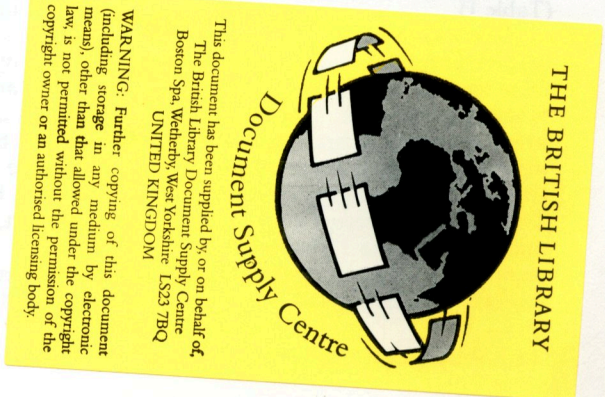

Table 4. Effect of benomyl and dip substrate on stem blight development caused by *Botryosphaeria dothidea* inoculated 3 and 6 mo after treatment across all benomyl rates

Substrate	Time of benomyl application (mo)	Lesion length (mm)		Survival (%)
		1 mo	11 mo	
Kaolin	3[a]	6[b]	17	100
Kaolin	6	19	98	62
Terra-sorb	3	26	45	100
Terra-sorb	6	44	132	38

[a] Months from benomyl treatment to inoculation.
[b] Each value represents the mean of eight observations rounded to the nearest whole number.

11 mo from plants the roots of which were immersed in 3,000 μg/ml of benomyl-kaolin clay 3 mo before inoculation were unsuccessful. By contrast, the fungus was reisolated from 100% of inoculations from the same treatment combination when a 6-mo dip-to-inoculation interval was used.

Although a number of treatments permitted 100% survival of inoculated test plants, lesion lengths varied widely. As stated, benomyl was most effective when used at a rate of 2,000–3,000 μg/ml of active ingredient; these rates of benomyl also were effective in limiting stem canker development caused by *B. corticis* (Demaree & M. S. Wilcox) Arx & E. Müller (7). Higher rates resulted in phytotoxicity and longer lesion lengths. Dipping plants in a substrate rather than set-in application was assumed at the outset of this test to be the better method, but there were no differences among the most efficacious treatments. Significant differences did occur when main effects of the dipping technique were combined across all other treatments, indicating that dipping generally provided a more uniform source of benomyl for uptake by plants. Benomyl root dips using these techniques are apparently effective only for a period of 3 to less than 6 mo. Infections occurring after that time period will progress in spite of these treatments.

Based on our results, it appears that benomyl root dips may be useful in limiting *B. dothidea* development but may not protect plants fully throughout a long growing season. In field studies, Creswell and Milholland (4) were able to collect spores of *B. dothidea* year-round with the exception of a few weeks in winter, which would seem to circumvent a control measure that is effective for only 3–5 mo, however, maximum infection of wounded plants exposed to natural inoculum occurred in June. If protective dip-treating could be timed to coincide with this period of highest disease probability, a significant reduction in the number of infected plants could be achieved.

Future efforts will concentrate on determining the usefulness of these dip treatments under field conditions. Benomyl retention in the root zone may be enhanced by the lower amount of leaching expected to occur in the field as opposed to container nursery bed regimes of daily overhead watering. Another possibility for extending the protected period would be the use of a clay base, which has a higher ionic capacity. This might be a way to retain benomyl within the dip solution, provided that ionic binding does not deactivate benomyl completely (5).

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Apparent Immunity and Tolerance to Tomato Big Bud Disease in *Lycopersicon peruvianum* and in Two of Its Tomato Hybrids

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ABSTRACT

Thomas, P. E., and Hassan, S. 1992. Apparent immunity and tolerance to tomato big bud disease in *Lycopersicon peruvianum* and in two of its tomato hybrids. Plant Dis. 76:139-141.

All plants in a seed lot of *Lycopersicon peruvianum*, U.S. Department of Agriculture Plant Introduction (PI) No. 128655, and in seed lots of two F_5 hybrid progenies of PI 128655 × tomato either did not become infected (apparent immunity) or were infected without symptoms (tolerance) after graft inoculation using tomato tissue infected with tomato big bud disease. The same germ plasm was previously shown to contain the same expressions of resistance and tolerance against three phloem-limited viruses—beet curly top, tomato yellow top, and potato leafroll.

A number of aster yellows type diseases of tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.) reported worldwide under a variety of names are believed to be caused by members of a group of mycoplasmalike organisms (MLOs) (3,9,12,21). These include tomato big bud diseases reported in the United States (5,6,8), Australia (1,13), and the Middle East (21); the stolbur diseases of tomato and potato in Europe and Russia (4,12,19); mal azul in Portugal (2); potato purple top wilt in India and Australia (1,9,13); and potato haywire in the United States (20). The exact relationships among the MLOs associated with these diseases are not known (4) but all are transmitted by one or more leafhopper species that vary in transmission efficiency for different strains of the pathogens in different areas of the world (4). Transmission from tomato plants with big bud or stolbur symptoms produced the potato syndrome called haywire, purple top wilt, or stolbur in different areas of the world (1,20).

Symptoms of tomato big bud in the western United States are identical to those described in detail by Samuel et al (13) in South Australia. They are characterized by teratological changes, particularly in the flowering structure. Calyx segments on flowers remain united almost to the tips, and the whole calyx enlarges to form a bladderlike structure 2–5 cm in diameter with a toothed opening at the top. Anther and ovary development stop and petals become virescent. Purple coloration develops, particularly along veins of the bladder structures and on the undersides of young leaves. Auxiliary buds proliferate, stems gradually thicken, and the plant finally assumes a tufted, rosette growth habit.

Golino (7) showed that the leafhopper-transmitted virescence agent (BLTVA), an MLO, has caused big bud symptoms in experimentally infected tomato. Furthermore, biological and molecular evidence has established that field-collected big bud symptomatic tomatoes from California are infected with BLTVA rather than other MLOs (14,15). Although it is not yet clear what relationship the BLTVA-MLO may have with the MLO that causes the disease in other parts of the world, BLTVA has become a prime suspect as the tomato big bud causal agent in the western United States within the geographic range of its vector, *Circulifer tenellus* (Baker).

Field infection rates approaching 100% in both tomatoes and potatoes have been reported for stolbur in Europe (4,19), and serious losses to purple top wilt in potato have been reported in Australia (1), but there is no record of serious economic losses to any MLO-induced disease of potato or tomato in the United States.

MLOs are phloem-limited pathogens. We previously discovered apparently complete tolerance and immunity to three phloem-limited viruses (beet curly top virus [BCTV], tomato yellow top virus [TYTV], and potato leafroll virus [PLRV]) in plants of *Lycopersicon peruvianum* (L.) Mill, USDA Plant Introduction (PI) No. 128655, and two selected F_5 hybrid progenies of PI 128655 × *L. esculentum* (10,11,16–18). The basis for resistance to the viruses appeared to be related to translocation in the phloem. This study was performed to determine whether PI 128655 was also resistant to a completely different but phloem-limited pathogen, the tomato big bud MLO.

MATERIALS AND METHODS

Source of germ plasm. *L. peruvianum*, PI 128655, was collected at Charanilla Tampaca, Peru, in 1938 by L. H. Blood (from original record of L. H. Blood) and increased by open-pollination at Ogden, UT, and later at Prosser, WA. Hybrid progenies were produced from interspecific crosses between a selected PI 128655 plant with immunity to BCTV (16,17) and tomato cv. Bonnie Best. F_1 and F_2 generations of the hybrids were increased in the field by open-pollination. Plants with immunity and complete tolerance to BCTV were selected in the F_3 generation, and plants with resistance to TYTV were selected in the F_4 generation. The F_5 hybrid progenies used in this study were derived from individual, open-pollinated F_3 plants selected for resistance to TYTV in the preliminary studies (11).

Inoculum source. A tomato plant with big bud symptoms typical of those described by Samuel et al (13) was selected in the field. Cuttings were rooted in a greenhouse, and the resulting plants were used as a source for graft inoculations. A second tomato plant from the field with typical big bud symptoms was used when the initial experiments were repeated. These two MLO lines both hybridized with a DNA probe specific for the BLTVA and did not hybridize with a probe specific for the aster yellows MLO, indicating that the tomato big bud symptomatic tomatoes used as the inoculum source for these experiments were infected by the BLTVA (hybridization assays conducted by Mary Shaw, Department of Plant Pathology, University of California, Davis, CA) (14,15). By ELISA, both sources of inoculum assayed negative for BCTV; beet western yellows virus (BWYV); PLRV; potato viruses A, M, S, X, and Y; and tobacco mosaic virus. Young tomato plants remained symptomless when rub-inoculated with

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extracts of the inoculum sources.

ft inoculation and indexing. Test ts and index plants (tomato cv. VF) were graft-inoculated by insert grafts nto the sides of the stem and by cleft grafts on excised terminal shoots. The grafts were wrapped with Parafilm, held in a mist chamber for 1 wk, and returned to the greenhouse.

RESULTS

Plants of *L. peruvianum*, PI 128655, the two hybrid progenies, and cultivated tomato were graft-inoculated with tissue from cuttings taken from the first field source of tomato big bud. Nine uniform plants from each category with successful graft unions were selected for use in these experiments.

The tomato scions on all of the selected plants grew and continued to express typical big bud symptoms. The inoculated tomato control plants developed typical big bud symptoms within 4–5 wk. However, all of the *L. peruvianum* and hybrid plants remained completely symptomless until discarded 24 wk after inoculation.

At 12 wk and again at 18 wk after inoculation, both the inoculated test and tomato control plants were indexed for the presence of the big bud agent by graft inoculation to healthy tomatoes. All of the tomato control plants, three of nine *L. peruvianum*, five of nine hybrid 1, and four of nine hybrid 2 plants produced positive indices (Table 1). The same indexing results were obtained with the same plants 18 wk after inoculation.

The resistance of *L. peruvianum*, PI 128655, and the two hybrid progenies was tested again 2 yr later. Groups of test plants from the same seed lots were graft-inoculated with a second field isolate of tomato big bud. In the second test, 18 plants in each test category (*L. peruvianum*, hybrid 1, and hybrid 2) and six cultivated tomato control plants with successful grafts were used.

Results were essentially identical to those obtained in the first test. Again, all tomato plants developed typical big bud symptoms within 6 wk, whereas all *L. peruvianum* and hybrid plants remained symptomless. At 12 wk after inoculation, big bud was detected by graft transmission to cultivated tomato in eight of 18 *L. peruvianum*, five of 17 hybrid 1, and seven of 18 hybrid 2, and in all inoculated tomato control plants (Table 1).

The plants from the second test were used in further experimentation to determine whether the big bud agent actually multiplied in the symptomless *L. peruvianum* and hybrid plants or, alternatively, whether it arrived there by passive transport from the infected scions. To do this, the infected graft scions were removed after the 12-wk indexing. The plants were maintained an additional 18 mo in a greenhouse and indexed again.

Table 1. Susceptibility of *Lycopersicon peruvianum* (PI 128655)[a] and two of its F_5 interspecific hybrid (*L. peruvianum* × *L. esculentum*) progeny populations[b] to tomato big bud disease transmitted by graft inoculation

	Number infected/number inoculated[c]			
Assessment	*L. peruvianum*	Hybrid 1	Hybrid 2	Tomato
1	3/9	5/9	4/9	9/9
2	8/18	5/18	7/18	6/6

[a]U.S. Department of Agriculture Plant Introduction Number.
[b]Fifth generation open-pollinated progeny of interspecific F_1 hybrids selected in the third generation for resistance to BCTV and in the fourth generation for resistance to TYTV. The *L. peruvianum* parent was resistant to BCTV.
[c]All infected *L. peruvianum* and hybrid plants remained symptomless, and all tomato plants developed big bud symptoms. Plants were indexed for infection 12 wk after graft inoculation.

During this interval, the plants were pruned several times to force growth of new vegetative stems. Results of the 18-mo index were identical to the 12-wk index. Plants that had indexed positive at 12 wk retained the big bud agent and remained symptomless.

DISCUSSION

About 60% of the *L. peruvianum*, PI 128655, and the hybrid progeny germ plasm populations assessed for resistance to tomato big bud were apparently immune, because they could not be infected by graft-inoculation methods that routinely infected susceptible plants. The remaining plants were infected with the big bud agent and were completely tolerant under our experimental conditions.

The question of whether the big bud agent failed to move from infected scions into the immune plants or, alternatively, whether it moved into but failed to multiply in the immune plants was not tested.

The expressions of resistance against tomato big bud obtained in the experiments reported here are essentially the same as those obtained with the same germ plasm populations when inoculated with BCTV, TYTV, and PLRV (10,16). Immunity to the viruses appeared to be based on resistance to virus translocation in the phloem. Because the tomato big bud MLO is phloem-limited, it seems possible that a basic mechanism in the phloem could account for resistance to movement of both the viruses and the MLO. However, neither these experiments nor the earlier experiments with viruses provided any information concerning the mechanisms responsible for tolerance (infection without symptoms) of plants that became infected. It seems highly improbable that tolerance to the viruses and to the MLO could be conditioned by the same mechanism.

We are not aware of any previous reports concerning resistance in tomato to big bud or similar MLO diseases. Because the MLO agents that cause tomato big bud, stolbur, potato purple top wilt, haywire, and similar diseases around the world are believed to be closely related, the resistances to big bud reported here may be effective against the other diseases. The reisolation in the hybrid progenies of the resistances identified in *L. peruvianum* suggests that the resistances may be incorporated into tomato by traditional breeding methods. These resistances may also be available for use in potato through advanced gene transfer methods.

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Superinfection of Orange Trees Containing Mild Isolates of Citrus Tristeza Virus with Severe Florida Isolates of Citrus Tristeza Virus

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ABSTRACT

Powell, C. A., Pelosi, R. R., and Cohen, M. 1992. Superinfection of orange trees containing mild isolates of citrus tristeza virus with severe Florida isolates of citrus tristeza virus. Plant Dis. 76:141-144.

The ability of four mild isolates of citrus tristeza virus (CTV) to suppress the spread of Florida severe isolates of CTV into Valencia sweet orange trees propagated on sour orange rootstock was assessed by symptom development over an 8-yr period. Decline symptoms occurred in some of the trees containing each of the mild isolates, as well as the unprotected (no mild isolate) control trees, within 3 yr after planting. After 5 yr, the percentage of decline in trees infected with the four mild isolates was 28, 22, 27, and 25, respectively, compared with 39% for the unprotected control trees. After 8 yr, the percentage of trees with symptoms (stunting or decline) was 75, 76, 74, and 73, respectively, compared with 86% for the control. Eight years after planting, a monoclonal antibody to CTV (MCA13) reacted with 100, 99, 92, and 19% of the extracts from trees with decline and stunting, stunting, decline, or no symptoms, respectively. A second monoclonal antibody (3DF1) reacted with extracts from all of the trees.

Citrus tristeza virus (CTV) causes economically important diseases wherever citrus is grown (2,8). The virus can cause stunting, slow decline, quick decline, stem pitting, or no symptoms depending on the virus isolate, environmental conditions, and citrus cultivar (7,11). In Florida, only mild isolates, which cause no obvious symptoms, and severe isolates, which cause stunting and/or decline, are currently present. Some Florida decline isolates of CTV will cause a moderate seedling yellows reaction (6). The Florida severe isolates affect only citrus on sour orange or *Citrus macrophylla* P. J. Wester rootstocks; they have not been reported to induce severe stem-pitting symptoms or affect other rootstocks.

Currently, the effect of CTV can be controlled in Florida by propagating citrus on rootstocks other than sour orange. However, there are several reasons why it would be beneficial to protect sour orange rootstock from disease induced by CTV. First, there are many older productive groves in Florida that are propagated on sour orange rootstock. Second, many growers feel that the combination of local environmental conditions and sour orange rootstock are responsible for the high quality of their fruit. Third, all of the other rootstocks currently grown in Florida have at least one major disease or horticultural problem not prevalent with sour orange.

One possible mechanism to protect citrus on sour orange rootstock from severe isolates of CTV is mild strain cross-protection. This approach has been successful in controlling stem-pitting isolates of CTV in both Brazil (4) and South Africa (5). There are indications that cross-protection of sour orange against decline-inducing isolates of CTV may be effective (3,9,13,14,17), but the long-term usefulness of this protection in the field has not been demonstrated.

One of the difficulties in monitoring cross-protection by mild isolates of CTV is the inability to easily distinguish the mild isolates from severe isolates. A breakdown in cross-protection could only be evaluated based on symptoms, and superinfection with severe isolates could not be confirmed serologically. Recently, a monoclonal antibody to CTV that reacts with most decline-inducing isolates of CTV, but not with mild isolates, has been produced (12). This antibody may provide the means to document the superinfection of trees containing mild isolates of CTV with severe isolates (17).

The purpose of this study was twofold. First, the ability of four mild strains of CTV to protect sweet orange trees on sour orange rootstocks from Florida severe strains of CTV was determined. Second, the reliability of monoclonal antibodies to differentiate between mild and severe isolates of CTV and to confirm superinfection of symptomatic trees containing mild isolates of CTV with severe isolates was evaluated.

MATERIALS AND METHODS

Virus isolates and tree propagation. The four mild isolates of CTV included in this study are DD102bb, Guettler HS, DPI 136-53-4, and DPI 1-12-5-X-E. DD102bb originated from a Valencia sweet orange (*Citrus sinensis* (L.) Osbeck) tree on sour orange rootstock (*C. aurantium* L.) in Winter Garden, FL, where CTV-induced disease was prevalent. The tree had remained symptomless for more than 20 yr. Guettler HS came from a symptomless Valencia sweet orange tree on sour orange rootstock from a grove in which 90% of the trees were in decline. DPI 136-53-4 and DPI 1-12-5-X-E were from symptomless old-line and nucellar Valencia sweet orange selections, respectively, on sour orange rootstock in Florida's budwood repository. Each isolate was obtained by graft inoculation of several greenhouse-grown, CTV-free Valencia sweet orange trees on sour orange rootstock with three scion bark chips from each source tree containing the mild isolates. The presence of CTV in the inoculated trees was

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