Graft Transmission of Systemic Resistance of Cucumber to Anthracnose Induced by *Colletotrichum lagenarium* and Tobacco Necrosis Virus

A. E. Jenns and J. Kuć

Graduate student and professor, respectively, Department of Plant Pathology, University of Kentucky, Lexington, 40546.
This research was supported in part by Cooperative State Research Service Grant 316-15-51, P.L. 89-106, of the U.S. Department of Agriculture.
Journal Series Paper 78-11-196 of the Kentucky Agricultural Experiment Station, Lexington, 40546.
Accepted for publication 31 January 1979.

ABSTRACT

JENNS, A. E., and J. KUĆ. 1979. Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus. Phytopathology 69:753-756.

Resistance to anthracnose, which was induced by infection of the first true leaf of cucumber cultivar SMR-58 with *Colletotrichum lagenarium* or tobacco necrosis virus (TNV), was transmitted to a scion of the same cultivar grafted onto the infected plant above the first true leaf. Resistance also was transmitted if grafting preceded the inducing inoculation. Resistance to anthracnose in susceptible cucumber was not transmitted by grafting onto uninoculated resistant cultivars but it was transmitted if the resistant rootstocks were inoculated with *C. lagenarium*. Susceptible cucumbers remained susceptible to anthracnose when grafted onto rootstocks of pumpkin and squash which were uninoculated or inoculated with *C. lagenarium* or TNV. *C. lagenarium* did not produce visible lesions on pumpkin or squash leaves, but TNV did so. Watermelons and muskmelons grafted onto susceptible cucumber rootstocks were protected against anthracnose by inoculating the rootstocks with *C. lagenarium*. The "signal" for induced systemic resistance is not cultivar-, genus-, or species-specific.

Additional key words: biological control.

Cucumbers (7), watermelons (2), and muskmelons (2) are systemically protected against anthracnose by inoculation with *Colletotrichum lagenarium,* and cucumbers can be systemically protected against anthracnose by inoculation with *Pseudomonas lachrymans* (1) or tobacco necrosis virus (5). The systemic resistance to carnation mosaic virus (CarMV), induced in certain clones of *Dianthus barbatus* by localized infection with CarMV, was reported to be transmitted through grafts between protected and healthy plants (8).

This study was conducted to determine whether a systemic "signal" for protection was graft transmissible from an inoculated to a noninoculated plant; whether this "signal" was cultivar-, genus-, or species-specific; and whether species which do not respond to inoculation with *C. lagenarium* with visible lesions transmit a "signal" to susceptible plants.

MATERIALS AND METHODS

Culture of host and pathogen. Race 1 of *Colletotrichum lagenarium* (Pass.) Ell. & Halst. (4) was maintained on bean pod agar at 24 C in the dark. Conidial suspensions were prepared from 4- to 9-day-old cultures as previously described (3). Tobacco necrosis virus (TNV) was obtained from Prof. R. W. Fulton (University of Wisconsin, Madison, 53706) and was maintained in cucumber plants. Cucumbers (*Cucumis sativus* L. 'Wisconsin SMR-58', 'Addis', 'Calypso', 'Sampson', and 'Poinsett'); watermelons (*Citrullus vulgaris* Schard. 'Iroquois'); muskmelons (*Cucumis melo* L. 'Sugarbaby'); pumpkins (*Cucurbita pepo* L. 'Sugarpie'); summer squash (*Cucurbita pepo* L. 'Summer Straight Neck') and winter squash (*Cucurbita moschata* Poir. 'Butternut') were used in these investigations. All plants were grown in 9- or 10-cm diameter plastic pots or 9-cm diameter clay pots containing Pro-Mix BX (Premier Brands Inc., Premier Peat Moss Corp., New York, NY 10036). A nutrient solution (Ra-Pid-Gro, Dansville, NY 14437) was applied twice weekly after seedling emergence. Plants were maintained in a greenhouse at 23–31 C under daylight supplemented with 14 hr of fluorescent and incandescent light.

Inoculation. The inducing and challenge inoculations were as described previously (5,6). It was previously shown that treating plants with sap from uninfected cucumber did not alter their resistance to *C. lagenarium* (5). Drops of water were applied to the first true leaf (leaf one) of control plants in experiments where *C. lagenarium* was applied as the inducing inoculation.

Grafting. Plants with the lowest internode at least 3 cm long were prepared for use as rootstocks by cutting off the tops with a sharp blade 3 cm above the lowest node and slitting the stump vertically to a depth of 1.5 cm. Scions were cut 3 cm below the lowest node and trimmed to wedges 1.5 cm from the cut base. After insertion of the wedges into the slits in the rootstocks, the grafts were bound with Parafilm (American Can Co., Marathon Products, Neenah, WI 54956). The grafted plants were placed in humidity chambers for 5 days, after which the chambers were partially opened to allow equilibration of the atmosphere in the chambers. In experiments with pumpkin and squash, survival of the plants after grafting was poor. Survival of grafted plants was improved when the scions were enclosed in individual plastic bags, (14 × 15 cm) which had been sprayed inside with water and were tied around the stem below the graft point. The grafted plants were kept shaded below the greenhouse bench for 5 days, then the bags were removed and the plants were returned to the bench. Lateral shoots that developed at the lowest node, and scion cotyledons, were removed 5–7 days after grafting.

RESULTS

Grafting after the inducing inoculation. Systemic resistance to anthracnose is induced 5 days after inoculation of the first true leaf of SMR-58 cucumbers with *C. lagenarium* or 2 days after inoculation with TNV (5,6). Therefore, untreated SMR-58 scions were grafted onto SMR-58 rootstocks 5 days after inoculation of leaf one of the rootstocks with 40 drops of a suspension of *C. lagenarium* spores (10^5/ml) and 2 days after inoculation with TNV. Seven days after grafting, the first leaves of the scions were inoculated with 40 drops of *C. lagenarium* spores (10^4/ml), and the

00031-949X/79/000136$03.00/0

numbers of lesions were recorded 7 days after inoculation. Scions showed increased resistance to anthracnose when grafted onto rootstocks inoculated with either *C. lagenarium* or TNV. Resistance was expressed as a reduction in lesion number (Table 1) and approximately a 30% reduction in lesion diameter. Ten lesions were measured per plant. The average diameter of lesions on control plants 7 days after inoculation was 3 mm.

Grafting before the inducing inoculation. Six days after grafting SMR-58 scions onto SMR-58 rootstocks, the first true leaves of the rootstocks were inoculated either with 40 drops of a suspension of *C. lagenarium* spores (5×10^5/ml) or with TNV; controls were treated with water or left untreated. Seven days later, the first and second true leaves of the scion were inoculated with 40 drops of a suspension of *C. lagenarium* spores (10^4/ml). Symptoms were recorded 7 days later. Scions showed increased resistance to anthracnose when rootstocks were inoculated with either *C. lagenarium* or TNV after grafting (Table 2). Resistance was expressed as a reduction in numbers of lesions (Table 2) and also a reduction in lesion diameter of about 33% on the first leaf of the scion and about 40% on the second.

Grafting susceptible cucumber scions onto resistant rootstocks. SMR-58 cucumber scions were grafted onto rootstocks of cucumber cultivars SMR-58, Addis, and Calypso. Cultivars Addis and Calypso are resistant to anthracnose. One week after grafting, the first leaves of the scions were inoculated with 40 drops of a suspension of *C. lagenarium* spores (5×10^4/ml). Symptoms were recorded 7 days after inoculation. The anthracnose-susceptible scions were equally susceptible when grafted onto resistant or susceptible rootstocks, and there was no significant difference in lesion number or in lesion size. In one experiment, scions of cultivars Addis, Calypso, and SMR-58 were grafted onto rootstocks of cultivar SMR-58. One week after grafting, the first true leaves of the scions were inoculated with 40 drops of a suspension of *C. lagenarium* spores (5×10^4/ml) and symptoms were recorded 7 days later. The mean numbers and sizes of lesions on the resistant scions, Addis and Calypso, were significantly smaller than the mean number and size of lesions on the susceptible scion, SMR-58, showing that the susceptible rootstocks did not induce susceptibility in the resistant scions.

Experiments also were conducted to test whether inoculating the rootstock of a resistant cultivar would influence the disease reaction of a susceptible scion. Susceptible SMR-58 scions were grafted onto SMR-58 rootstocks or rootstocks of the resistant cultivars Poinsett and Sampson. Five days after grafting, the first leaves of the rootstocks were inoculated with 40 drops of a suspension of *C. lagenarium* spores (5×10^5/ml) or treated with water. After inoculation, susceptible rootstocks developed many spreading necrotic lesions which eventually coalesced, killing the inoculated leaf and its petiole. Lesions on resistant rootstocks developed more slowly than those on susceptible rootstocks, and did not coalesce during the experiments. One week after the first inoculation, the first and second true leaves of the scion were inoculated with 40 drops of a suspension of *C. lagenarium* spores (10^5/ml) and symptoms were recorded 7 days later. Inoculation of rootstocks of cultivars Sampson and Poinsett with *C. lagenarium* induced resistance to anthracnose in SMR-58 scions (Table 3). The resistance induced in scions on infected resistant rootstocks was less marked than that in scions on infected susceptible SMR-58 rootstocks.

Grafting susceptible cucumber scions onto pumpkin and squash rootstocks. SMR-58 cucumber scions were grafted onto rootstocks of pumpkin cultivar Sugar Pie, summer squash cultivar Summer Straight Neck, squash cultivar Butternut, and cucumber cultivar SMR-58. Five days after grafting, the first leaves of the rootstocks were either inoculated with 40 drops of a suspension of *C. lagenarium* spores (5×10^5/ml), inoculated with TNV or left untreated. Seven days later, the first true leaves of the scions were inoculated with 40 drops of a suspension of *C. lagenarium* spores (10^5/ml). Lesions were counted 7 days after inoculation. Inoculation of the rootstocks of pumpkin, summer squash, and butternut squash with *C. lagenarium* did not produce lesions, whereas inoculation with TNV produced necrotic lesions. Neither treatment, however, affected the resistance of the scions to anthracnose (Table 4). Inoculation of the SMR-58 rootstocks with *C. lagenarium* or TNV produced necrotic lesions, and scions on inoculated SMR-58 rootstocks were significantly more resistant to

TABLE 1. The anthracnose-resistance of SMR-58 cucumber scions grafted onto SMR-58 cucumber rootstocks infected with *Colletotrichum lagenarium* or tobacco necrosis virus

Exp. no.	Treatment of first true leaf of rootstock	Interval in days between treatment of rootstock and grafting	Mean number of lesions on first true leaf of scion[a]
1	TNV inoculation	2	14 (± 1.53)[b] ***[c]
	Untreated	2	28 (± 1.44)
2	*C. lagenarium* 1×10^5	5	8 (± 1.23) ***
	Water	5	16 (± 1.34)

[a] First true leaf of scion was inoculated 7 days after grafting with 40 drops of a suspension of *C. lagenarium* spores (10^4/ml). Lesions were counted 7 days after inoculation.
[b] Means and standard errors of a total of 40 plants in three trials.
[c] Triple asterisks indicate a very highly significant ($P = 0.001$) difference between the mean and that of the appropriate control.

TABLE 2. The effect of inoculating SMR-58 cucumber rootstocks with *Colletotrichum lagenarium* or tobacco necrosis virus on the resistance to anthracnose of previously grafted SMR-58 cucumber scions

Exp. no.	Treatment of first true leaf of rootstock 6 days after grafting	Mean number of lesions per scion leaf[a]	
		First true leaf	Second true leaf
1	*C. lagenarium* 5×10^5 spores/ml	15 (± 0.89)[b] ***[c]	12 (± 0.87) ***
	Water	25 (± 0.89)	29 (± 0.87)
2	TNV	13 (± 0.69) ***	7 (± 0.99) ***
	Untreated	20 (± 0.69)	27 (± 0.99)

[a] Scion leaves were inoculated 13 days after grafting with 40 drops of a suspension of *C. lagenarium* spores (5×10^4/ml). Lesions were counted seven days after inoculation.
[b] Means and standard errors of a total of 45 plants in three trials.
[c] Triple asterisks indicate a very highly significant ($P = 0.001$) *t*-test difference between the mean and that of the appropriate control.

anthracnose than scions on untreated SMR-58 rootstocks.

Inducing resistance in watermelon and muskmelon scions grafted onto susceptible SMR-58 rootstocks. Scions of watermelon cultivar Sugar Baby, muskmelon cultivar Iroquois, and cucumber cultivar SMR-58 were grafted onto rootstocks of cucumber cultivar SMR-58. Five days after grafting, the first true leaves of the rootstocks were either inoculated with 40 drops of a suspension of *C. lagenarium* spores (5×10^5/ml) or treated with water. Twelve days after grafting, the first three true leaves of the scion were inoculated with 20 drops of a suspension of *C. lagenarium* spores (10^5/ml). Inoculation of SMR-58 rootstocks with *C. lagenarium* induced resistance to anthracnose in watermelon and muskmelon scions (Table 5). The muskmelon scions were less susceptible than the watermelon and cucumber scions under the conditions tested.

DISCUSSION

Systemic resistance to anthracnose induced in cucumber rootstocks after inoculation with *C. lagenarium* or TNV can be transmitted to untreated cucumber scions. Therefore, the rootstock

TABLE 3. The effect of inoculating resistant cucumber rootstocks with *Colletotrichum lagenarium* on the resistance to anthracnose of SMR-58 cucumber scions

Exp. no.	Rootstock	*C. lagenarium* inoculum or water control treatment of first true leaf of rootstock 5 days after grafting (spores/ml)	Mean lesion number per scion leaf[a]	
			First true leaf	Second true leaf
1	Poinsett (resistant)	5×10^5	20 (± 2.23)[b] *[c]	21 (± 2.68) *
		0 (Water)	31 (± 2.08)	32 (± 1.61)
	SMR-58 (susceptible)	5×10^5	14 (± 2.50) ***	6 (± 1.95) ***
		0 (Water)	34 (± 1.16)	36 (± 1.05)
2	Sampson (resistant)	5×10^5	21 (± 2.74) **	11 (± 2.10) ***
		0 (Water)	34 (± 1.39)	27 (± 2.04)
	SMR-58 (susceptible)	5×10^5	13 (± 2.81) **	7 (± 2.53) ***
		0 (Water)	29 (± 2.32)	28 (± 2.47)

[a] Scion leaves were inoculated 12 days after grafting with 40 drops of a suspension of *C. lagenarium* spores (10^5/ml). Lesions were counted 7 days after inoculation.
[b] Means and standard errors of a total of 40 plants in two trials.
[c] Asterisks *, **, and *** indicate that the *t*-test means differ significantly from that of the appropriate control at $P = 0.05$, 0.01, or 0.001, respectively.

TABLE 4. The effect of inoculating pumpkin and squash rootstocks with *Colletotrichum lagenarium* and tobacco necrosis virus on the resistance of SMR-58 cucumber scions to anthracnose

Treatment of first true leaf of rootstock 5 days after grafting	Mean number of lesions on 1st true leaf of scion[a]			
	Rootstock			
	Pumpkin 'Sugarpie'	Summer squash 'Summer Straight Neck'	Winter squash 'Butternut'	Cucumber 'SMR-58'
C. lagenarium 5×10^5 spores/ml	36 (± 2.15)[b]	31 (± 2.57)[c]	39 (+ 0.53)[b]	24 (± 2.17)[b] ***[d]
TNV	37 (± 0.84)	30 (± 3.48)	39 (± 0.36)	26 (± 3.22) ***
Untreated	36 (± 1.35)	35 (± 1.96)	39 (± 0.44)	39 (± 0.44)

[a] Scion leaf was inoculated 12 days after grafting with 40 drops of a suspension of *C. lagenarium* spores (10^5/ml). Lesions were counted 7 days after inoculation.
[b] Means and standard errors of 16–23 plants in three trials.
[c] Means and standard errors of 16 plants in two trials.
[d] Triple asterisks indicate a very highly significant ($P = 0.001$) *t*-test difference between the mean and that of the untreated control.

TABLE 5. The effect of inoculating SMR-58 cucumber rootstocks with *Colletotrichum lagenarium* on the resistance of muskmelon and watermelon scions to anthracnose

Scion	*C. lagenarium* inoculum or water control treatment of first true leaf of rootstock 5 days after grafting (spores/ml)	Mean lesion number on scion leaves[a]		
		leaf 1[b]	leaf 2[b]	leaf 3[c]
Watermelon 'Sugar Baby'	5×10^5	1 (± 0.43) **[d]	3 (± 0.55) **	9 (± 1.00) **
	0 (Water)	7 (± 0.84)	11 (± 0.87)	16 (± 1.08)
Muskmelon 'Iroquois'	5×10^5	2 (± 0.46) **	2 (± 0.36) **	3 (± 0.55) **
	0 (Water)	6 (± 0.77)	8 (± 0.73)	8 (± 0.94)
Cucumber 'SMR-58'	5×10^5	5 (± 0.90) **	4 (± 0.93) **	4 (± 0.94) **
	0 (Water)	14 (± 0.80)	14 (± 0.71)	13 (± 1.19)

[a] Scion leaves were inoculated 12 days after grafting with 20 drops of a suspension of *C. lagenarium* spores (10^5/ml). Lesions were counted 7 days after inoculation.
[b] Means and standard errors of at least 36 plants in four trials.
[c] Means and standard errors of at least 24 plants in three trials.
[d] Double asterisks indicate a highly significant ($P = 0.01$) *t*-test difference between the mean and that of the appropriate control.

of an induced plant, consisting of the inoculated leaf, the cotyledons, hypocotyl, and roots, is alone capable of producing a protective factor which passes via a graft to a scion from an untreated plant. This factor continues to be produced by the rootstock until at least 10 days after inoculation with *C. lagenarium,* since the graft, which is made 5 days after inoculation, is not established for another 5 days. Because the inoculated leaf can be removed after 4 days without reducing protection in the leaf above (6), the protective factor might be produced in a part of the rootstock other than the inoculated leaf.

The protective factor passes through an established graft between two susceptible cucumber plants. However, based on previous observations (5,6,7), induced resistance generally was greater in intact, than in grafted, plants.

Susceptible cucumber scions do not become resistant to anthracnose when grafted onto uninoculated resistant cucumber rootstocks. However, when resistance is induced by inoculating the resistant rootstocks with *C. lagenarium,* the susceptible scions do become resistant. This shows that a graft-transmissible signal for systemic resistance is not present in the noninfected resistant cultivars Poinsett, Sampson, Calpyso, and Addis. The resistant cultivars Poinsett and Sampson developed smaller lesions after inoculation with *C. lagenarium* than did the susceptible cultivar SMR-58, and the resistance induced in SMR-58 scions by inoculation of resistant rootstocks was less marked than that induced by inoculation of susceptible rootstocks. This suggests that the extent of necrosis caused by the inducing inoculation affects the extent of resistance induced systemically.

Inoculation of pumpkin and squash rootstocks with *C. lagenarium* at 5×10^5 spores per milliliter did not produce lesions and resistance was not induced in cucumber scions. However, pumpkin, squash, and cucumber rootstocks inoculated with TNV all developed similar necrotic lesions, although resistance to anthracnose was induced only in scions on TNV-inoculated cucumber rootstocks and not in scions on TNV-inoculated pumpkin and squash rootstocks. Either the localization of TNV infection in necrotic lesions in pumpkin and squash does not lead to the production of a systemic "signal" for resistance to anthracnose or this "signal" is not transmissible to or is ineffective in cucumbers.

Systemic induced resistance to anthracnose is not unique to cucumbers, but also has been demonstrated in watermelons and muskmelons (2). The induction of anthracnose resistance in watermelon and muskmelon scions by inoculation of cucumber rootstocks with *C. lagenarium* demonstrates that the "signal" for induced systemic resistance is not genus- or species-specific.

LITERATURE CITED

1. CARUSO, F. L. 1977. Protection of cucumber against Colletotrichum lagenarium by Pseudomonas lachrymans. (Abstr.) Proc. Am. Phytopathol. Soc. 4:159.
2. CARUSO, F. L., and J. KUĆ. 1977. Protection of watermelon and muskmelon against Colletotrichum lagenarium by Colletotrichum lagenarium. Phytopathology 67:1285-1289.
3. ELLISTON, J. E., J. KUĆ, and E. B. WILLIAMS. 1976. Protection of Phaseolus vulgaris against anthracnose by Colletotrichum species nonpathogenic to bean. Phytopathol. Z. 86:117-126.
4. GOODE, M. J. 1958. Physiological specialization in Colletotrichum lagenarium. Phytopathology 48:79-83.
5. JENNS, A. E., and J. KUĆ. 1977. Localized infection with tobacco necrosis virus protects cucumber against Colletotrichum lagenarium. Physiol. Plant Pathol. 11:207-212.
6. KUĆ, J., and S. RICHMOND. 1977. Aspects of the protection of cucumber against Colletotrichum lagenarium by Colletotrichum lagenarium. Phytopathology 67:533-536.
7. KUĆ, J., G. SHOCKLEY, and K. KEARNEY. 1975. Protection of cucumber against Colletotrichum lagenarium by Colletotrichum lagenarium. Physiol. Plant Pathol. 7:195-199.
8. WEINTRAUB, M., and W. G. KEMP. 1961. Protection with carnation mosaic virus in Dianthus barbatus. Virology 13:256-257.