Comparison of Methods for Inoculation of Muskmelon with *Fusarium oxysporum* f. sp. *melonis*

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ABSTRACT

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Differences in Fusarium wilt resistance of three muskmelon cultivars were determined from three methods of inoculation. In the standard method, roots of uprooted seedlings were dipped in an inoculum suspension and seedlings transplanted into noninfested soil; differences among the cultivars were demonstrated when inoculated 6 and 11 days after planting. Other methods, which require considerably less labor and space, involve pipetting inoculum to undisturbed seedlings or dipping modified plastic trays containing seedlings into inoculum suspensions. The pipette inoculation method resulted in inconsistent ranking of cultivars for resistance to Fusarium wilt. The tray-dip inoculation method resulted in a consistent ranking of cultivars, but differences were not as clearly defined as those that resulted from the standand inoculation method. For all inoculation methods, less disease was observed when seedlings were inoculated 11 days after planting than 6 days after planting.

Additional key words: *Cucumis melo*

Fusarium wilt, caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *melonis* Leach & Currence, is an economically important disease of muskmelon (*Cucumis melo* var.

reticulatus) in Indiana. Once the disease is established in a field, the pathogen is likely to remain indefinitely because subsequent crops of susceptible melon cutivars increase the pathogen population (1,9), and stable population levels have been detected after rotation with nonhost crops (1).

Because of the persistence of the pathogen in soil, the disease is best managed by using wilt-resistant muskmelon genotypes. Standard inoculation procedure to evaluate genotypes of muskmelon or watermelon for resistance to Fusarium wilt involves uprooting seedlings, washing roots, dipping them in an inoculum suspension, and transplanting seedlings into noninfested soil (4,10). A similar method was used to test different isolates of *F. oxysporum* for pathogenicity (8). Other

procedures include seeding or transplanting directly into infested soil (2). With the exception of field tests, methods generally employ a range of inoculum concentrations so that resistance or susceptibility is not masked by extraordinarily high or low inoculum levels.

Tests for wilt resistance that employ multiple inoculum concentrations are accompanied by significant resource constraints. The standard inoculation procedure is time consuming, and transplant containers require considerable greenhouse space. Alternative procedures that require less labor and space would hasten the identification of wilt-resistant genotypes. Plastic growing trays reduce space requirements and afford the opportunity to appraise resource-saving procedures. The objective of this research was to determine the effectiveness of standard and alternative inoculation procedures to evaluate muskmelon genotypes for resistance to Fusarium wilt.

MATERIALS AND METHODS

Inoculation procedures were evaluated on seedlings of three muskmelon cultivars: Burpee Hybrid (W. Atlee Burpee Company, Warminster, PA), Summet (Asgrow Seed Company, Kalamazoo, MI), and Superstar (Harris Moran Seed Company, Rochester, NY). These cultivars were selected because they represent the wide range of resistance we observed in infested fields. Superstar is the most resistant; Burpee Hybrid is very susceptible. Summet is

more resistant than Burpee Hybrid but slightly less resistant than Superstar. Plants were raised in soilless bark-peat-perlite substrate (JPA, West Chicago, IL) in plastic trays in a greenhouse, where temperature was maintained at 26 ± 2 C. Inoculum was prepared from isolate 8318, race 2 (7) of *F. oxysporum* f. sp. *melonis*, collected locally from wilted muskmelon vines. Mycelial plugs (3 mm in diameter) from a 5-day-old colony growing on acidified potato-dextrose agar were transferred to 500-ml flasks containing 100 ml of a potato-dextrose broth (10 g/L). Flasks were placed on a shaker operating at 96 rpm and maintained at 23 C. After 5 days, contents of the flasks were combined and filtered through two layers of cheesecloth. The filtrate (more than 95% microconidial) was diluted with distilled water to obtain inoculum concentrations of 10^3, 10^4, 10^5, 10^6, and 10^7 microconidia per milliliter. A hemactyometer was used to quantify inoculum.

Table 1. Comparison of probit lines obtained from regression of probit disease incidence on log inoculum concentration for each combination of cultivar, inoculation method, and inoculation time

Inoculation method	Cultivar	Inoculation time (days after planting)				F-ratio[y]
		6		11		
		Probit line[x]		Probit line		
		Intercept	Slope	Intercept	Slope	
Standard	Burpee Hybrid	1.04	0.95 a[z]	0.09	1.08 a	0.89
	Summet	−2.13	1.38 b	0.55	0.72 b	45.51*
	Superstar	−2.09	1.10 c	−0.10	0.60 c	33.34*
Pipette	Burpee Hybrid	0.34	0.77 a	0.94	0.61 a	5.21*
	Summet	0.57	0.68 a	2.10	0.30 b	19.74*
	Superstar	−2.46	1.02 a	2.62	0.18 b	10.09*
Tray-dip	Burpee Hybrid	1.43	0.59 a	2.78	0.41 a	5.13*
	Summet	0.26	0.63 b	2.12	0.38 b	4.40*
	Superstar	1.40	0.40 b	0.17	0.53 c	4.79*

[x]The probit lines include intercept and slope parameters for each cultivar inoculated by the three methods 6 and 11 days after planting.

[y]F-ratio for comparison of disease incidence 6 and 11 days after planting for each combination of cultivar and inoculation method. * = Significant F-ratio, $P = 0.05$.

[z]Lines within each inoculation method followed by the same letter are not significantly different according to a general linear test method (6).
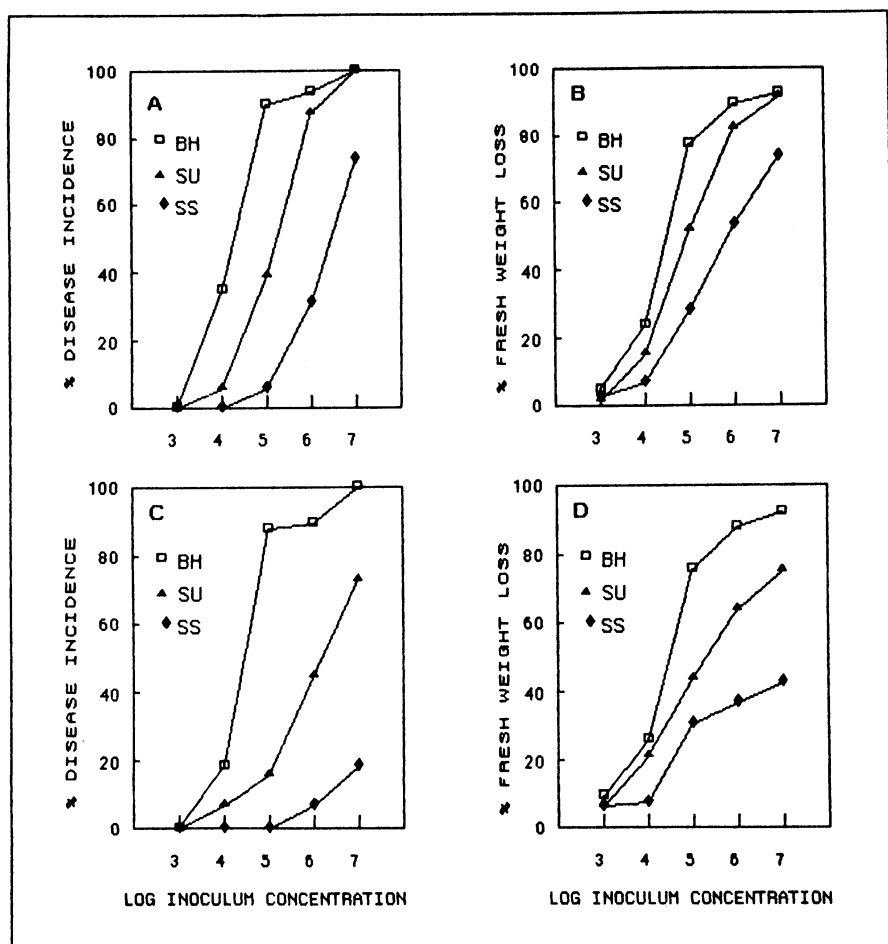

Fig. 1. Inoculum-dose vs. disease-response (ID-D) curves for three muskmelon cultivars inoculated using the standard method. Cultivars are Burpee Hybrid (BH), Summet (SU), and Superstar (SS). **(A)** Disease incidence and **(B)** fresh-weight loss for inoculation 6 days after planting; **(C)** disease incidence and **(D)** fresh-weight loss for inoculation 11 days after planting.

Seedlings were inoculated by three methods 6 days (cotyledon stage) or 11 days (expansion of first true leaf) after planting. Replicated, noninoculated treatments were included in all experiments. Ten or 12 days after inoculation, disease was assessed and fresh weights were determined for all seedling shoots in each replicate. In preliminary experiments, disease incidence did not increase significantly after that time.

Standard method. Seedlings were raised in soilless substrate in plastic trays (JPA, West Chicago, IL) containing 196 cells each. Six or 11 days after planting, the seedlings were uprooted and the roots washed gently in water, then dipped in the appropriate inoculum suspension for about 1 min. Six-day-old seedlings were transplanted into 50-cell plastic trays and 11-day-old seedlings were transplanted into waxed-paper cups (300 ml). Wilt was assessed 10 days after inoculation for 30 seedlings (three replicates of 10) of each cultivar and for each inoculum concentration.

Pipette method. Seedlings remained undisturbed throughout the experiment in plastic trays containing 50 cells each (TLC Polyform, Inc., Minneapolis, MN). Five milliliters of the appropriate inoculum suspension was delivered to the substrate around each seedling with an automatic pipetting machine (Oxford Laboratories Inc., Foster City, CA). Twelve days after inoculation, wilt incidence was assessed for 50 seedlings (five replicates of 10) of each cultivar and for each inoculum concentration.

Tray-dip method. Seedlings were raised in soilless substrate in modified plastic trays containing 196 cells each. Plastic was cut from the base of each cell to enlarge the drain openings to a diameter of 1.4 cm. The trays were gently pressed into a sand-covered greenhouse bench so that the base of each cell was buried 3–5 mm in the sand bed. The cells were filled with substrate and seeded without disturbing the trays. Six or 11 days after planting, the trays were lifted from the sand bed. Roots protruding from the cell bottoms were rinsed with water before the entire tray was placed in a shallow basin of the appropriate inoculum concentration for 5 min. The tray was then returned to its original position on the sand bed and left undisturbed for 12 days.

Disease incidence was estimated as $I =$

$(X + 0.5)/(N + 1.0)$, where I = disease proportion, X = number of seedlings with symptoms in each replicate, and N = total number of seedlings in each replicate. Although this estimate of disease incidence is slightly biased, it allowed for treatments that resulted in no disease in some replicates to be included in statistical analyses of the dosage-mortality functions. Treatments that resulted in no disease for all replicates were not included in the analyses.

Proportions of estimated disease incidence were transformed to probits (3) and regressed on log-transformed inoculum dose values. The probit lines for the three cultivars for each of the six combinations of inoculation method and inoculation time were tested for differences with a general linear test approach (6).

RESULTS

The methods differed in their time and greenhouse space requirements. The standard inoculation method required about 32 hr; the pipette and tray-dip methods required 8 and 4 hr, respectively. Seedlings in paper cups required three times more greenhouse bench space than seedlings in plastic trays.

In probit line analyses, the methods differed as a means to demonstrate differences in Fusarium wilt resistance of the three cultivars. Differences in resistance among cultivars were clearly distinguished with the standard root-dip method. Significantly different probit lines resulted for seedlings inoculated 6 and 11 days after planting (Table 1). This is reinforced by inspection of inoculum-dose vs. disease-response (ID-D) curves for disease incidence and fresh-weight loss at both inoculation times (Fig. 1A–D). Probit lines representing disease response for inoculation at 6 and 11 days were significantly different for both Summet and Superstar but were not different for Burpee Hybrid (Table 1).

The intermediate resistance of Summet was not distinguished from Burpee Hybrid when inoculated 6 days after planting by the pipette method and was not distinguished from Superstar when inoculated 11 days after planting (Table 1). This is evident in ID-D curves (Fig. 2A,C). The curves for disease incidence compare well with those for fresh-weight loss for seedlings inoculated 11 days after planting (Fig. 2C,D), but different patterns of ID-D curves resulted for seedlings inoculated 6 days after planting (Fig. 2A,B). Also, differences in disease response for seedlings inoculated at different times for each cultivar were significant (Table 1).

Differences in resistance between Summet and Superstar were not distinguished with the tray-dip method for inoculation at 6 days; however, significantly different probit lines resulted for the three cultivars for inoculation at 11 days (Table 1).

Different trends were apparent for ID-D curves representing fresh-weight loss and disease incidence. Curves for the cultivar Summet more closely resemble those for Burpee Hybrid in terms of fresh-weight loss for both inoculation times (Fig. 3B,D), but in terms of disease incidence, Summet was not different from Superstar for inoculation at 6 days (Fig. 3A), and the three curves are distinct for inoculation at 11 days (Fig. 3C). Probit lines representing disease response for inoculation at 6 and 11 days were significantly different for each cultivar (Table 1).

DISCUSSION

Differences among cultivars were best identified using the standard root-dip method based on appearance of ID-D curves and probit analyses. The ID-D curves for disease incidence were visually distinct, the probit lines differed significantly, and the ID-D curves for fresh-weight loss resembled those for disease incidence. These criteria were not satisfied for other methods.

Regardless of inoculation method, less disease was observed when seedlings were inoculated 11 days after planting than 6 days after planting. This was indicated by

the ID-D curves for disease incidence and fresh-weight loss. One exception to this occurred with the cultivar Burpee Hybrid when inoculated using the standard method. The relative lack of resistance in this cultivar was emphasized by similarities in disease incidence and fresh-weight loss between the two inoculation times. The importance of differences in disease response at different inoculation times is that one inoculation time may provide an accurate reflection of resistance. In these experiments, results from inoculation at 11 days are more consistent with observations in commercial fields. This supports other results that indicate that resistance may not be expressed in very young seedlings (5).

The standard method resulted in more disease and greater weight loss than the other methods. This was probably due to increased wounding and direct exposure of the entire root system to the inoculum. Differences among cultivars were not clearly distinguished using the pipette method. The relative lack of root injury and uncertainty about the amount of effective inoculum reaching the root surfaces may be responsible for this result.
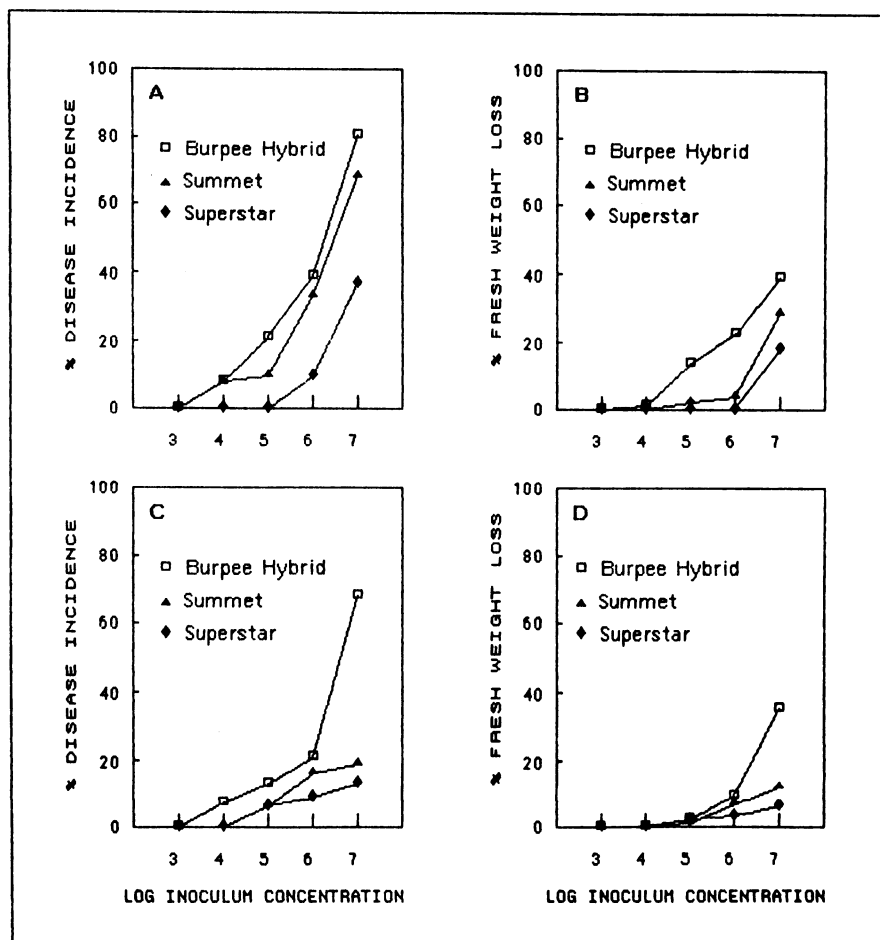
Although the tray-dip method included

Fig. 2. Inoculum-dose vs. disease-response (ID-D) curves for three muskmelon cultivars (Burpee Hybrid, Summet, and Superstar) inoculated using the pipette method. (A) Disease incidence and (B) fresh-weight loss for inoculation 6 days after planting; (C) disease incidence and (D) fresh-weight loss for inoculation 11 days after planting.
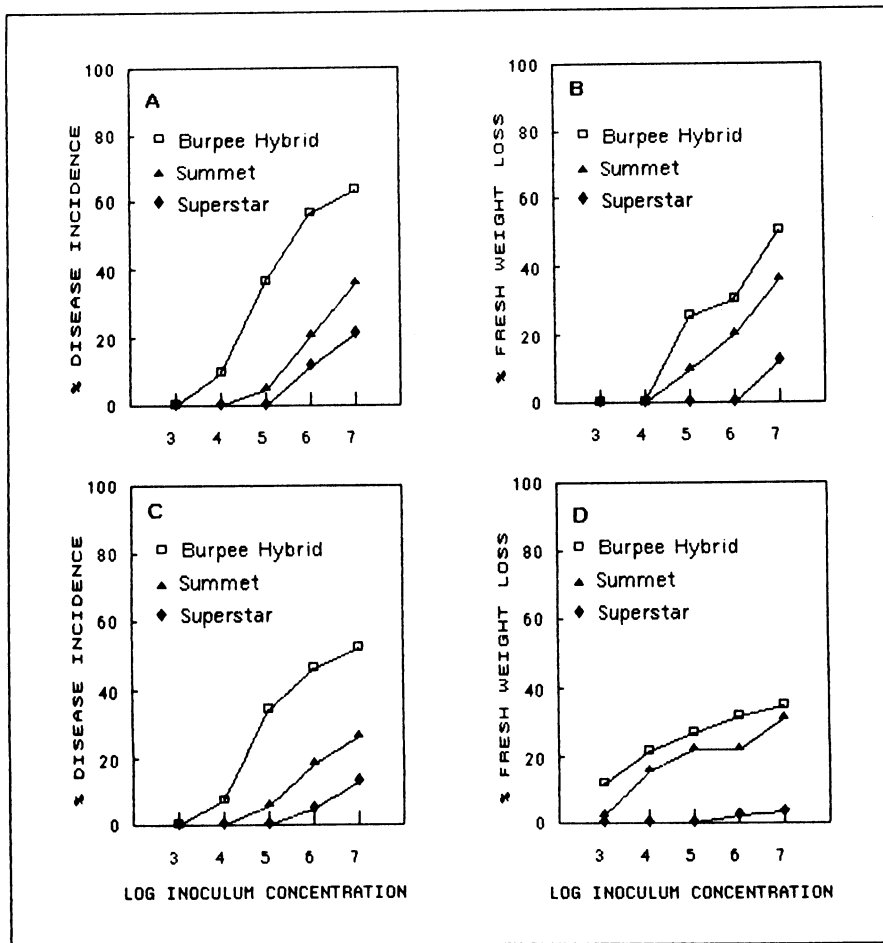
Fig. 3. Inoculum-dose vs. disease-response (ID-D) curves for three muskmelon cultivars (Burpee Hybrid, Summet, and Superstar) inoculated using the tray-dip method. **(A)** Disease incidence and **(B)** fresh-weight loss for inoculation 6 days after planting; **(C)** disease incidence and **(D)** fresh-weight loss for inoculation 11 days after planting.

substantial root injury and direct exposure to inoculum, the ID-D curves for disease incidence and fresh-weight loss were diverse. More confidence could be placed in the tray-dip method if the curves had similar shapes. This is especially important when seedlings remain in plastic trays, because nutrient imbalances that might occur in trays often mimic initial symptoms of Fusarium wilt. Nutrient stress might be relieved with supplemental, periodic treatment with a nutrient solution.

Inoculation by the tray-dip method of whole trays of a variety of commercial cultivars might be acceptable for evaluation of their relative resistance but could not be used to evaluate a diverse population of genotypes with different time requirements for seed germination. Evaluations would be biased for genotypes with seed germination times at least 2 days greater than the mean germination time. If germination times were known and genotypes were sorted accordingly, then the tray-dip method would result in considerable resource savings.

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