Induced Resistance from Simultaneous Inoculation of Tomato with *Fusarium oxysporum* Sacc. and *Verticillium albo-atrum* Reinke & Berth[1]

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Abstract. **Induced resistance to verticillium wilt occurs in fusarium-resistant tomato cultivars from dip inoculation with mixed cultures of Race 1 of *Fusarium oxysporum* f. *lycopersici* (F) and *Verticillium albo-atrum* (V). This induced resistance decreases as F level in mixed inoculi is reduced. Simultaneous screening for resistance to V and F can be achieved by inoculation with V and reinoculating 48 to 72 hours later with F.**

Induced resistance by prior inoculation with nonpathogenic organisms has been demonstrated for various plant diseases (1, 2, 3, 4, 5). Attempts to simultaneously screen individual tomato plants for fusarium and verticillium resistance revealed fusarium-induced resistance to verticillium in fusarium-resistant cultivars (6). In this study, we examine the effects of fusarium inoculum levels and time of inoculation on expression of this induced resistance.

In preliminary studies, several cultivars with fusarium resistance (FR), verticillium resistance (VR) or both (VFR) were inoculated with mixed inoculi containing approx equal propagules of each pathogen. F induced resistance to V in all FR cultivars; V did not induce resistance to F in VR cultivars.

The present studies involved 'Kokomo' (FR) and 'Bonny Best' (F susceptible) to examine the effects of inoculum level and time of inoculation on this induced resistance. Three-day-old filtered Tochinai cultures of F and 9-day V cultures on PDA were diluted with sterile distilled water to give standard inoculum.

To examine the effect of F inoculum level on induced resistance, mixed inoculi were prepared in which V level was maintained constant at 6.6×10^7 propagules/ml and F reduced by multiples of 10 from 6.6×10^7 to 6.6×10^3 propagules/ml (see Table 1). Single organism inoculi were prepared by appropriate dilution of the standard inoculi. Two week old seedlings were dip inoculated, transplanted into 450 g (16 oz) plastic pots containing a sterile 2 soil:1 peat:1 sand mixture and grown in the greenhouse for approx 4 weeks prior to disease rating. Each treatment contained 64 plants (16 plants/rep × 4 reps) in a randomized complete block design. Individual plants were scored for vascular discoloration using a rating scale (1 = none; 6 = dead).

Similar procedures were followed in time of inoculation studies. Seedlings were inoculated with V, clump planted, and reinoculated with fresh standardized F cultures 24, 48 and 72 hr later. Controls included inoculation with V alone, F alone and mixed inoculum at the time of initial V treatment (Table 2).

F-induced resistance to V in 'Kokomo' (FR) decreased as F levels in mixed inoculi were reduced (Table 1). Equal propagules for each pathogen resulted in disease ratings similar to that of the F-inoculated control. Vascular discoloration similar to the V-inoculated control occurred only when F levels in mixed inoculi were reduced to extremely low levels (6.6×10^3). This F level is inadequate for F-resistance screening in seedlings of susceptible 'Bonny Best' as only moderate disease development occurred after 4 weeks. Varying V and F levels does not appear to be a feasible approach to simultaneously screen for V and F resistance.

Pre-inoculation with V followed by F inoculation 24, 48 or 72 hr later (Table 2) resulted in a decrease in induced resistance and disease ratings similar to the V inoculated controls. Optimal disease development occurred when F inoculation was delayed until 48 or 72 hr after V inoculation.

Simultaneous screening of individual plants and/or progenies for V and F resistance is possible by inoculation at different times. This procedure is useful to eliminate susceptible plants during generation advance with "single seed descent" breeding where separate tests for each disease must be made on the same plant. Breeding populations are inoculated with V, clump planted and 2 days later reinoculated with F. Individuals susceptible to V or F would be eliminated before completion of the reproductive cycle.

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Table 1. Effects of *Fusarium* inoculum level on induced resistance to *Verticillum*.

Cultivar	Inoculum level (propagules/ml) Verticillium	Fusarium	Disease rating[z]
Kokomo (FR)	0	0	1.2a
"	0	6.6×10^7	1.8ab
"	6.6×10^7	6.6×10^7	1.8ab
"	6.6×10^7	6.6×10^6	2.5bcd
"	6.6×10^7	6.6×10^5	2.8cde
"	6.6×10^7	6.6×10^4	3.5ef
"	6.6×10^7	6.6×10^3	4.1fg
"	6.6×10^7	0	4.2g
Bonny Best (FS)	6.6×10^7	0	4.8g
"	0	6.6×10^7	4.9g
"	0	6.6×10^3	2.9de

[z]1 = low; 6 = dead. Mean separation by Duncan's multiple range test, 5% level. FR = fusarium resistant; FS = fusarium susceptible.

Table 2. Effect of interval between *Verticillium* and *Fusarium* inoculation on *Fusarium*-induced resistance to *Verticillium*.

Cultivar	Inoculum level (propagules/ml) Verticillium	Fusarium	Time between V and F treatment (hr)	Disease rating[z]
Kokomo (FR)	0	0	–	1.0a
"	0	7.9×10^7	–	1.5a
"	7.9×10^7	0	–	4.5b
"	7.9×10^7	7.9×10^7	0	1.7a
"	7.9×10^7	7.9×10^7	24	3.7b
"	7.9×10^7	7.9×10^7	48	4.2b
"	7.9×10^7	7.9×10^7	72	4.3b
Bonny Best (FS)	7.9×10^7	0	–	4.5b
"	0	7.9×10^7	–	5.5c

[z]1 = low; 6 = dead. Mean separation by Duncan's multiple range test, 5% level. FR = fusarium resistant; FS = fusarium susceptible.

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Pectinesterase, Polygalacturonase, Cx-Cellulase Activities and Softening of the *rin* Tomato Mutant[1]

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Abstract. **Pectinesterase (PE), polygalacturonase (PG), and cellulase (Cx form) activities and softening of 4 physiological maturities were compared in normal and *rin* tomatoes (*Lycopersicon esculentum* Mil cv. Rutgers). PG, PE, and Cx-cellulase activities increased during ripening of normal fruits. In *rin* fruits, PG activity was not detected, PE activity remained relatively constant, and Cx-cellulase activity increased during ripening. The lack of softening in *rin* fruits appears to be associated with the lack of PG activity.**

The fruit ripening inhibitor (*rin*) mutant in tomato lacks the normal climacteric (5), does not develop characteristic carotenes and fails to soften (12). Other characteristics of fruit ripening affected by this mutation have not been investigated. The long storage life associated with this recessive gene is of particular interest. In this report we compare changes in PE, PG and Cx-cellulase, and softening during ripening of normal and *rin* tomato fruits.

Isogenic normal and *rin* stocks were developed by 5 successive backcrosses with 'Rutgers' as the recurrent parent. Flowers from 'Rutgers' and isogenic *rin* plants were tagged at anthesis and one fruit was allowed to develop per cluster. Field-grown fruits were harvested 41, 46, 49, and 54 days from anthesis, intervals which corresponded to distinct maturity stgages (mature-green to ripe) of 'Rutgers'. Harvested fruit were washed in water containing 0.1% sodium hypochlorite, air-dried and separated into 3 uniform lots of 10 fruits each. An Asco Firmness Meter set at 1 kg prestress and 1.5 kg linear stress for 30 sec was used for determining firmness of each fruit (1). Enzymes were extracted by homogenizing tomato sections in aqueous solution containing 1% polyvinylpyrrolidone and 1 M NaCl and by filtering through 8 layers of cheese-cloth. Cx-cellulase was desorbed from the cell walls prior to filtering as described by Dickinson and McCollum (3). The action of PE on citrus pectin, PG on pectic acid, and Cx-cellulase on carboxymethyl-cellulose (hercules 7HP) was determined by the methods described by Rouse and Atkins (13), Kertesz (10), and Dickinson and McCollum (3), respectively. Cannon-Fenske No. 200 viscometers were used for PG and Cx-cellulase assays. Enzyme units are presented as microequivalents/g fresh wt/min for PE, % change in viscosity of 15 ml 1% pectic acid/2.5 g fresh wt/15 min for PG and % change in viscosity of 15 ml 1% carboxymethyl-cellulose/2.5 g fresh wt/3 hr for Cx-cellulase.

A close association has been established between normal tomato softening and activities of PE and PG (2, 6, 7, 8). We show this relationship for 'Rutgers' and *rin* (Fig. 1). Softening increased concomitantly with increased PE and PG activity in 'Rutgers'. In contrast, *rin* fruits remained firm, no PG activity was detected, and PE activity remained relatively constant. Incubation of predetermined levels of active PE and PG with homogenates of *rin* fruits did not affect the enzyme activities (unreported data) which indicates that lower PG and PE activities in *rin* fruits are not a result of the presence of inhibitors.

Lowered PG activity has also been reported in tomato fruit tissue affected

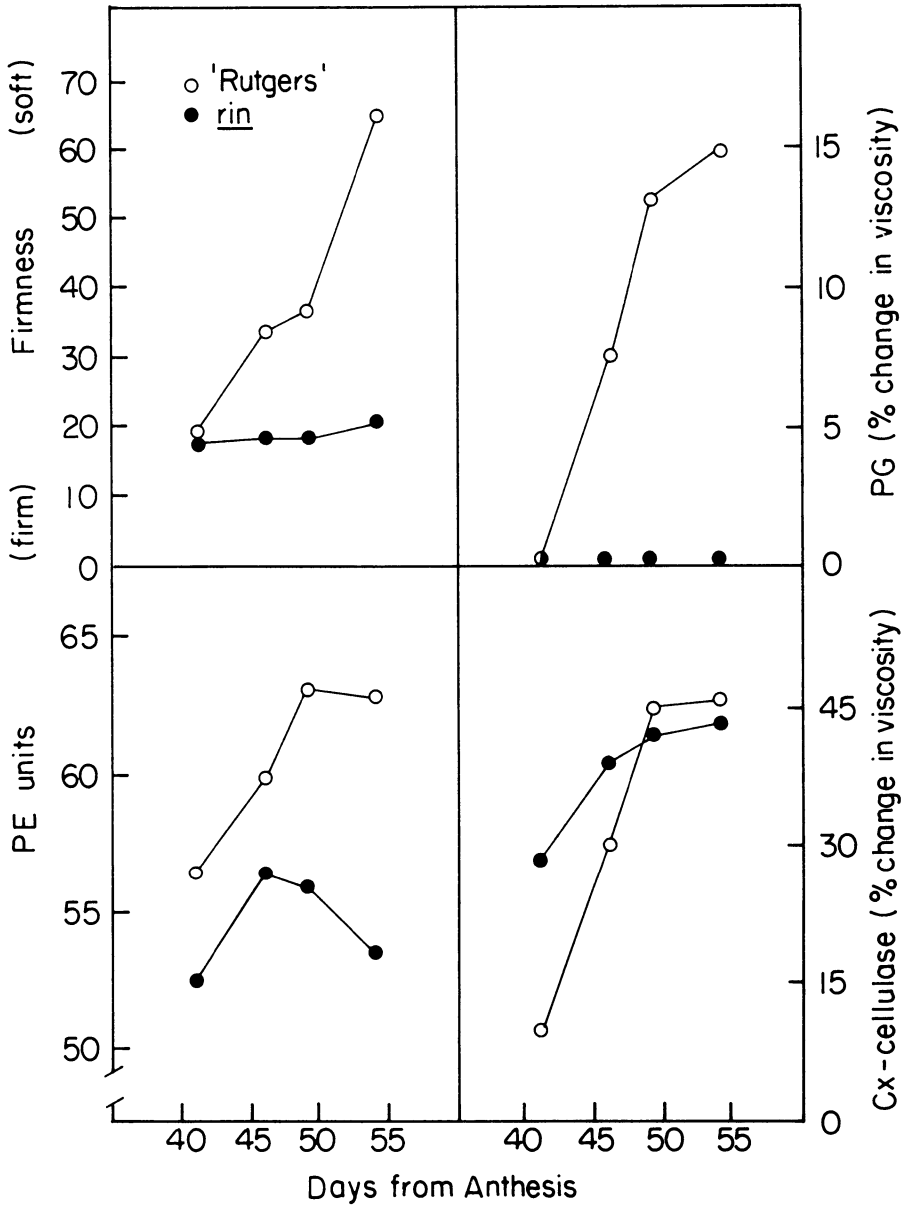

Fig. 1. Changes in firmness (Asco units), PG, PE, and Cx-cellulase activity of 'Rutgers' and *rin* tomatoes as related to physiological maturity.

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