound solution to this dilemma is the development of nematode resistant rootstocks.

Objectives:

1. Use standardized procedures to screen 40 candidate rootstocks for resistance/tolerance to root lesion nematode.

2. Challenge four candidate rootstocks with high levels of sugar flotation centrifugation extracted ring nematodes for short term greenhouse test.

3. Hybridize germplasm identified as root lesion resistant/tolerant with known sources of root knot resistance during the 1990 bloom.

Plans and Procedures:

Standardized procedures for lesion nematode resistance/tolerance screening were developed by Dr. Dennis Culver in 1986. These procedures will be used to screen 40 candidate rootstocks during 1990. Materials identified as being resistant/tolerant to the lesion nematode in previous greenhouse screens will be crossed with root-knot resistant stocks during bloom, 1990. Candidate rootstocks that appear especially promising in the greenhouse screen will be field tested. Evaluation criteria for the lesion nematode screen include: nematodes/g root (fw), as well as active growth of shoots and roots. Evaluation occurs as 90 and 250 s after inoculation. Plants inoculated with lesion nematode will be compared with non-inoculated controls using standard ANOVA procedures.

1990 Budget Summary (Proposal)

I. Breakdown of expenditures

A. Salaries

student help - 1 person, full time, 12 weeks @ $6.00/hr — **$2880.**

B. Supplies

1. Greenhouse pots - 1.5, 4 and 6" — **$500.**
2. Soil supplies (perlite, peat, sand) — **$500.**
3. Chemicals (fungi., insecta., fert., disinfect.) — **$300.**
4. Photography and reproduction — **$100.**

C. Services

1. Nematode sampling
   - a) lesion screen
     (40 accessions)(2 dates)(8 plant/date)($10./plant) — **$6400.**
   - b) ring screen development
     (4 accessions)(1 date)(10 plants/date)($20./plant) — **$800.**
   - c) lesion inoculum
     (40 accessions)(2 dates)(8 plant/date)(150 nematodes/plant)=960,000 nematodes
     can obtain ~3000 nematodes/host plant
     960,000/3000=320 host plants needed
     (320 hosts)($10./host) — **$3200.**
   - d) ring inoculum
     (4 accessions)(10 plants/accession)(10,000 nematodes/plant)=400,000 nematodes
     can obtain ~ 8000 nematodes/liter soil
     400,000/8000=50 liters soil needed
     (50 L)($20./L - sample) — **$1000.**

D. Equipment

1. structural plastic benchtops for shadehouse
   10, 3' X 8' sections @ $30. each — **$300.**

E. Travel

work group meetings, annual conferences. — **$400.**

**Total 1990 proposed budget $16,380**
**($16500)**

II. Carryover funds

A trust agreement has been set-up with the Almond Board of California, California Prune Board and California Tree Fruit Agreement. The California Prune Board committed $2300. during FY89 to this project. However, the monies are not yet in place. This money has been earmarked for nematode sampling of candidate rootstocks in the 1989 lesion nematode screen.

# GENETIC IMPROVEMENT OF STONE FRUIT ROOTSTOCKS FOR RESISTANCE TO NEMATODES

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## ABSTRACT

Sufficient plant materials were collected during the summer of 1989 to screen 40 candidate rootstocks for resistance/tolerance to root lesion nematode (*Pratylenchus vulnus*) using standardized procedures developed during 1986. In 1988, eight candidate rootstocks were successfully screened for resistance/tolerance to this pest. PI442380, an evergreen peach, appears to have a high degree of tolerance to root lesion nematode. Screening procedures for ring nematode (*Criconemella xenoplax*) have not yet been finalized; however, it was demonstrated that sugar flotation - centrifugation is the most effective extraction procedure for establishing ring nematode populations on candidate rootstocks. Forty-three specific crosses were performed at bloom, 1989 to combine root knot and root lesion nematode resistance. Some hybridizations have been successful.

## OBJECTIVES

(1) Utilize existing standardized procedures to 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 (Culver *et al*, 1989) were collected during the summer of 1989. Specific names of accessions or cultivars are listed, by category, 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°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 clones of a candidate rootstock are necessary in the lesion nematode screen. Half the plants are used as non-inoculated controls. The remainder of the plants are each 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.

Plants are destructively sampled at the designated harvest period. Total root and shoot fresh weights are obtained. Root systems are mist extracted for seven 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. Resistant, tolerant and susceptible candidate rootstocks are defined as follows:

Resistant: Root and shoot weights of inoculated plants are not significantly different from untreated controls. Nematodes not present in roots.

Tolerant: Nematodes present in roots. No significant difference in root or shoot weight between inoculated and untreated controls.

Susceptible: Nematodes present in roots. Root and shoot weight of inoculated plants are significantly less than untreated controls.

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

| *Prunus* sp. | Peach germplasm | Plum germplasm |
|---|---|---|
| *P. blirieana* | 95-17 | 3-4 |
| *P. minutiflora** | Fla. 2-1-11* | Bruce* |
| *P. orthosepala** | Fla. 2-3-16* | Convoy |
| *P. spinosa** | Fla 4-3-16* | GF 43* |
| *P. dasycarpa** | PI442378* | K41-10* |
| *P. texana* | Pillar #67 | PI304928 |
| | Pillar #70 | |

| Standard rootstocks | Interspecific hybrids |
|---|---|
| GF8.1* | Alf 44-18 |
| Harrow Blood | Alf 46-13 |
| M2624* | Alf 46-19 |
| Pisa #2* | K187-1* |
| S3400 | 68-971* |
| St. Julian 53.7 | 69-1637P |
| | 7788-147* |

| Apricot germplasm | PTSL materials |
|---|---|
| GF58.7 | SL1089* |
| PI418541 | SL1090* |
| PI418552 | SL2009* |
| PI506389 | SL2760* |
| PI506392 | |

* Indicates that the accession has been inoculated with lesion nematode

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*Development of Ring Nematode Screening Procedures*

A large greenhouse experiment was performed during 1989 to examine the effects of inoculum level and extraction procedure on 'Nemaguard' peach. Specifically, ring nematode inoculum sources included: 1) sugar flotation - centrifugation, 2) water elutriation, 3) infested soil. Inoculum levels included an untreated control as well as 100, 1,000 and 10,000 nematodes per plant. Treatments were evaluated for root and shoot fresh weight as well as numbers of nematodes present per 250 cc of soil. Plants were harvested at 4 months and again at 8 months. Due to physical limitations in the experimental design, not all inoculum levels were used for each inoculum source at each harvest period.

*Hybridization to Incorporate Root Knot and Root Lesion Resistance*

Many interspecific crosses were planned for the 1989 bloom. Table 2 identifies accessions or cultivars used as either parent in crosses designed to incorporate multiple nematode resistance. Large pollen samples of late blooming accessions were collected, dried and stored for use in crosses during the 1990 bloom. This will make future hybridizations possible between early and late blooming accessions.

Table 2. Cultivars or germplasm accessions used in crosses to incorporate root knot and root lesion resistance during the 1989 bloom.

| Root knot resistant | | Root lesion resistant/tolerant |
|---|---|---|
| Nemaguard | Nemared | *P. japonica* |
| S2535 | GF557 | *P. tomentosa* 'Orient' |
| Red Ran Sdlng. | P115-102 | SL1665 |
| Rutgers Red Leaf | Okinawa | B5-13 |
| P60-100 | K41-10 | B10-13 |

RESULTS

*Lesion Nematode Screen*

Results from the long term harvest of 1988 candidate rootstocks screened for lesion nematode resistance were obtained in April, 1989. Non-inoculated controls were not planted for comparison at the onset of this test. Therefore, direct comparisons in growth parameters between treated and control plants could not be made. Three separate ANOVA were performed to compare shoot weight, root weight, and nematodes per gram of root for the eight candidate rootstocks. Results are presented in Table 3.

Table 3. Averages of growth parameters and *Pratylenchus vulnus* counts for eight candidate rootstocks at 2 dates after inoculation. Plants were inoculated with 150 live nematodes on 5 August 1988. Fresh weights (FW) are expressed in grams.

| | 90 days after inoculation | | | 250 days after inoculation | | |
|---|---|---|---|---|---|---|
| Candidate | FW shoots | FW roots | # Nemas[y] | FW shoots | FW roots | # Nemas |
| S60-1 | 1.4 b[z] | 5.5 | 51 b | 1.8 c | 20.6 c | 59 |
| PI442380 | 5.5 a | 5.6 | 75 b | 227.8 a | 96.5 a | 85 |
| TN R2 | 0.6 b | 3.6 | 143 ab | 2.5 c | 12.2 c | 128 |
| Nemaguard | 1.9 b | 5.9 | 157 ab | 1.9 c | 13.7 c | 55 |
| Lovell #2 | 1.5 b | 4.8 | 89 ab | 3.3 c | 16.6 c | 44 |
| 14DR51 | 1.4 b | 3.4 | 185 a | 1.4 c | 9.0 c | 144 |
| SL1410 | 1.9 b | 4.7 | 48 b | 3.1 c | 11.3 c | 103 |
| SL1665 | 2.3 b | 4.2 | 75 b | 34.4 b | 48.4 b | 103 |

[y] Data expressed as live nematodes per gram FW of roots

[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. Columns without letters denote no significance between treatment means within the column.

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Although comparisons of inoculated vs non-inoculated plants are not possible, the candidate rootstock PI442380 appears tolerant of root lesion nematode. Shoot weights were significantly higher than all other accessions at both 90 and 250 days after inoculation. Roots weights were also significantly higher than the other accessions tested at the long term harvest. There were significant differences in nematode counts between candidate rootstocks at 90 days, but not at 250 days. Heavy emphasis will be placed on this candidate rootstock with regard to hybridization during the 1990 bloom period.

Twenty-one of the 40 candidate rootstocks to be screened during 1989 have already been inoculated with lesion nematode. Results from the short term harvest period are available for six of these 21 accessions (Table 4). Significant differences between inoculated plants and controls have been noted for shoot weight of PI442378, as well as root weight of Pisa #2.

*Development of Ring Nematode Screening Procedures*

Results pertaining to the development of ring nematode screening procedures were somewhat disappointing. Table 5 shows shoot fresh weights and nematode populations infesting 'Nemaguard' roots after 4 and 8 months. Even at the highest rate of inoculum, there were no significant differences in shoot weights between inoculated plants and controls.

Positive results were obtained, however, in determining the most effective means of establishing ring nematode populations on candidate rootstocks. We believed that sugar flotation-centrifugation treatment recommended by Nyczepir *et al* (1987) was harsh and led to nematode mortality during extraction. It was inferred that water elutriation or the use of infested soil as inoculum would yield a lower mortality rate. This would allow the inoculation of fewer nematodes per plant, reduce costs and produce a more efficient screening technique.

Table 4. Initial results for shoot and root fresh weight (g) at 90 day harvest of six candidate rootstocks being screened for lesion nematode resistance. Inoculated plants had 150 live *Pratylenchus vulnus* applied to soil at the onset of test.

| Accession | Shoot FW | Root FW | Nematodes /g root FW |
|---|---|---|---|
| PI442378 | | | |
| inoculated | 4.0±1.7 * | 6.7±1.8 | 72±55 |
| control | 6.1±1.5 | 7.4±0.8 | – |
| K187-1 | | | |
| inoculated | 4.4±3.2 | 6.4±2.1 | 113±75 |
| control | 8.9±3.5 | 9.1±3.0 | – |
| Pisa #2 | | | |
| inoculated | 3.7±0.5 | 7.6±0.9 * | 171±107 |
| control | 4.2±0.9 | 9.0±0.8 | – |
| *P. spinosa* | | | |
| inoculated | 13.1±2.2 | 14.8±2.2 | unavailable |
| control | 12.6±3.4 | 14.0±2.7 | – |
| Fla. 2-3-16 | | | |
| inoculated | 4.2±1.9 | 10.9±4.6 | unavailable |
| control | 4.1±1.2 | 10.6±2.9 | – |
| 68-971 | | | |
| inoculated | 11.4±1.2 | 24.8±3.9 | unavailable |
| control | 10.3±1.3 | 22.7±1.6 | – |

* Indicates significance at 0.05 level.

Results from this test clearly indicate that inoculum from the sugar flotation - centrifugation procedure is superior to inoculum from water elutriation or infested soil in establishing ring nematode populations on roots of 'Nemaguard' peach (Table 5). The sugar flotation inoculum increased population levels three and thirteen-fold over initial inoculum levels (1000 nematodes) at the 4 and 8 month harvest, respectively. Population level differences were significant at the 0.01 level. The sugar flotation - centrifugation extraction method will be used exclusively in future ring nematode experiments.

Table 5. Shoot FW (g) of 'Nemaguard' peach and ring nematode populations per 250 cc soil at 4 and 8 months as affected by type and level of inoculum.

| Inoculum type | Initial inoculum level 0 | 100 | 1,000 | 10,000 |
|---|---|---|---|---|
| | | Shoot FW - 4 month harvest | | |
| Sugar flotation | 3.9±0.9 | – | 3.1±0.4a* | – |
| $H_2O$ elutriation | 3.2±0.7 | 2.7±0.7 | 3.1±0.5a | 2.6±0.5 |
| Infested soil | 4.4±0.9 | 4.0±1.0 | 3.1±0.5a | – |
| | | Nematode population - 4 month harvest | | |
| Sugar flotation | 0 | – | 3,038±2,683a | – |
| $H_2O$ elutriation | 1.7±2.7 | 663±295 | 1,089±460 b | 2,769±970 |
| Infested soil | 0.1±0.3 | 12±9 | 168±117 c | – |
| | | Shoot FW - 8 month harvest | | |
| Sugar flotation | 141±41 | – | 61±56a | – |
| $H_2O$ elutriation | 157±39 | 70±70 | 101±70a | 88±68 |
| Infested soil | 73±65 | 127±55 | 103±71a | 103±77 |
| | | Nematode population - 8 month harvest | | |
| Sugar flotation | 2.6±3.0 | – | 13,892±16,953a | – |
| $H_2O$ elutriation | 0.2±0.4 | 412±300 | 1,019±825 b | 2,386±1,237 |
| Infested soil | 0.3±1.0 | 236±180 | 783±395 b | 1,877±2,097 |

* Means and standard deviations followed by the same letter within a shoot FW or nematode population block are not significantly different at the 0.05 level according to a Duncan Multiple Range test.

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*Hybridization to Incorporate Root Knot and Root Lesion Resistance*

Forty-three specific crosses were performed at bloom, 1989 to incorporate root knot and root lesion resistance. *Prunus tomentosa* and *P. japonica* were used as a source of root lesion resistance. Many standard rootstocks and advanced selections were used as parents for their root knot nematode resistance. Table 6 lists the specific crosses performed during the 1989 bloom.

Observation of fruit set, and numbers of hybrid seed obtained from these crosses have given us an idea of their efficiency as parents. *Prunus tomentosa* appears to be an unsuitable female parent for hybridization with peach germplasm. Trees having *P. tomentosa* as a background genotype in their pedigree were also unreceptive to the various applied peach pollens. Hybrid seed was obtained, however, in reciprocal crosses where *P. tomentosa* pollen was placed on stigmatic surfaces of peach. Hybrid seed was obtained from many *P. japonica* X peach crosses. Frequencies of set were extremely low but the crosses were successful and many more hybridizations of this type are planned in 1990. One hybrid plant has survived through the seedling stage and will be planted in the field next spring. The pedigree of this hybrid is *P. japonica* X GF557. All other hybrid seed has just been planted (root knot resistant peach X *P. tomentosa*) and it will be several weeks before we are able to determine whether or not surviving plants are in fact hybrids.

Table 6. Crosses performed at USDA station in Fresno, CA during 1989 bloom to incorporate root knot and root lesion resistance.

| | |
|---|---|
| *P. japonica* X Nemared | *P. tomentosa* X Nemared |
| *P. japonica* X Rutgers Red Leaf | *P. tomentosa* X Nemaguard |
| *P. japonica* X Red Ran Sdlng. | *P. tomentosa* X Lovell |
| *P. japonica* X S2535 | *P. tomentosa* X K41-10 |
| *P. japonica* X GF557 | *P. tomentosa* X Red Ran Sdlng. |
| *P. japonica* X Lovell | *P. tomentosa* X Rutgers Red Leaf |
| *P. japonica* X Okinawa | *P. tomentosa* X S2535 |
| *P. japonica* X P60-100 | *P. tomentosa* X Okinawa |
| | *P. tomentosa* X GF557 |
| Red Ran Sdlng X *P. tomentosa* | P60-100 X Lovell |
| Red Ran Sdlng X GF557 | P60-100 X Nemared |
| Red Ran Sdlng X K41-10 | P60-100 X K41-10 |
| Rutgers Red Leaf X *P. tomentosa* | Nemaguard X *P. tomentosa* |
| Rutgers Red Leaf X Okinawa | Nemaguard X SL1665 |
| Rutgers Red Leaf X GF557 | Nemared X *P. tomentosa* |
| Rutgers Red Leaf X Lovell | Nemared X SL1665 |
| P115-102 X *P. tomentosa* | B5-13 X K41-10 |
| P115-102 X SL1665 | B5-13 X *P. tomentosa* |
| B10-13 X *P. tomentosa* | Lovell X Nemared |
| B10-13 X Nemared | Lovell X *P. tomentosa* |
| B10-13 X Rutgers Red Leaf | Lovell X SL1665 |
| | Lovell X GF557 |
| | Lovell X K41-10 |

## CONCLUSIONS

One of eight candidate rootstocks screened for lesion nematode resistance/tolerance during 1988 appears tolerant of this pest. This rootstock, identified as 'PI442380', will be used in hybridizations during the 1990 bloom to incorporate lesion nematode tolerance into existing root knot nematode resistant rootstocks. Twenty-one of the 40 candidate rootstocks to be screened during 1989 have been inoculated with lesion nematode. Six of these accessions have completed their short term screening period. Active root lesion nematodes were extracted from the root systems of each of these candidate rootstocks. During 1989, it was demonstrated that inoculum from the sugar flotation - centrifugation method is most effective for establishing ring nematode populations on candidate rootstocks. This method will be used exclusively in the future. Planned hybridizations during the 1989 bloom included 43 specific crosses to incorporate root knot and root lesion nematode resistance into a single rootstock.

## REFERENCES


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