Methods for Inoculating Muskmelon with *Erwinia tracheiphila*

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ABSTRACT

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Inoculation of muskmelon seedlings with *Erwinia tracheiphila* using a 15-pin dispenser with reservoir was superior to other methods because of ease of manipulation, speed of operation, distribution of inoculum, and transmission rate on susceptible seedlings.

Additional key words: bacterial wilt, *Cucumis melo*, resistance

Erwinia tracheiphila (Smith) Dye, 1968, the causal agent of bacterial wilt of cucurbits, occurs in North America, Europe, Asia, and Africa (1). It is particularly destructive to muskmelons (*Cucumis melo* L.) and cucumbers (*C. sativus*) in the north central and northeastern United States (4). Although losses from bacterial wilt are reduced by insecticide control of the vectors (5), muskmelon growers in southwestern Indiana often experience losses of 5–25% of plant populations to bacterial wilt (Reed, *unpublished*). Muskmelon cultivars and hydrids resistant to bacterial wilt would reduce plant mortality, insecticide application, and thus production costs.

Screening of muskmelon seedlings for wilt resistance requires plant inoculations that provide ease of manipulation, minimal and uniform inoculum volumes, minimal inoculation time, successful inoculation of susceptible plants, and consistent and repeatable results. Bacterial wilt inoculations using leaf rubs, pin pricks, needle punctures, and hypodermic needle injections have met with varying degrees of success (2). In a thesis on identification of *E. tracheiphila*, G. E. Evans (*personal communication*) stated that multiple prick systems have the advantage of causing minimal mechanical damage to the cotyledons. Some of these methods result in less than adequate transmission success with susceptible seedlings, whereas others are slow and tedious. Prend and John (2) considered a multineedle inoculating pad with 25–30 pins in a padded rubber stopper to be the most effective method. The pad was saturated with inoculum, then pressed against a cotyledon supported with a pot label. This method gave a high percentage of successful inoculations, but the continual use of the label was time-consuming. It also appeared that the volume of inoculum was variable.

MATERIALS AND METHODS

Leaf rub, multiple prick, artist airbrush, punch, and needle puncture methods of inoculation were compared in experiment I to determine the simplest and most efficient method for inoculations of large numbers of muskmelon seedlings with *E. tracheiphila*. Leaf rub inoculation was made by lightly rubbing the Carborundum-dusted upper surface of both cotyledons with inoculum-saturated gauze. The multiple prick inoculation was made using a No. 1 cork through which eight randomly placed pins protruded a distance of 1 mm (Fig. 1). Prior to each inoculation, the cork was dipped in inoculum and the pins pressed into the upper surface of the cotyledons, which were supported by fingertip. The artist airbrush inoculation was made by spraying a narrow jet of inoculum from the reservoir onto the center of each cotyledon until the surface appeared abraded. The punch inoculation was

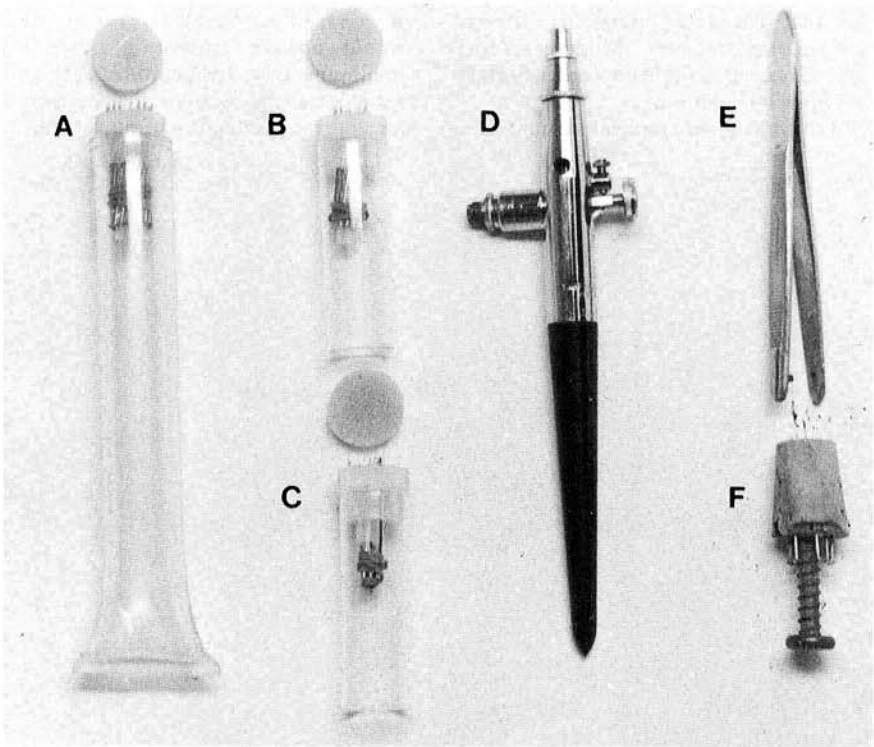

Fig. 1. Instruments tested for inoculation of muskmelon seedlings with bacterial wilt: **(A)** 15-pin dispenser, **(B)** 12-pin dispenser, **(C)** 8-pin dispenser, **(D)** artist airbrush, **(E)** punch, and **(F)** 8-pin cork.

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made using a punch constructed from forceps with the tips removed and the alignment peg shortened to 2.5 mm. Prior to inoculation, the punch was dipped in inoculum to fill the alignment hole, and a hole was then punched in the midvein of each cotyledon. The needle puncture inoculation was made using a standard dissecting needle with a band of masking tape 2 mm above the point to control depth. Prior to each inoculation, the needle was dipped in inoculum and then pressed into the stem at the base of the cotyledons.

Inoculum was prepared from infected Perlita muskmelon seedlings that had been inoculated at the fully expanded cotyledon stage 5 days prior to use and exhibited wilting of both cotyledons. Stems from approximately 350 (50 g) symptomatic seedlings were rinsed in distilled water, sectioned, added to 4 ml of distilled water per gram of stem tissue, macerated in a microblender, and strained through a single gauze layer. Inoculum used at room temperature was stirred regularly to prevent settling, and inoculations were completed within 2 hr after preparation.

We have found that bacterial wilt cultures lose virulence unpredictably and that bacteria apparently occur as a mixture of virulent and avirulent individuals. We established the virulence of our inoculum, as is routinely done in resistance research, on the response of susceptible cultivars (Perlita and Charentais Improved) rather than on bacterial cell counts.

Muskmelon cvs. Perlita, Charentais Improved, and Burpee Hybrid were used to evaluate inoculation procedures. Perlita and Charentais Improved are highly susceptible, whereas Burpee Hybrid is moderately susceptible to wilt. Seedlings were planted in Jiffy-64 trays using Jiffy Plus potting medium. Uniform germination, which is often difficult to obtain with muskmelon, was assured by pressing the medium into the tray until it was depressed 5 mm from the tray top, saturating the medium with water, placing seeds loosely on the pressed surface, filling the remainder of the cell with dry medium, and lightly (to avoid seed displacement) watering with warm water. The seedlings were greenhouse-grown at 30 C with constant light from 40-watt Luxor, Vita-Lite lamps suspended 27 cm above the tray with one bulb per 0.28 m^2 of bench space. Seedlings were watered daily and received no fertilization other than that provided in the Jiffy Plus.

Seedlings were inoculated at the fully extended cotyledon stage when the primordia of the first leaf became visible (generally 5 days after planting). Inoculations were centered on the midvein near the base of the cotyledon or in the stem (needle puncture) immediately below the cotyledon. Seedlings were observed daily for symptoms, and those with completely wilted cotyledons were counted and removed from the trays.

All experiments were designed as randomized complete blocks for statistical interpretation. Experiment I included six treatments, eight replicates and 12 plants per treatment per replicate; experiment II included eight treatments, seven replicates, and 16 plants per treatment per replicate; experiment III included four treatments, eight replicates, and 16 plants per treatment per replicate.

After experiment I, we attempted to make the Prend and John (2) system faster and more efficient. Two multiple prick, dispenser-type, inoculation tools were constructed (Fig. 1). The dispensers were made from Kimble 60965-L, 1-dram shell vials with plastic caps. The vial was used as a reservoir, and pins were pushed 2 mm through the plastic cap and wrapped tightly inside with a rubber band to prevent slipping. The first tool had eight pins arranged in a "plus" design, and the second had 12 pins arranged in a circle. Three small holes were made with a heated dissecting needle in each cap to allow inoculum to flow out of the reservoir. A 3-mm sponge wafer was impaled on the pins to aid spread of the inoculum and prevent injury to the fingertips that supported the cotyledons.

Experiment II demonstrated that the dispensers were faster than the other methods, but neither the release of inoculum from the dispenser nor the volume of the dispenser was totally satisfactory. In experiment III, we substituted plastic tubing (1.27 cm i.d., sealed at one end) for the reservoir and increased the pin number to 15 (Fig. 1). The pins were better supported in this tool by placing a 20-mm section of 6-mm-diameter disposable pipette in the center of the pins (inside cap) and wrapping them tightly with a rubber band. A 1-mm hole was placed in the center of the plastic cap to allow flow of inoculum, and the pins were again covered with a 3-mm sponge wafer. The greater volume and flexibility of the tube gave a more uniform flow of inoculum into the sponge. The dispenser tube, which held 12.5 ml of inoculum, was refilled when half empty to maintain uniform flow of inoculum.

RESULTS

Experiment I. The highest percentage of infection after 5 and 10 days was by the punch method, although the eight-pin cork method gave good infection after 10 days (Table 1). All methods produced good infection 15 and 20 days after inoculation on the highly susceptible Perlita seedlings, but only the punch and eight-pin cork methods at 15 days and the punch, eight-pin cork, and needle puncture methods at 20 days gave good transmission on the less susceptible Burpee Hybrid seedlings. The punch technique was superior in percentage of transmission but much slower to manipulate than the eight-pin cork, the next most effective method.

Burpee Hybrid seedlings developed symptoms more slowly and had fewer wilted plants 20 days after inoculation than Perlita seedlings. Also, 11% of the uninoculated Perlita and 2% of the uninoculated Burpee Hybrid seedlings developed symptoms 20 days after inoculation. Transmission to uninoculated plants possibly occurred during daily observations through abrasions and breakage of plants growing in contact with inoculated plants.

Experiment II. Experiment II was conducted to determine whether the inoculation methods in experiment I could be improved and whether inoculation at two sites on the seedling was necessary, based on transmission success and rate of symptom development. Inoculations at two sites were superior to inoculations at one site. Contrast between the two methods in percentage of wilted plants was greater with Burpee Hybrid than with Perlita seedlings (Table 1).

The punch method on both Perlita and Burpee Hybrid, the needle puncture on Burpee Hybrid, and the artist airbrush on Perlita seedlings produced the highest percentages of wilted seedlings. The punch, needle puncture, eight-pin cork, and 12-pin dispenser methods all gave good transmission 10, 15, and 20 days after inoculation. Similar results were recorded for all techniques 15 and 20 days after inoculation with Perlita seedlings.

Overall, greater transmission was obtained with the punch method, whereas the dispenser method was faster than other techniques.

Experiment III. Experiment III was conducted to determine whether a dispenser could be modified to produce as high a percentage of infection as the punch method. After 5 days, the percentage of infection was higher with the punch method than either the new 15-pin dispenser with reservoir or the needle puncture methods; however, all inoculation techniques gave excellent infection 10, 15, and 20 days after inoculation.

DISCUSSION

The punch method of inoculation produced the most rapid symptom development and the highest frequency of transmission of all methods tested. This method used minimal and uniform inoculum volumes and gave consistent and repeatable results. It was not, however, easy to manipulate, and it took considerable time. The 15-pin dispenser method produced a slower rate of symptom development but a high frequency of infection at 10, 15, and 20 days after inoculation. The 15-pin dispenser was easy and fast to use, used a small and constant amount of inoculum,

Table 1. Comparison of inoculation methods for infection of muskmelon seedlings with *Erwinia tracheiphila*

Inoculation method	Number of inoculation sites	Infection (%) days after inoculation							
		5		10		15		20	
		Cultivar[a]							
Experiment I		P	BH	P	BH	P	BH	P	BH
Leaf rub	2	5	0	55	20	87	47	92	57
Eight-pin cork	2	14	7	85	79	91	86	94	89
Artist airbrush	2	13	0	82	53	94	67	98	74
Punch	2	34	5	93	84	96	91	96	91
Needle puncture	1	17	7	70	54	92	77	97	86
Uninoculated	...	0	0	4	0	8	1	11	2
LSD 5%		10.4	NS[b]	10.4	10.8	9.6	12.3	8.7	11.5
Experiment II		P	BH	P	BH	P	BH	P	BH
Leaf rub	1	10	1	40	14	78	28	92	39
	2	7	1	57	15	86	32	95	54
Eight-pin cork	1	18	17	58	62	93	75	96	79
	2	26	24	96	67	100	86	100	92
Eight-pin dispenser	1	5	8	66	40	88	60	90	65
	2	20	14	86	61	96	75	100	79
12-pin dispenser	1	14	8	83	40	97	66	97	73
	2	17	16	92	75	99	86	100	91
Artist airbrush	1	22	13	80	48	96	72	100	76
	2	54	18	82	60	96	79	96	83
Punch	1	22	16	69	54	94	73	94	79
	2	52	38	93	80	98	91	100	93
Needle puncture	1	24	26	72	65	89	91	92	94
	2	30	44	89	76	95	94	98	96
Uninoculated	...	0	0	0	0	0	0	6	3
	...	0	0	0	0	0	0	0	1
LSD 5%		18.5	10.7	18.2	13.9	12.0	12.9	8.5	12.9
Experiment III		CI	BH	CI	BH	CI	BH	CI	BH
15-pin dispenser	2	5	6	95	95	99	99	100	99
Punch	2	28	13	98	93	100	98	100	98
Needle puncture	2	5	2	86	88	96	94	100	97
Uninoculated	...	0	0	0	0	1	1	1	2
LSD 5%		3.5	4.6	14.5	13.6	10.0	12.2	8.5	12.0

[a] P = Perlita, BH = Burpee Hybrid, and CI = Charentais Improved.
[b] Not significant.

and gave consistent and repeatable results. Because our goal was to develop a method suitable for screening large numbers of seedlings for resistance, the 15-pin dispenser proved to be the superior method. It combined the best aspects of the method used by Prend and John (2) (multiple pricks and storage of inoculum) with ease of manipulation and better control of inoculum volume. Symptom development was more rapid with the punch than other methods, probably because the inoculation punctured the midvein of the cotyledon. Introduction of inoculum directly into the vascular system with the punch and needle puncture methods could result in overcoming mechanisms that block movement of bacteria from leaves to the primary vascular system. Although both the needle puncture and eight-pin cork methods resulted in good transmission of the bacterium, neither was rapid nor gave the ease of manipulation accomplished with the 15-pin dispenser.

The use of muskmelon seedlings to evaluate wilt resistance allows the plants to be tested at their most susceptible stage of growth (2,3). The 15-pin dispenser provides an efficient tool for screening large numbers of seedlings for resistance research; however, seedlings must be evaluated 10 days after inoculation to ensure recognition of susceptible plants. Although slight differences in susceptibility might not be recognized, the technique provides excellent identification of resistant lines.

The 15-pin dispenser has been successful for evaluations of several hundred breeding lines. As many as 7,500 seedlings have been inoculated by one person in a day. Screening for resistance can now be completed in 26 days, a process that has taken as many as 45 days. The 15-pin dispenser is being used to screen for bacterial wilt resistance in a recurrent selection program and in germplasm from the National Muskmelon Research Group.

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