A Leaf Disk Assay for Detection of Resistance of Melons to *Sphaerotheca fuliginea* Race 1

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ABSTRACT

Cohen, R. 1993. A leaf disk assay for detection of resistance of melons to *Sphaerotheca fuliginea* race 1. Plant Dis. 77:513-517.

The resistance of 10 entries of melons (*Cucumis melo*) to powdery mildew, caused by *Sphaerotheca fuliginea* race 1, was evaluated by means of a leaf disk assay. Disks (9 mm in diameter), removed from fully expanded leaves, were placed in petri dishes containing water agar amended with 25 μg/ml of benzimidazole and maintained at 25 C. Disks were inoculated by applying a 10-μl drop of a water suspension of conidia or by blowing air over an infected plant toward the test disk. Differences in inoculation efficacy between the two methods were insignificant. Infection efficacy and sporulation were greater for the adaxial position of the disk. Powdery mildew development and sporulation were reduced following exposure to high light intensity, compared with lower light intensities. The percentage of sporulating disks originating from the cotyledons of resistant plants was low (0–5%), indicating that preliminary selection can be done as early as the cotyledonary stage. The response of the host to powdery mildew, based on the inoculation of disks originating from the third leaf, correlated well with results obtained with the whole plant, indicating that a disk assay using this leaf may accurately predict the response of melon plants to *S. fuliginea*.

Powdery mildew, caused by *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci, limits the production of melons (*Cucumis melo* L.) throughout the world (14). Fungicide application and the use of resistant cultivars are the major means of disease control in cucurbits (5,7,11, 14).

Breeding for resistance is based on a system of inoculation of plants with the pathogen and selection of the resistant individuals (5,14). Many difficulties arise when selection for resistance is conducted in the field. Occasionally, melon plants grown in the field exhibit powdery mildew infections at a relatively late stage of their growth, mostly after fruit set. Selection of resistant plants at this stage forces the breeder to self-pollinate the whole population to be tested. Moreover, the infection in the field is not uniform, and different pathogens and various races of the pathogens may also infect the plant. Powdery mildew development in the field is dependent to a great extent on environmental conditions. Excessively high temperature limits the development of the disease (1,13), whereas cool temperatures and shading enhance it. Therefore, screening for resistance under greenhouse conditions, where relatively young plants (three to four leaves) are used for selection, is common (5). This method is more accurate than selection in the field because it allows controlled inoculation. However, it requires a costly controlled environment. Therefore, developing a rapid, accurate, and reproducible method for the evaluation of melon response to powdery mildew is important.

The use of leaf disks in plant pathology

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, 3586-E, 1992 series.

Accepted for publication 28 December 1992.

Table 1. Characteristics of melon (*Cucumis melo*) entries tested

Entry (cultivar name)	Abbreviation	Common name	Variety	Source	Response to powdery mildew[w] (disease reaction)
...	NY × 212	Muskmelon	*reticulatus*	Israel	Resistant
Noy Yizre'el	NY	Muskmelon	*reticulatus*	Israel	Resistant
...	NY × P202	Muskmelon	*reticulatus*	Israel (EH[x])	Resistant[y]
Dulce	DUL	Muskmelon (cantaloupe)	*reticulatus*	USA	Resistant
Gil'ad	GIL	Casaba	*inodorous*	Israel	Resistant
...	212	Muskmelon	*reticulatus*	Israel (BL[z])	Susceptible
Persia 202	P202	Muskmelon	*reticulatus*	Iran (BL)	Susceptible
Ananas Yoqne'am	AY	Muskmelon	*reticulatus*	Israel	Susceptible
Rochet	TPRB	Casaba	*inodorous*	Spain	Susceptible
Piel de Sapo	PPSA	Casaba	*inodorous*	Spain	Susceptible

[w] Based on field observations at the mature fruit stage.
[x] Experimental hybrid.
[y] Tested in a greenhouse, not in the field.
[z] Breeding line.

Table 2. Effect of leaf disk position of levels of infection and sporulation of *Sphaerotheca fuliginea*

Entry	Disease response[x]	Infected disks[y] (%)		Sporulated disks (%)	
		Adaxial	Abaxial	Adaxial	Abaxial
NY × 212	R	17	0	0	0
NY	R	0	0	0	0
NY × P202	R	11	0	0	0
DUL	R	0	0	0	0
212	S	100[z]	44	100[z]	28
P202	S	100	83	100	83
AY	S	94	72	94[z]	44
TPRB	S	100	72	100	72
PPSA	S	100	91	91	70

[x] R = resistant, S = susceptible.
[y] Powdery mildew was detected 5 days after inoculation on disks which originated from the second leaf.
[z] Denotes a significant difference ($P = 0.05$) in a paired t test.

research, such as in breeding programs for resistance, has been reported previously (2–4,6,10). Indeed, Bertrand and Pitrat (2) described a leaf disk method for powdery mildew of melons; however, with their methodology the results of leaf disk and whole-plant inoculation often differed. The purpose of this study was to improve the method for selecting melon plants resistant to *S. fuliginea* race 1 based on detached leaf disk inoculation. A brief report has been published (4).

MATERIALS AND METHODS

Plant material and growth conditions. Ten melon genotypes with varying levels of resistance to *S. fuliginea* race 1 were tested (Table 1). Plants were grown in 250-ml plastic pots containing vermiculite and peat (1:1 v/v) in the greenhouse. Day length ranged from 10.5 to 12 hr, and temperature was maintained at 25 ± 5 C by day and 15 ± 5 C by night. At different stages of plant development, either the whole plant was inoculated or leaves were removed for the leaf disk assay.

The pathogen. An isolate of *S. fuliginea* race 1 isolated from melon (*C. melo* 'Ananas Yoqne'am') was used for inoculation in all tests. The isolate was maintained on Ananas Yoqne'am in a growth chamber at 23 C with a 12-hr photoperiod (300 $\mu E \cdot m^{-2} \cdot s^{-1}$). In addition, melon plants of cultivar Noy Yizre'el, which is resistant to race 1 of the pathogen but susceptible to race 2, were placed among the inoculated plants for race identification and for detection of possible contamination of the pathogen with race 2. All of the Noy Yizre'el plants remained free of powdery mildew throughout the course of the experiments.

Leaf disk preparation, inoculation, and environmental conditions. Leaf disks (9 mm in diameter) were removed from cotyledons or leaves by means of a cork borer. The disks (10 per replication) were placed in petri dishes containing 0.16% water agar amended with 25 μg/ml of benzimidazole (Sigma B 9131; A. Dinoor and N. Eshed, *personal communication*). Benzimidazole is nontoxic to the pathogen and was used to prevent early senescence of the leaf disks. Inoculum was prepared by rinsing a powdery mildew-infected leaf with water containing 0.01% Tween 20. The conidial suspension concentration was evaluated with the aid of a hemocytometer. Leaf disks were inoculated by placing a 10 μl drop of suspension containing conidia of *S. fuliginea* (2×10^4/ml unless otherwise indicated) on the adaxial side of each leaf disk. In the experiment conducted to test the effect of leaf disk position on infection severity (Table 2), another set of leaf disks was inoculated on the abaxial side. The maximum elapsed time from inoculum preparation until the actual inoculation was 15 min. After inoculation, the petri dishes with the disks were placed in a growth chamber at 25 C with a 12-hr photoperiod at a light intensity of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$.

To test the effect of light on mildew development, various light intensities were obtained by placing the petri dishes at different distances from the light source. Light intensity was measured with a quantum meter. The last two tests were repeated twice.

Disease-severity evaluation. The development of powdery mildew hyphae and conidia on the leaf disks was examined 5–8 days after inoculation. The frequency of different stages of powdery mildew development on the disks was determined with a stereoscope and rated as follows: 0 = noninfected disk; 1 = only hyphae present; 2 = hyphae with up to 50 conidiophores per disk; and 3 = more than 50 conidiophores per disk (heavy sporulation). For statistical analysis, the total number of infected disks includes disks ranked 1, 2, and 3; and sporulating disks include disks ranked 2 and 3 only.

To compare the response of the whole plant to leaf disks, whole plants were inoculated by blowing air from an infected plant as the inoculum source. Disease severity in whole plants was determined by visually estimating the percent leaf area covered by the pathogen using a standard key (14). In one experiment, leaf disks were inoculated either by a conidial suspension or by blowing air from an infected plant.

Statistical analysis. The significance ($P = 0.05$) of differences in each category of fungal development was determined by Duncan's multiple range test.

RESULTS

Effect of disk position on infection. Powdery mildew mycelia and conidia developed on either the adaxial or abaxial side of inoculated leaf disks (sec-

Table 3. Effect of light intensity on levels of infection and sporulation of *Sphaerotheca fuliginea*[x]

		Light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)									
		200	130	90	45	30	200	130	90	45	30
Entry	Disease response[y]	Infected disks (%)					Sporulated disks (%)				
NY × 212	R	17 a[z]	17 a	22 a	28 a	22 a	0 a	6 a	0 a	17 a	17 a
NY	R	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
NY × P202	R	0 b	22 ab	17 b	47 a	22 ab	0 a	6 a	6 a	0 a	6 a
DUL	R	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
GIL	R	6 b	33 ab	28 ab	50 a	39 ab	0 b	0 b	6 ab	22 a	17 ab
212	S	83 a	89 a	100 a	72 a	83 a	72 a	55 a	72 a	72 a	55 a
P202	S	55 c	61 bc	94 ab	100 a	100 a	33 b	61 ab	83 a	94 a	94 a
AY	S	50 b	100 a	94 a	100 a	100 a	44 b	83 a	94 a	77 a	78 a
PPSA	S	50 b	100 a	94 a	100 a	77 a	39 b	100 a	83 a	94 a	44 b

[x] Leaf disks were originated from the first true leaf. Data were recorded 7 days after inoculation.
[y] R = resistant, S = susceptible.
[z] Within rows, values with a common letter do not differ significantly ($P = 0.05$).

ond leaf) originating from susceptible entries. However, in some cases significantly higher percentages of infection were observed on the adaxial side of the disks (Table 2). The level of infection or sporulation on the adaxial side of disks from susceptible entries ranged from 91 to 100%, compared with 0–17% on disks from resistant entries.

Effect of light intensity on infection of leaf disks. Disks from the first leaf of susceptible entries (PPSA, P202, 212, and AY) were already infected to some degree 5 days after inoculation. Infection was observed on those entries on disks exposed to 90, 45, and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$, but not on those exposed to 130 or 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Two days later, powdery mildew developed at various levels on all the entries tested (except for NY and DUL) under all light conditions (Table 3). Infection and sporulation rates on NY × 212 and on 212 were not affected by light intensity, but in all other entries powdery mildew development was related to light intensity. High light intensity inhibited pathogen development, whereas low light intensity favored it. Exposure of inoculated leaf disks to 45–90 $\mu E \cdot m^{-2} \cdot s^{-1}$ was usually optimal for powdery mildew growth and sporulation.

Response of disks from cotyledons. Percentages of infected and sporulating disks originating from the cotyledons of susceptible entries were 60 and 97%, respectively (Fig. 1). Within this group, the difference between levels of infected and sporulated disks was small (5–16%). Sporulation on the resistant entries, NY × 212, NY, and DUL, was low (0–5%). Except for NY × 212, infection level was also low (0–15%). Infection and sporulation levels in the resistant hybrid NY × P202 were extremely high (97%).

Effect of inoculum concentration and inoculation method. Except for one entry (TPRB), infection levels on disks from the first leaf inoculated with blown air were similar to those inoculated with the suspension at 2×10^4 conidia per milliliter (Table 4). This was true for both susceptible and resistant entries. With a lower inoculum concentration (2×10^3 conidia per milliliter), infection was observed only on the susceptible entries, P202 and PPSA.

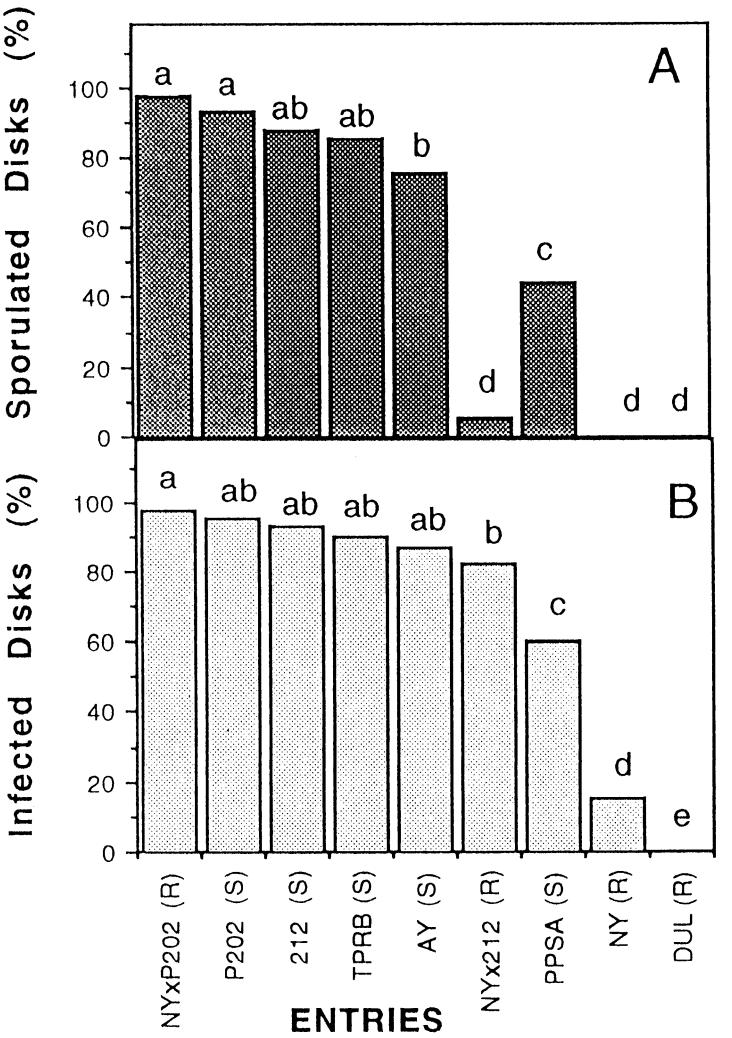

Fig. 1. Severity of **(A)** sporulation and **(B)** infection of *Sphaerotheca fuliginea* on cotyledon disks. Values with a common letter do not differ significantly ($P = 0.05$).

Effect of leaf order and comparison between leaf disks and the whole plant. The percentage of infected leaf disks harvested from the first three leaves of the susceptible entries (except for the first leaf of TPRB) ranged between 75 and 100% (Table 5). In the resistant entries NY and DUL, no infection was observed on disks from any of the leaves. The levels of infection of the resistant entries on disks from the second and third leaves were low (0–8%); levels on disks from the first leaf were higher (up to 50%). No sporulation was observed on disks originating from any of the resistant entries, as compared with 75–100% on disks

from the second and third leaves of susceptible entries.

The severity of powdery mildew in the whole plant was examined at the five-leaf stage. No disease was detected on resistant entries, confirming previous field observations (R. Cohen, *unpublished*). Disease severity of the susceptible entries ranged from 76 to 100%.

DISCUSSION

Powdery mildew develops well on both the adaxial and abaxial sides of leaves of cucurbit plants grown in the field or in the greenhouse. In the summer, when the plants are exposed to high temperatures and light intensity, powdery mildew symptoms are observed first on shaded leaves or on the adaxial leaf surface (14). In the leaf disk assay, hyphae developed more rapidly and sporulation was easier to observe on the adaxial side of the leaf (Table 2). The rough surface and the trichomes on the abaxial side of the leaf may be conducive to a more humid microenvironment, which probably inhibits pathogen development (1,13). Thus, tests with leaf disks should be conducted on the adaxial side.

Photosynthesis, which is affected by light intensity, has been demonstrated to be a prerequisite for the sporulation of several obligatory parasites (12). An increase in spore production by *Erysiphe cichoracearum* DC. on squash (*Cucurbita pepo* L. var. *ovifera* (L.) Alef.) was reported when the photoperiod was extended and the light intensity was increased (12). In contrast, powdery mildew growth and sporulation on leaf disks were favored in certain cases by low light intensity (Table 3). Leaf disks exposed to high light intensity seemed to turn yellow somewhat more rapidly than those incubated under the lower light intensity, which may possibly lead to poor pathogen development.

The response of melon cotyledons to powdery mildew does not necessarily represent the response of the whole plant to the pathogen (8,9). Indeed, cotyledon infection of resistant melon plants is well known in commercial greenhouses, especially when light intensity is low. Cotyledon disks originating from the hybrid NY × 212 and its resistant parental cultivar NY were infected with powdery mildew, but the percentages of sporulating disks were low (0 and 5%, respectively). DUL, another resistant cultivar, exhibited no infection on its cotyledon disks. Unexpected high sensitivity to powdery mildew was observed in NY × P202 cotyledons. In contrast to other entries tested in this study, the response of NY × P202 in the field is still unknown. It is possible that the susceptible reaction observed with the hybrid cotyledon disks is due to P202, a very susceptible line. Levels of cotyledon infection may serve as a first step in a preliminary selection of resistant individuals out of a large plant population. This selection method is reliable, because a powdery mildew-free cotyledon ensures a resistant plant. However, selection at the cotyledon stage may lead to a loss of plants which exhibit resistance at a later stage (9). Except for TPRB, no significant differences were observed between inoculation with blown air and with the highly concentrated conidia suspension (Table 4). Inoculating plants by blowing air is easier and faster than placing conidial suspension droplets on each leaf disk. However, a large number of infected leaves is required for this method of inoculation compared with the relatively small number required to obtain a conidial suspension. Field observation in Israel has shown that PPSA and P202 are extremely susceptible to powdery mildew. Indeed, these were the only entries that were infected following inoculation with the low (2×10^3) conidial suspension (Table 4).

Disease severity observed on whole plants was similar to that observed with infection levels on disks from the third leaf (Table 5). This finding indicates that leaf disks from the first two leaves might respond differently from the same leaves on the intact plant. Resistance response in the disk assay seems to be more reliable when carried out using the third leaf.

The results of this study indicate that with the use of this leaf disk method one can identify, with a high degree of accuracy and dependability, melon genotypes that are resistant or susceptible to race 1 of *S. fuliginea*. Bertrand and Pitrat (2) used a leaf disk method over a wide range of genetic backgrounds in combination with several pathotypes, but they observed differences in leaf disk and whole-plant responses. The present results, together with those of Bertrand and Pitrat, suggest that leaf disk methodology needs to be modified and calibrated to fit the desired combination of host genetic background and pathotype.

In previous studies of early blight and powdery mildew using leaf disks (3,6,10), the disks were floated on water. Placing the disks on solid agar in petri dishes enabled the maintenance of constant humidity and made the system easier to handle.

The assay described in this paper enabled mass selection of resistant plants from a large segregating population, thus saving greenhouse or field space and labor.

Table 4. Effect of inoculation method and inoculum concentration on infection and sporulation of *Sphaerotheca fuliginea* on disks from the first leaf

		Infected disks (%)			Sporulated disks (%)		
			Suspension (conidia/ml)			Suspension (conidia/ml)	
Entry	Disease response[y]	Air blow	2×10^4	2×10^3	Air blow	2×10^4	2×10^3
NY × 212	R	67 a[z]	46 ab	0 b	29 a	13 a	0 a
NY	R	0 a	0 a	0 a	0 a	0 a	0 a
NY × P202	R	8 a	4 a	0 a	4 a	0 a	0 a
DUL	R	0 a	0 a	0 a	0 a	0 a	0 a
GIL	R	25 a	8 a	0 a	8 a	0 a	0 a
212	S	100 a	79 a	0 b	100 a	67 a	0 b
P202	S	83 a	87 a	17 b	83 a	79 a	17 b
AY	S	100 a	96 a	0 b	96 a	85 a	0 b
TPRB	S	100 a	71 b	0 c	96 a	50 b	0 c
PPSA	S	100 a	87 a	8 b	100 a	70 a	8 b

[y] R = resistant, S = susceptible.
[z] Within rows, means with a common letter do not differ significantly ($P = 0.05$).

Table 5. Severity of infection and sporulation of *Sphaerotheca fuliginea* on leaf disks and on whole plants

		Infected disks (%)			Sporulated disks (%)			Whole plant infected leaf area[x] (%)
		Leaf order			Leaf order			
Entry	Disease response[v]	I[w]	II	III	I	II	III	
NY × 212	R	50 a[y]	4 a	0 a	0 a	0 a	0 a	0
NY	R	0 a	0 a	0 a	0 a	0 a	0 a	0
NY × P202	R	17 a	8 a	0 a	0 a	0 a	0 a	0
DUL	R	0 a	0 a	0 a	0 a	0 a	0 a	0
GIL	R	37 a	0 b	0 b	0 a	0 a	0 a	0[z]
212	S	100 a	100 a	87 a	100 a	100 a	83 a	76
P202	S	100 a	100 a	100 a	100 a	100 a	100 a	85
AY	S	87 a	100 a	100 a	79 a	76 a	100 a	100
TPRB	S	25 c	87 b	100 a	25 c	87 b	100 a	80
PPSA	S	92 ab	75 b	100 a	92 ab	75 b	100 a	86

[v] R = resistant, S = susceptible.
[w] I = first true leaf, II = second true leaf, III = third true leaf.
[x] Average of first three leaves.
[y] Within rows, means with a common letter do not differ significantly ($P = 0.05$).
[z] Hyphae without conidia on the first leaf.

ACKNOWLEDGMENTS

I thank D. Shtienberg of the Department of Plant Pathology, Agricultural Research Organization, Volcani Center, for his critical review of the manuscript and S. Schreiber for expert technical assistance.

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