Techniques

Improved Mechanical Transmission of Tomato Ringspot Virus to *Prunus* Seedlings

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ABSTRACT

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The efficiency of different methods to transmit tomato ringspot virus (TmRSV) to *Prunus* seedlings (mainly peach) was compared in the greenhouse. Virus infection was determined by enzyme-linked immunosorbent assay (ELISA). Treatments varied as to virus isolate, inoculum source and preparation, seedling age, environmental conditioning of seedlings before inoculation, and inoculation technique. The highest and most consistent transmission rate was obtained with the "knife-slash" technique, which involved slashing stems of 3- to 10-mo-old seedlings 100–150 times with a contaminated razor blade. This method was repeatable with three biologically different isolates of TmRSV. Leaf rubbing combined with heat treatment of seedlings before inoculation was generally more effective than leaf rubbing alone, but the transmission rate was not consistent. The knife-slash method enables the evaluation of a wide range of TmRSV isolates on peach, which is essential in the development of cross-protection as a strategy to control peach stem pitting, as well as the rapid screening of *Prunus* germ plasm for TmRSV resistance. The Peach Yellow Bud Mosaic, Apricot, and Staff isolates induced symptoms on peach (oak-leaf pattern on leaves, leaf distortion, and stunted growth), whereas the Chickadee isolate did not.

Additional key words: knife-cut inoculation, stem-cut inoculation.

Tomato ringspot virus (TmRSV) is one of the most damaging viruses to fruit trees and berry crops in North America (1,2,15,19,20,22–24,26,27). TmRSV belongs to the nepovirus group, has a wide host range, and is vectored by *Xiphinema* species (25).

TmRSV is readily transmitted mechanically to herbaceous plants and by graft inoculation among woody plants (4,8,13,16,18,21). However, mechanical transmission of TmRSV from herbaceous hosts or from purified virus preparations to fruit trees is difficult, and the infection rate is usually low and inconsistent (6,23,28). Because of this difficulty, Koch's postulates (5) have not been unequivocally fulfilled for most of the diseases associated with TmRSV in fruit trees.

A simple and reliable method of experimental transmission of TmRSV to peach is essential for the study of the control of peach stem pitting by cross-protection that is currently under way in our laboratory. It would facilitate the evaluation of potentially mild TmRSV isolates on peach, in particular, wild-type isolates and chemically induced mutants (M. W. Bitterlin, *unpublished*) that are established only on herbaceous host plants. Furthermore, resistance or immunity in *Prunus* has been reported (9,13). Rapid and reliable transmission of TmRSV is necessary for evaluation of additional *Prunus* germ plasm for resistance to TmRSV and for the evaluation of seedlings resulting from hybridizations of

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resistant and susceptible germ plasm.

In this paper, we compare several methods of experimentally transmitting TmRSV to peach. We show that the "knife-slash" method, adapted from Garnsey et al (10,12), most reliably transmitted TmRSV to peach seedlings.

MATERIALS AND METHODS

Twenty-one inoculation treatments (Table 1), most of them replicated several times, were carried out over a 3-yr period. The treatments varied as to TmRSV isolate, inoculum preparation, seedling age, conditioning of plants before inoculation, and inoculation technique.

Virus isolates. Four isolates of TmRSV were used: a Peach Yellow Bud Mosaic isolate (PYBM) from California that systemically infects peach trees in the field (M. W. Bitterlin, *unpublished*), an apricot isolate (Apricot) from Maryland, an apple isolate (Chickadee) from Oregon, and a grape isolate (Staff) from Ontario, Canada.

Inoculum preparation. Inocula were prepared from different sources and by different methods. Crude sap ("crude" in Table 1) was obtained from cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) cultivar California Blackeye, cucumber (*Cucumis sativus* L.) cultivar Marketer, *C. metuliferus* (Naud.) Mey, *Nicotiana benthamiana* Domin, and peach (*Prunus persica* (L.) Batsch) ground 1:2 (w/v) in different buffers (0.01 M potassium phosphate [phosphate], pH 7.0; phosphate + 0.8% bentonite; phosphate + 0.01 M cysteine HCl + 3% nicotine alkaloid; or distilled H_2O only). For the knife-slash method (Table 1, treatments 18, 19, and 21), tissue was ground 1:2 (w/v) in 0.1 M K_2HPO_4 + 0.01 M EDTA, pH 7.0, pressed through cheesecloth, and clarified and concentrated by one cycle of low- and high-speed centrifugation. Partially purified virus (p. purif.) for treatments 8–13 was clarified by grinding tissue 1:2 (w/v) in 0.5 M sodium citrate + 0.1% thioglycollic acid, pH 6.5, to which chloroform was added 1:1 (w/v) while grinding, followed by polyethylene glycol precipitation and two cycles of differential centrifugation. The final resuspended high-speed pellets were diluted before inoculation to obtain virus concentrations of 0.04–0.22 mg/ml. The infectivity of the inocula was verified by assay on *Chenopodium quinoa* Willd. and averaged 190 local lesions per leaf.

Test plants and plant conditioning. A total of 841 *Prunus* seedlings were inoculated; 87% were seedlings of peach cultivars Boone County, BR 76, Halford, Nemaguard, Siberian C, Tennessee Natural, and selection 16-167; the others were *P. davidiana* Franch., *P. mahaleb* L., and *P. tomentosa* Thunb. The seedlings were grown in 10-cm pots (Table 1, treatments 1–14) or in 15-cm pots (treatments 15–21) in artificial soil mix (containing Osmocote 14-14-14, a controlled-release fertilizer) in the greenhouse with 14 hr of supplemental fluorescent lighting at about 25 C. Older seedlings were fertilized with a liquid 15-30-15 fertilizer. The 10- to 60-day-old seedlings (treatments 1–14) were between 10 and 30 cm tall. The 3- to 10-mo-old seedlings (treatments 15–21) had a diameter of at least 8 mm. As far as possible, the seedlings were inoculated just after they had started new growth, either after they had been cut back, or the leaves had been removed, or after a dormancy period of 6–8 wk at 3–10 C. About 2 wk after inoculation (and again later if necessary), the plants were pruned to force growth and prevent terminal bud set.

TABLE 1. Mechanical transmission of tomato ringspot virus to *Prunus* seedlings[a]

Treatment no.	TmRSV isolate and inoculum preparation[b]	Inoculation procedure and special treatment[c]	Replicates[d]	Transmission		
				Rate[e]	%[f]	Range[g]
Transmission to 10- to 60-day-old seedlings						
1	PYBM, crude	Leaf rubbing	8	2/66	3	0–20
2	PYBM, crude	Leaf rubbing, dark	23	6/180	3	0–33
3	PYBM, crude	Leaf rubbing, water	8	10/44	23	0–40
4	PYBM, crude	Leaf rubbing, dark, water	5	5/29	17	0–40
5	PYBM, crude	Leaf rubbing, dark, 28–32 C	8	1/78	1	0–5
6	Apricot, crude	Leaf rubbing, dark, 32 C	4	2/22	9	0–20
7	Chickadee, crude	Leaf rubbing, dark, 32 C	8	0/105	0	–
8	PYBM, p. purif.	Leaf rubbing, dark	2	1/12	8	0–17
9	PYBM, p. purif.	Leaf rubbing, water	2	0/14	0	–
10	PYBM, p. purif.	Leaf rubbing, dark, water	1	0/6	0	–
11	PYBM, p. purif.	Root dip and root rubbing	1	0/6	0	–
12	PYBM, p. purif.	Stem and leaf injection	1	0/6	0	–
13	PYBM, p. purif.	Knife-slash (<10 cuts)	2	0/16	0	–
14	Staff, leaf piece	Tissue graft	1	1/40	3	–
Transmission to 3- to 10-mo-old seedlings						
15	PYBM, leaf piece	Tissue graft	4	0/24	0	–
16	Staff, leaf piece	Tissue graft	2	3/120	3	0–5
17	Apricot, stem	Tissue graft	2	0/16	0	–
18	Apricot, conc.	Knife-slash (100+ cuts)	1	12/12	100	–
19	PYBM, conc.	Knife-slash (100+ cuts)	3	24/31	77	46–100
20	PYBM, crude	Knife-slash (100+ cuts)	1	3/6	50	–
21	Chickadee, conc.	Knife-slash (100+ cuts)	1	4/8	50	–

[a] Peach (*Prunus persica*) cultivars Boone County, BR 76, Halford, Nemaguard, Siberian C, and Tennessee Natural and *P. davidiana, P. mahaleb,* and *P. tomentosa.*

[b] Tomato ringspot virus (TmRSV) isolates: Peach Yellow Bud Mosaic (PYBM) from peach, California; Apricot from apricot, Maryland; and Chickadee from apple, Oregon. Inoculum preparations: Crude sap was obtained from different herbaceous host plants and from peaches by grinding infected tissue in different buffers (see text); concentrated (conc.) preparations by one low-speed and one high-speed centrifugation; partially purified preparations (p. purif.) by chloroform extraction followed by two cycles of differential centrifugation, then diluted to a concentration of 40–220 μg/ml.

[c] Inoculation procedure: Rub-inoculations (leaf and root) were done with cotton swab or with fingers after dusting with corundum; tissue graft with double-bladed knife; leaf and stem injection with syringe and hypodermic needle; and knife-slash inoculation with contaminated razor blade. Special treatment = treatment of seedling before inoculation (other than normal greenhouse conditions): dark = placing seedlings in darkness for 2–4 days; water = dipping seedling in water of 45–55 C for 60–120 sec; 28 and 32 C treatments were done in controlled chambers.

[d] Number of replicates of treatment. Each replicate within a treatment consisted of 5–60 seedlings (av. 9.6).

[e] Numerator is number of TmRSV-infected seedlings, denominator is number of seedlings inoculated (seedlings were assayed by ELISA).

[f] Percentage transmission of all replicates per treatment combined.

[g] Range of transmission per replicate (in precentages).

Inoculation techniques. Inoculations were usually carried out during evening hours in the greenhouse or in a cool place, and the plants were covered with newspaper, cardboard boxes, or were placed under mist the night after inoculation to minimize desiccation of plant tissue injured by inoculation.

In the leaf-rubbing procedure, the inoculum was applied with a cotton swab, a pestle, or fingers on both sides of the leaves and upper portions of the stem, which had been dusted with 600-mesh corundum. Because reports from other researchers (6,28) and preliminary results in our laboratory had suggested that dark and temperature treatments of young peach seedlings before inoculation would increase the susceptibility of plants to virus infections, we attempted to optimize the environmental conditions. Treatments consisted of placing the seedlings in darkness for 2–4 days before inoculation, dipping them in water of 45–55 C for 60–120 sec, and keeping them in a dark mist chamber at 28–32 C.

In other treatments, roots were slightly injured by rubbing and were dipped in partially purified virus solution (treatment 11), and partially purified virus was injected into leaves and stems of young seedlings (treatment 12).

Treatments that involved the grafting of herbaceous tissue to peach seedlings were tried. In treatments 15 and 17, leaf pieces of cucumber and cowpea and stem pieces of TmRSV-infected *Gomphrena globosa* L. and *Vinca rosea* L. were cut with a double-bladed knife and inserted under bark flaps of seedlings (11). In treatments 14 and 16, infected *Gomphrena* leaf sections about 10× 5 mm were cut so that each section contained a portion of the leaf midvein. For inoculating very small seedlings (1 mo old), *Gomphrena* leaf sections were smaller and did not include the midvein. Two T-shaped cuts were made in the stem of each peach seedling and a *Gomphrena* leaf section placed under the open bark flap. Bark flaps were then closed and the grafts sealed with rubber or latex tape. The tape was removed after about 3 wk.

The knife-slash inoculation was performed as described by Garnsey and Whidden (12) and Garnsey et al (10). Immediately before inoculation, a 10% sucrose solution (in 0.01 M sodium phosphate + 0.001 M EDTA + 0.001 M sodium azide, pH 7.0) was added to the inoculum so that it would stick better to the razor blade (0.5–1.0 ml of sucrose to 1.0 ml of virus solution). Typically, about 0.3 ml of inoculum was needed per plant and was equivalent to about 0.7 g of TmRSV-infected inoculum tissue. The inoculum was placed on the razor blade with a pasteur pipet. Cuts penetrating to the wood were made on two sides of about the lower two-thirds of the stem (avoiding the buds) where the leaves had been removed. Fewer than 10 cuts per plant were made on 29-day-old seedlings (treatment 13) and 100–150 cuts on 3- to 10-mo-old seedlings (treatments 18–21). The stems were not wrapped, but the plants were usually left for 12–15 hr in a cool place (about 15–20 C) before returning to the greenhouse.

Young (10- to 60-day-old) and old (3- to 10-mo-old) seedlings were repeatedly tested for infection up to 3–4 and 6–11 mo, respectively, after inoculation. Transmission of TmRSV was determined by enzyme-linked immunosorbent assay (ELISA) and symptoms (22) and, in some cases, by bioassay on *C. quinoa*. The direct double-antibody sandwich ELISA procedure (7) was followed, except in one test, where an indirect method was used (17). Antiserum to the PYBM or Staff isolate was used to detect the PYBM, Apricot, and Staff isolates, and antiserum to the Grape Yellow Vein or Chickadee isolates of TmRSV (3) was used to detect the Chickadee isolate.

RESULTS

The transmission efficiency of different treatments, as determined by ELISA, is summarized in Table 1. The highest and most consistent transmission rate was obtained with the knife-slash method on 3- to 10-mo-old seedlings (treatments 18–21), with an average of 75% transmission (range 46–100%) in six replicates (and 100% in three of them), carried out with three isolates of TmRSV. Cutting stems of young seedlings (10- to 60-day-old) only a few times (treatment 13) failed to transmit the virus. Because of the small size of these young seedlings, it was impossible to slash their stems 100–150 times. Leaf rubbing with dark and/or heat treatments (45–55 C water or 28–32 C air) of seedlings before inoculation (treatments 2–10) improved the transmission rate compared with leaf rubbing alone (treatment 1), but the results were inconsistent. In the 13 replications of treatments 3 and 4, for example, the transmission rate varied between 0 and 40%. Root dipping and rubbing (treatment 11) and stem and leaf injection (treatment 12) were not successful in transmitting the virus. Four of 200 seedlings inoculated by tissue grafting (treatments 14–17) were infected. Bioassay of 27 ELISA-positive and 15 ELISA-negative peach seedlings on *C. quinoa* confirmed the ELISA diagnoses.

The influence of the peach cultivar of *Prunus* species on the transmission rate was not systematically studied. Because no transmission was achieved to young (10- to 60-day-old) seedlings of *P. davidiana*, *P. mahaleb*, and *P. tomentosa* in initial trials of treatments 1–14 with 11–63 seedlings per species, experiments with these species were not continued. The overall infection rates of different cultivars of peach seedlings varied between 0 and 10% in the same treatments (Halford 10% [14 infected of 136 inoculated], Siberian C 5% [9/177], Nemaguard 3% [2/60], BR 76 2% [2/121], and Tennessee Natural 0% [0/5]). The three cultivars Boone County, Tennessee Natural, and 16-167 were used for tissue grafts in treatments 15–17, where only three of 160 seedlings became infected. Four cultivars were used for the knife-slash inoculations of treatments 18–21, but again, no attempt was made to evaluate any cultivar-virus strain interaction. Transmission rates of 88, 88, 76, and 45% were obtained with BR 76 (15/17), Nemaguard (7/8), Boone County (16/21), and Halford (5/11), respectively. Treatments 18 and 20 as well as one replicate of treatment 19 were carried out the same day; treatment 21 and two replicates of treatment 19 were done at another date.

TmRSV infections were detected by ELISA about 1 mo after leaf-rub inoculation and 6–8 wk after knife-slash inoculation. With the Chickadee isolate (treatment 21), however, transmission was not detected until 2–11 mo after inoculation. Similarly, three of four successful transmissions to seedlings inoculated by tissue grafting with the Staff isolate (treatments 14 and 16) were detected only after a 6-wk dormancy of the seedlings. Seedlings infected with the PYBM, Apricot, and Staff isolates of TmRSV developed distorted leaves and irregularly shaped chlorotic blotches along the veins, typical of yellow bud mosaic (22), about the time when infection was detectable by ELISA. No symptoms were produced by the Chickadee isolate.

DISCUSSION

The objective of this study was to develop an efficient and consistently repeatable method for mechanical transmission of TmRSV to peach. The knife-slash method proved reliable in six trials (Table 1, treatments 18–21) with three biologically very distinct isolates of TmRSV (3). This involved inoculating seedlings with 100–150 cuts after the leaves had been removed and/or they had gone through dormancy. It is conceivable that an even higher transmission rate might be obtained with optimal conditions in terms of ideal age and physiological stage of the seedlings, number of cuts made, and the environment in which the seedlings are grown. On the other hand, it seems unlikely that TmRSV is accidentally transmitted with contaminated pruning tools from infected to healthy trees in the nursery or orchard, because slashing young seedlings a few times with a razor blade contaminated with partially purified virus (treatment 13) failed to transmit the virus.

A conditioning of young seedlings by heat and/or dark exposure before leaf-rub inoculation increased the transmission rate to some extent, confirming previous reports (6,14, 28), but in each of these treatments (2–10 in Table 1), there were numerous replicates with no transmission at all (even in the most successful treatments, 3 and 4). The inoculum tissue source and the buffer for inoculum preparation (especially for crude sap) was not crucial as had been suggested (29), because transmission was obtained with inocula from cowpea, cucumber, *C. metuliferus*, and *N. benthamiana*

prepared in different buffers.

The inoculation technique, i.e., applying the inoculum to the proper plant part and/or the right amount of cell injury applied at inoculation, is probably more important than inoculum source. The amount of wounding of the 29-day-old seedlings in treatment 13 might have been too limited to establish infection, even though the inoculum was quite concentrated (0.22 mg/ml). On the other hand, leaf damage might have been too serious when seedlings were air-heated before inoculation (treatments 5–7). The nearly complete failure of the tissue grafts (treatments 14–17) might be explained by an amount of TmRSV inoculum (in the inserted tissue pieces) too limited to establish infection in the peach seedling.

As far as we are aware, this is the first study that extensively compared the efficiency of different inoculation procedures for mechanical transmission of TmRSV to *Prunus* seedlings. We did not attempt to evaluate the susceptibilty of different peach cultivars or *Prunus* species, but we wanted to find an efficient method to transmit TmRSV to peach. The knife-slash technique enables us now to do cross-protection studies on peach seedlings. The fact that high and repeatable transmission results were obtained with biologically different TmRSV isolates substantiates the value of the knife-slash method. This method was first shown by Garnsey et al (10,12) to be effective in transmitting citrus exocortis viroid and citrus tristeza virus from citrus to citrus. The somewhat longer incubation time with this method compared with the leaf-rub inoculation is outweighed by its superior reliability. This method enables us to advance our studies on the cross-protection of peach stem pitting, in which the transmission of TmRSV isolates from herbaceous plants or from in vitro sources to peach is essential. The procedure will also be valuable for the development of other control strategies, e.g., resistance, where a high number of plants must be inoculated. Furthermore, the fulfillment of Koch's third postulate (5) can now positively establish the virus etiology in the case of the TmRSV-associated virus diseases and might be useful for etiological studies of other fruit tree virus diseases as well.

LITERATURE CITED

1. Allen, W. R., and van Schagen, J. G. 1982. Tomato ringspot virus in European hybrid grapevines in Ontario: A re-evaluation of the incidence and geographic distribution. Can. J. Plant Pathol. 4:272-274.
2. Barrat, J. G., Mircetich, S. M., and Fogle, H. W. 1968. Stem pitting of peach. Plant Dis. Rep. 52:91-94.
3. Bitterlin, M. W., and Gonsalves, D. 1985. Biological and serological comparison of tomato ringspot virus isolates. (Abstr.) Phytopathology 75:1293.
4. Bitterlin, M. W., Gonsalves, D., and Cummins, J. N. 1984. Irregular distribution of tomato ringspot virus in apple trees. Plant Dis. 68:567-571.
5. Bos, L. 1981. Hundred years of Koch's Postulates and the history of etiology in plant virus research. Neth. J. Plant Pathol. 87:91-110.
6. Cadman, C. H., and Lister, R. M. 1962. Relationships and particle sizes of peach yellow bud mosaic and tomato ringspot viruses. Plant Dis. Rep. 46:770-771.
7. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
8. Cummins, J. N., and Gonsalves, D. 1982. Recovery of tomato ringspot virus from inoculated apple trees. J. Am. Soc. Hortic. Sci. 107:798-800.
9. Cummins, J. N., and Gonsalves, D. 1986. Constriction and decline of 'Stanley' prune associated with tomato ringspot virus. J. Am. Soc. Hortic. Sci. 111:315-318.
10. Garnsey, S. M., Gonsalves, D., and Purcifull, D. E. 1977. Mechanical transmission of citrus tristeza virus. Phytopathology 67:965-968.
11. Garnsey, S. M., and Whidden, R. 1970. A rapid technique for making leaf-tissue grafts to transmit citrus viruses. Plant Dis. Rep. 54:907-908.
12. Garnsey, S. M., and Whidden, R. 1973. Efficiency of mechanical inoculation procedures for citrus exocortis virus. Plant Dis. Rep. 57:886-889.
13. Hoy, J. W., and Mircetich, S. M. 1984. Prune brownline disease: Susceptibility of prune rootstocks and tomato ringspot virus detection. Phytopathology 74:272-276.
14. Kassanis, B. 1952. Some effects of high temperature on the susceptibility of plants to infection with viruses. Ann. Appl. Biol. 39:358-369.
15. Keplinger, J. A., Braun, A. J., and Provvidenti, R. 1968. Crumbly berry of red raspberry caused by tomato ringspot virus. Plant Dis. Rep. 52:386-390.
16. Lister, R. M., Allen, W. R., Gonsalves, D., Gotlieb, A. R., Powell, C. A., and Stouffer, R. F. 1980. Detection of tomato ringspot virus in apple and peach by ELISA. Acta Phytopathol. Acad. Sci. Hung. 15:47-55.
17. Lommel, S. A., McCain, A. H., and Morris, T. J. 1982. Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology 72:1018-1022.
18. Mircetich, S. M., Civerolo, E. L., and Fogle, H. W. 1971. Relative differential efficiency of buds and root chips in transmitting the causal agent of peach stem pitting and incidence of necrotic ringspot virus in pitted trees. Phytopathology 61:1270-1276.
19. Mircetich, S. M., and Fogle, H. W. 1976. Peach stem pitting. Pages 77-87 in: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U.S. Dep. Agric. Agric. Handb. 437. Government Printing Office, Washington, DC.
20. Mircetich, S. M., and Hoy, J. W. 1981. Brownline of prune trees, a disease associated with tomato ringspot virus infection of Myrobalan and peach rootstocks. Phytopathology 71:30-35.
21. Rosenberger, D. A., Cummins, J. N., and Gonsalves, D. 1985. Development of apple union necrosis and decline in apple trees inoculated with tomato ringspot virus. (Abstr.) Phytopathology 75:1312.
22. Schlocker, A., and Traylor, J. A. 1976. Yellow bud mosaic. Pages 156-165 in: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U.S. Dep. Agric. Agric. Handb. 437. Government Printing Office, Washington, DC.
23. Smith, S. H., Stouffer, R. F., and Soulen, D. M. 1973. Induction of stem pitting in peaches by mechanical inoculation with tomato ringspot virus. Phytopathology 63:1404-1406.
24. Stace-Smith, R. 1984. Red raspberry virus diseases in North America. Plant Dis. 68:274-279.
25. Stace-Smith, R. 1984. Tomato ringspot virus. No. 290. Descriptions of plant viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 6 pp.
26. Stouffer, R. F., and Uyemoto, J. K. 1976. Association of tomato ringspot virus with apple union necrosis and decline. Acta Hortic. 67:203-208.
27. Uyemoto, J. K. 1975. A severe outbreak of virus-induced grapevine decline in Cascade grapevines in New York. Plant Dis. Rep. 59:98-101.
28. Yarwood, C. E. 1956. Mechanical transmission of peach yellow bud mosaic virus. Plant Dis. Rep. 40:299.
29. Yarwood, C. E. 1957. Juice transmission of viruses to peach. (Abstr.) Phytopathology 47:38.