An Improved Method for Screening Cucumbers for Scab Resistance

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The technical assistance of Mary Fisher is gratefully acknowledged.

ABSTRACT

Germination of conidiospores of *Cladosporium cucumerinum* exposed to 1% Czapek-Dox broth (CDB) was increased compared to that in water. The addition of 1% CDB also widened the temp range optimum (15.5 - 25 C) and decreased the incubation time under high humidity (24 h) required for disease development in susceptible cucumber seedlings.

Phytopathology 64:565-567.

Additional key words: spore germination, *Cladosporium cucumerinum*.

Scab or spot rot of cucumbers caused by *Cladosporium cucumerinum* Ell. & Arth. is a serious disease of cucumbers (2, 4, 5, 10, 12), and varieties resistant to the disease have been developed (1, 2, 11, 12). Screening for resistance is usually done at the first true leaf stage using the method developed by Walker (10) by spraying the inoculum on the young seedlings and incubating the plants at 17 C and high relative humidity (RH). At temp from 20 to 25 C, the distinction between the resistant and the susceptible plants is reduced (10). This is in contrast to the reports of German workers (3, 7) who obtained the best results at 25 C. Although Walker (10) did not report the exact duration of seedling incubation at 17 C and high RH for screening assay, it is assumed to be about 48 - 72 h (1, 6). Andeweg (1) reported he had used Walker's method (10); however, he kept the inoculated seedlings at 15 - 20 C and at 100% RH for 6 h and then at a lower humidity for 5 days before the readings were taken. Strider and Konsler (9) used 2×10^4 to 4×10^4 spores per ml of *C. cucumerinum* for inoculating plants of *Cucurbita* spp.; however, no references were found regarding spore concn for *Cucumis* spp. inoculation. The exact mode of collecting the spores is also vague. This could be an important factor because Stoddard (8) found that spores of *C. cucumerinum* which lost germinability after two transfers could be stimulated to germinate by the addition of nutrients.

This study shows that the spores of *C. cucumerinum* germinate better in 1% Czapek-Dox broth and that the inoculum thus prepared is a more efficient inoculum to screen cucumber seedlings for resistance at a wide range of temp.

MATERIALS AND METHODS.—*C. cucumerinum* grown on potato-dextrose agar (PDA) for 8 days was used for all studies. The spores were collected by scraping them off in water to which a drop of Tween-20 (0.5%) per plate was added. The spore suspension was filtered through glass wool and adjusted to 4×10^6 spores per ml. It was further diluted 1:1 with water (Sp-W) or with a presterilized solution of 2% Czapek-Dox broth (Sp-CDB) to give a final spore concn of 2×10^6 ml.

Drops of spore suspensions were placed on a large glass slide in a petri plate over a moist paper towel and incubated along with the inoculated seedlings in an incubator (Sherer-Gillett, Model RT46B-SE) at 15.5 C. Each treatment was replicated with five glass slides, each with eight drops. After 24 h of incubation, 100 spores from each drop were counted at random to estimate germination. The experiment was repeated at 20 and 25 C, and in a plastic chamber under greenhouse conditions. The max temp inside the plastic chamber was 23.3 C, and the min 14.4 C during the 24-h incubation.

Cucumber seedlings of 'WSMR-18' (Wisconsin) and 'Gy-3' (S. Carolina), scab-resistant and susceptible cultivars, respectively, were germinated in presterilized soil in peat pots and grown for 8 days in a greenhouse until the cotyledons were fully expanded. The seedlings were inoculated by spraying to run off with the Sp-W or Sp-CDB inoculum and kept at 15.5 C in the incubator. Plants sprayed with only water or 1% CDB served as controls. To maintain high RH, an empty flat was inverted over the plants and covered with cloth bags which were kept moist. Seedlings were removed 1, 2, and 3 days after incubation and maintained under greenhouse conditions. Disease readings were taken on the 6th day after inoculation, and the number of dead plants was recorded. The survivors were either partially diseased with a few lesions on the stem and cotyledons, or were healthy. All surviving plants were grown for another 2 wk when the total number of dead plants was recorded. Twenty plants were used for each treatment with three replicates. The experiment was repeated at 20 and 25 C in the incubator, and also in the greenhouse. For the greenhouse experiment, flats were covered with a 15-cm-high plastic chamber. The top and sides were covered with cloth bags which were kept moist. This prevented overheating of the plants on a sunny day and also maintained high RH inside the chamber. The max/min temp during first, second, and third day were 23.3/14.4, 22.7/14.0, and 22.2/14.4 C, respectively.

RESULTS.—The germination of spores ranged from 0 to 14% in water and 40 to 98% in 1% CDB at different temp (Table 1). The results obtained when the plants were inoculated with Sp-W or Sp-CDB and incubated at 15.5, 20, and 25 C are presented in Table 2. 'Gy-3' plants inoculated with Sp-W did not all die in any combination

TABLE 1. Germination of spores of *Cladosporium cucumerinum* in water and in 1% Czapek Dox broth on glass slides when incubated at different temp for 24 h

Temperature (C)	Germination (%)[a]	
	Water	1% CDB
15.5	0	40
20.0	5	96
25.0	14	98
23.3/14.4[b]	5	69

[a]For each treatment a total of 4,000 spores were counted to estimate the germination.

[b]The spores were incubated in a greenhouse where the maximum recorded temp was 23.3 C and the minimum 14.4 C.

TABLE 2. Effect on scab development of resistant (WSMR-18) and susceptible (Gy-3) cucumber seedlings when inoculated with *Cladosporium cucumerinum* spores suspended in water or 1% Czapek-Dox broth and incubated at 15.5, 20, and 25 C first at high humidity and then at low humidity

Incubation Temp	Cultivar[a]	Incubation at high humidity	% Dead plants			
			Spores in water		Spores in 1% CDB	
			6 days	20 days	6 days	20 days
15.5 C	'Gy-3'	1	70	92	100	100
		2	83	97	100	100
		3	63	97	100	100
	'WSMR-18'	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
20 C	'Gy-3'	1	42	93	100	100
		2	47	90	100	100
		3	17	88	100	100
	'WSMR-18'	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
25 C	'Gy-3'	1	55	90	100	100
		2	37	70	80	100
		3	37	65	90	100
	'WSMR-18'	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0

[a]For controls, either water or 1% CDB solution without spores were used to spray both cultivars; none of the plants was infected.

of temp and incubation at high humidity (Fig. 1). In all cases more plants were dead 20 days after inoculation compared to 6 days. WSMR-18 plants were all healthy in all cases when inoculated with Sp-W.

When Sp-CDB was used as the inoculum, 100% of the Gy-3 plants were killed within 6 days in all cases except at 25 C for 2 and 3 days of incubation (Fig. 1). However, after 20 days of inoculation, Gy-3 plants were all dead and WSMR-18 plants were all healthy, irrespective of temp and incubation period.

Under greenhouse conditions, both treatments (Sp-W and Sp-CDB) resulted in 100% killing of Gy-3 within 6 days irrespective of the incubation time. WSMR-18 plants were healthy and free of disease, except those inoculated with Sp-CDB and incubated for 3 days. These plants showed necrotic lesions on the cotyledons and 12% were dead within 6 days. The killing observed was not typical of the susceptible reaction in that the stems were intact and showed no lesions. They did not collapse as did Gy-3. When the survivors were grown for 20 days, some stunting was observed.

DISCUSSION.—Walker (10) suggested that the optimum temp for scab screening of cucumber seedlings is 17 C at high RH and the plants should be incubated for more than 48 h. Others (3, 7) have reported 25 C to be the optimum for testing scab resistance. Since the germinability of spores, the spore concn, and the nutritional status of the inoculum was not mentioned, it is difficult to explain this discrepancy. In this experiment, at a spore concn of 2×10^6, addition of 1% CDB enhanced the germination of the spores at 15.5, 20, and 25 C. When such a spore suspension was used for inoculation, irrespective of the temp, all the susceptible plants incubated for only 1 day under high RH were dead 6 days after inoculation and the resistant plants were all healthy.

At 15.5 C, spores of *C. cucumerinum* failed to germinate after 24 h in water and germination was only 40% in 1% CDB. However, at this temp, Sp-W killed 70% and Sp-CDB 100% of Gy-3 plants. This suggests the presence of available nutrients on the plant surfaces. However, Sp-CDB was superior to Sp-W in every instance in inducing the disease.

Under greenhouse conditions, Sp-W and Sp-CDB

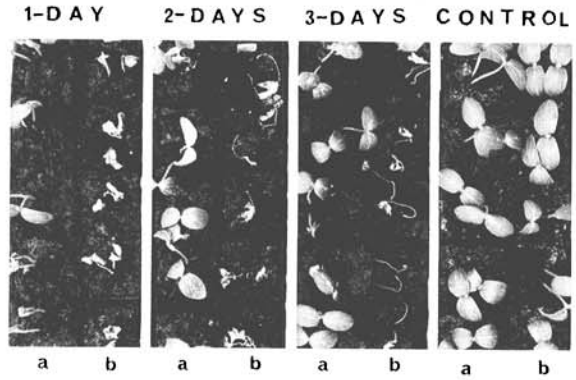

Fig. 1. Cultivar 'Gy-3' cucumber seedlings 6 days after inoculation with spores of *Cladosporium cucumerinum* suspended in (a) water, or (b) 1% Czapek-Dox broth (CDB) and incubated at 25 C and high humidity for 1, 2, and 3 days. Control was treated with only water or 1% CDB.

inoculum resulted in 100% killing of susceptibles within 6 days, although spore germination on glass slides was only 5% and 69%, respectively.

It is clear that the addition of 1% CDB increased infection by *C. cucumerinum* over a wider range of temp and a shorter (24 h) duration at high RH than water alone as the spore carrier. The effect of 1% CDB was more obvious when the plants were incubated under nonoptimum conditions for disease development. Successful scab testing has been done under greenhouse conditions during both hot summer days and cool winter days using 1% CDB as the spore carrier.

LITERATURE CITED

1. ANDEWEG, J. M. 1956. The breeding of scab-resistant frame cucumbers in the Netherlands. Euphytica 5:185-195.
2. BAILEY, R. M., and I. M. BURGES. 1935. Breeding cucumbers resistant to scab. Proc. Am. Soc. Hortic. Sci. 32:474-476.
3. BEHR, L. 1948. Histologische Untersuchungen an kratzekranken Gurken (Cucumis sativus L.) unter besonderer Berucksichtigung des Krankheitsverlaufes der Kratze (Cladosporium cucumerinum Ell. & Arth.) an Fruchten. Phytopathol. Z. 15:92-123.
4. GREELMAN, D. W. 1960. A summary of the prevalence of plant diseases in Canada in 1960. Can. Plant Dis. Surv. 4:31-121.
5. HOPEN, J. H., and D. J. DE ZEEUW. 1962. Reduction of susceptibility to cucumber scab by cucumber mosaic virus. Plant Dis. Rep. 46:93-97.
6. PIERSON, C. F., and J. C. WALKER. 1954. Relations of Cladosporium cucumerinum to susceptible and resistant cucumber tissue. Phytopathology 44:459-465.
7. SCHULTZ, H. 1939. Untersuchungen uber das Verhalten von Gurkensorten bei kunstlicher Infektion mit Cladosporium cucumerinum Ell. & Arth. sowie einige morphologische Eigneschaften des Pilzes. Gartenbauwissenschaft 13:605-618.
8. STODDARD, E. M. 1954. Acceleration of spore germination in Cladosporium cucumerinum. Phytopathology 54:507 (Abstr.).
9. STRIDER, D. L., and T. R. KONSLER. 1965. An evaluation of the cucurbita for scab resistance. Plant Dis. Rep. 49:388-391.
10. WALKER, J. C. 1950. Environment and host resistance in relation to cucumber scab. Phytopathology 40:1094-1102.
11. WALKER, J. C. 1958. Two new pickling cucumber varieties resistant to scab and mosaic. Plant Dis. Rep. 42:1337-1338.
12. WALKER, J. C., C. F. PIERSON, and A. B. WILES. 1953. Two new scab-resistant cucumber varieties. Phytopathology 43:215-217.