were absent since there were no other peach or fruit orchards blooming at the same time. Orchard size (170 trees) was large enough to attract bees. Synchrony of flowering between different genotypes and the occurrence of cleistogamy (20) or of male sterility (3, 17) in peach are other factors that could influence the rate of outcrossing. Variation in flowering date was observed between cultivars, possibly reducing the maximum possible outcrossing rate. Male-sterile cultivars were excluded from the study.

The results from the present study suggest that substantial amounts of outcrossing can occur in peach orchards. Our corrected outcrossing estimates of 33% and 15%, respectively, for the 1984 and 1985 seasons were similar to Fogle's estimates of 14% and 22% outcrossing (6, 7). These estimates are substantially larger than earlier ones (10, 18). The obvious implication for peach breeding is that open-pollinated peach seed cannot be assumed to be selfed seed if the orchard contains several genotypes. Hesse has suggested eliminating the petals as an alternative to bagging flowers to prevent outcrossing (10). Since no significant differences in outcrossing rates for showy vs. nonshowy flowers or for wide vs. narrow petals was observed, petal removal (alteration of floral morphology) to ensure selfing may not prevent outcrossing. The procedure should be examined.

Outcrossing in peach should result in the retention of a considerable amount of heterozygosity. This was not found to be the case for various isozymes (2). Pedigree analysis of U.S. peach cultivars showed a substantial amount of inbreeding (16). These observations are probably the result of a very limited germplasm base from which modern cultivars were developed. The question of how much heterozygosity is present in peach is not clear, however, since several authors found segregation of morphological characters in progeny after several generations of selfing (4, 12, 21). (They may have been observing outcrossing events if they failed to bag the parent flowers.) The occurrence of significant levels of outcrossing implies that wild peaches in China should have a considerable amount of heterozygosity and within-population variation (14). Measuring heterozygosity in wild peaches from China should permit verification of this hypothesis.

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Resistance to Tomato Spotted Wilt Virus in Lettuce

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Additional index words. *Lactuca sativa*, thrips, disease resistance

Abstract. **Tomato Spotted Wilt Virus (TSWV), a thrips-transmitted virus disease, has become a limiting factor in lettuce (*Lactuca sativa* L.) production throughout Hawaii and several other areas of the world. In this study, two partially resistant lettuce cultivars, Tinto and PI 342517 ('Ancora'), were crossed with the susceptible check cultivar Green Mignonette and to each other. Results of resistance tests of the F_1 and F_2 plants suggest that 'Tinto' and PI 342517 have the same genes for resistance and that this resistance is partially dominant.**

Tomato Spotted Wilt Virus (TSWV) is the causal agent of a thrips-transmitted disease first identified on tomatoes (Brittlebank, 1919). It has a wide host range of more than 200 species of plants, including economically important crops such as pepper, peanuts, and pineapple, as well as lettuce (Best, 1968; Cho et al., 1987a). This disease has recently become a major limiting factor in lettuce production in Hawaii (Cho et al., 1987b), probably in association with the practice of continuous cropping with overlapping plantings. Other areas in which TSWV has been reported on lettuce include California (Harris, 1939), South Australia (Moller and Rogers, 1960), Chile (Docampo and Nome, 1970), and South Africa (Boelma and Bolton, 1984).

TSWV is transmitted naturally only by adult or larval thrips that have fed on infected plants while still in the larval stage (Bald and Samuel, 1931). When fields are replanted before all the thrips that have pupated in the soil after acquiring TSWV from the previous crop have emerged, heavy damage to the young plants can occur. Initial symptoms appear as

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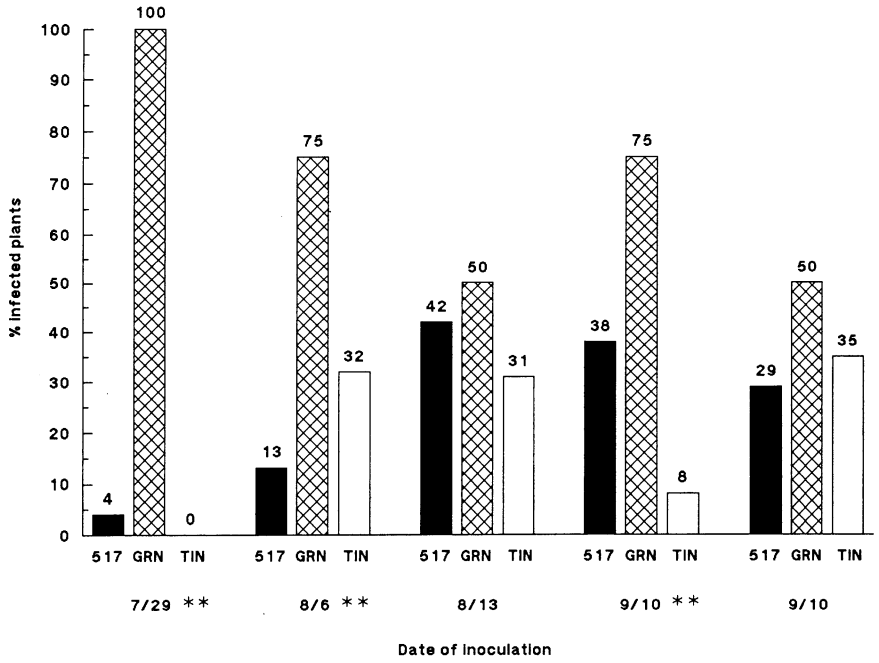

Fig. 1. Tomato Spotted Wilt Virus infection trials of PI 342517 (517), 'Green Mignonette' (GRN), and 'Tinto' (TIN) at Kula, Maui, July–Aug. 1986.
**Significant difference between 'Green Mignonette' and other cultivars at ($P < 0.01$).

Table 1. Infection rates of PI 342517, 'Tinto,' 'Green Mignonette', and their F_1 and F_2 families inoculated with Tomato Spotted Wilt Virus on 8 Sept. 1986 at Kula, Maui.

Lettuce line or cross	No. plants	Infected plants (%)
PI 342517 (Ancora)	36	11.1 A[z]
Tinto	36	30.6 ABC
Green Mignonette	36	72.2 D
PI 342517 x Green Mignonette		
F_1	7	28.6 ABC
F_2	36	38.9 BC
F_2	36	47.2 CD
Green Mignonette x Tinto		
F_1	34	44.1 BC
F_2	36	31.4 ABC
F_2	36	36.1 ABC
F_2	35	38.9 BC
Tinto x PI 342517		
F_1	20	20.0 AB
F_2	36	25.0 ABC
F_2	36	25.0 ABC
F_2	36	33.3 ABC

[z]Mean separation by Waller–Duncan multiple range test, 5% level.

small brown necrotic spots on young leaves, followed by stunting of the meristem, systemic wilting of the entire plant, and premature death within 2 weeks after the appearance of initial symptoms.

Control of TSWV is difficult to achieve for several reasons, such as the large host range and the large number of infectious thrips that emerge from the soil following a TSWV-infected crop. Hence, this study was initiated to identify sources of resistance to the virus and to evaluate any such resistance.

The resistant parents used in this study were 'Tinto' and PI 342517, both of which showed significantly higher resistance levels than the susceptible check in some, although not all, trials (O'Malley, 1987). 'Tinto' was among 112 lettuce lines received from E.J. Ryder (USDA Agricultural Research Station, Salinas, Calif.). PI 342517 was received from the Western Regional Plant Introduction Station, Pullman, Wash. Subsequently, it was discovered that 'Tinto' (also known as PI 342444 and incorrectly listed in the Plant Inventory as 'Trinto') and PI 342517 ('Ancora') both originated from the same seed company, Rijk Zwaan B. V., De Lier, Netherlands (I.W. Boukema, personal communication). 'Green Mignonette' was used as the susceptible parent. It is a semi-heading-type lettuce grown widely in Hawaii, where it is called 'Manoa', and is severely affected by TSWV.

Test plants were mechanically inoculated with TSWV in a greenhouse at the Univ. of Hawaii farm, Kula, Maui (elev. 900 m), above the elevation at which the thrips vector is found. The inoculum was prepared by macerating three to four infected lettuce leaves in a mortar containing 20 ml of chilled buffer. The buffer consisted of 0.1 M potassium phosphate, pH 7, containing 0.1 g sodium metabisulfite/ml (Best, 1968). Seedlings ≈3 weeks old were inoculated by stroking three or four carborundum-dusted leaves with a soft bristle brush dipped in freshly prepared inoculum. TSWV symptoms would start to appear anytime from about 1 week to sometimes 6 or more weeks after inoculation. Resistant cultivars generally showed a delay in the appearance of symptoms, even though often all eventually succumbed. Plants were individually evaluated 3 weeks after inoculation for TSWV symptoms. Data were expressed as percent infected for each entry tested.

The two resistant and one susceptible lines were tested in five separate trials planted on 9, 16, 24 July and 14 and 20 Aug. 1986 and inoculated 20 to 27 days later. Plants, 24 of each parent, were grown in one 72-compartment Speedling tray for each trial.

Both resistant lines were crossed with 'Green Mignonette' and with each other. The parents and their F_1 and F_2 families were planted 19 Aug. 1986 and inoculated 20 days later. Each entry was grown in a separate 72-compartment Speedling tray. Each F_2 family originated from a different F_1 plant.

Averaged across the five trials, 'Tinto' (19.8%) and PI 342517 (25.0%) were significantly more resistant than 'Green Mignonette' (70.0%), but were not different from each other. The results varied, however, from trial to trial (Fig. 1); in the third and fifth trials, there were no significant differences, while, in the first, second, and fourth trials, differences were highly significant. Variation in percentage of plants infected could be caused by such factors as fluctuations in the environment, titre of the inoculum, and age of the plants. This last factor may have caused the difference between the fourth and fifth trials, in which the plants were inoculated on the same day with the same inoculum, but the plants in the fourth trial were one week older.

There were significant differences among the three parents and their F_1 and F_2 families (Table 1). All the F_1 and F_2 families were significantly different from 'Green Mignonette', except the one F_2 family of PI 342517 x 'Green Mignonette'. The 'Tinto' x PI 342517 progenies were generally the most resistant, at a level equal to their parents. The 'Green Mignonette' x 'Tinto' progenies were intermediate. These results agree with preliminary tests with smaller numbers of plants (O'Malley, 1987) and thus suggest the following conclusions: 'Tinto' and PI 342517 have resistance to TSWV that can be demonstrated under the present testing conditions. The F_1 and F_2 progeny of crosses between these two lines are also resistant, with no evidence for segregation in the F_2. They could be allelic for resistance genes.

When 'Tinto' and PI 342517 are crossed with 'Green Mignonette', the F_1 families are more like the resistant parents than the susceptible ones. The F_2 families have a high degree of resistance, but less than the F_2 families of 'Tinto' x PI 342517. Thus, resistance seems to be partially dominant.

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Virulence Studies and Resistance to *Clavibacter michiganensis* ssp. *michiganensis* in Tomato Germplasm

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Additional index words. *Lycopersicon esculentum*, bacterial canker, resistance screening, virulence

Abstract. **Six strains of bacterial canker (*Clavibacter michiganensis* ssp. *michiganensis*), isolated from tomato plants from greenhouses or fields near Cleveland, Ohio, were tested for virulence. The most virulent of the strains was used to evaluate 13 cultivars reported to be resistant to bacterial canker. Eleven of the cultivars were resistant at a high inoculum level (8.5×10^8 cells per plant) and the other two only at a lower level (8.5×10^2 cells per plant). It was demonstrated that it is possible to identify plants with intermediate resistance using a dilute inoculum of a virulent strain of *C. michiganensis*.**

Bacterial canker (Cmm) is responsible for severe losses in tomato-growing areas of the world. This disease has been reported in 25 states in the United States (Strider, 1969) and crop losses as high as 70% to 80% have occurred in western North Carolina. As recently as 1985, crop losses of 17% to 20% occurred in southern Michigan; some growing areas in Canada had an 84% yield reduction at harvest (Stephens, 1987). Severe crop losses from this disease in Ohio occurred in 1986 and 1987.

To determine the degree of resistance or susceptibility of a crop to a pathogen, inoculation technique plays an important role. From several techniques tested for Cmm, stem inoculation was shown by DeJong and Honma (1976) to have the lowest environmental variation and was suggested for use in genetic studies. Tomato seedlings can become infected by Cmm with as few as five cells/plant when introduced directly into xylem tissue (Thyr, 1968). Forster and Echandi (1973) had found that inoculum levels of 10^7 cells/ml and 10^9 cells/ml responded similarly in differentiating between resistant and susceptible tomato cultivars.

Variation in the virulence among Cmm strains has been reported (Baines, 1947; Fawcett and Bryan, 1934; Strider, 1969; Thyr, 1971). Strider and Lucas (1970) showed variation on the tomato cultivar Manapal, while Thyr (1968) described differences in virulence of seven strains from six geographical areas of the United States. To ensure an acceptable level of resistance, a highly virulent strain should be used to evaluate selections in a breeding program (Thyr, 1969; Thyr, 1972).

Since Elenkov (1965) produced the resistant cultivar Bulgaria 12, a number of tomato cultivars with resistance to Cmm have been released, including 'H2990' (Emmatty and John, 1973), 'MR4', 'Monense' (Laterrot, Brand and Daunay, 1978), '$CmVF_{232}$', and 'Okitsu Sozai No. 1-20' (Kuriyama and Kuniyasu, 1974). Differences in the virulence of strains of the pathogen used to evaluate these lines have resulted in contradictory reports on their relative resistance. Boelema (1980) found that the resistant 'Bulgaria 12' (PI 330272) was no more resistant than 'Roma VF' (a susceptible cultivar) in South Africa; however, 'H2990', 'Okitsu Sozai No. 1', and 'UC134' were resistant.

This study was undertaken to investigate the relative virulence of strains isolated in Ohio and to evaluate resistance in a collection of tomato germplasm from a broad geographical area.

Bacterial strains used in this study were all from the Cleveland, Ohio area. Strains Cmm1, Cmm2, Cmm3, and Cmm6 were obtained from diseased greenhouse tomato plants and Cmm4 and Cmm5 were isolated from stems and diseased tomato plants in the field.

Inoculum preparation. A loopful of Cmm cells from each strain was transferred from yeast extract dextrose calcium carbonate (Schaad, 1980) agar plates to 5 ml of nutrient broth yeast extract (Schaad, 1980) and incubated overnight in an incubator shaker at

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Table 1. Probit analysis of the quantal responses of tomato cultivars to graded inoculum doses of six strains of *C. michiganensis*.

Cultivar	Strain	Slope(b)	ED_{50} value[z] (cells/plant)	Virulence (rank)
Moneymaker	Cmm1	0.759	59 c	2
	Cmm2	0.801	1.1×10^4 a	4
	Cmm3	0.674	8.9×10^3 a	4
	Cmm4	0.932	5.7×10^2 b	3
	Cmm5	0.578	8.9 d	1
	Cmm6	0.712	7.6×10^2 b	3
Plovdiv 8/12	Cmm1	0.477	1.3×10^4 c	2
	Cmm2	0.262	1.2×10^5 b	3
	Cmm3	0.645	9.6×10^4 b	3
	Cmm4	0.654	3.8×10^4 b	2
	Cmm5	0.596	2.2×10^2 d	1
	Cmm6	0.379	2.5×10^6 a	4

[z]The ED_{50} values followed by the same letter(s) are not significantly different according to the calculated Z values.