Physiological Specialization in *Pseudoperonospora cubensis*

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ABSTRACT

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Controlled inoculation studies were conducted using local isolates of *Pseudoperonospora cubensis* in Israel, Japan, and the United States. Inoculations to 26 cultivars representing 13 species and subspecies within seven genera of the family Cucurbitaceae revealed distinct physiological specialization among isolates. Five pathotypes of *P. cubensis* could be distinguished based on high compatibility with specific hosts. All five pathotypes were highly compatible with *Cucumis sativus* and *C. melo* var. *reticulatus*. Pathotype 1 was highly compatible with them only. Pathotype 2 was highly compatible with these hosts and *C. m.* var. *conomon*. Pathotype 3 was highly compatible with these three species and subspecies plus *C. m.* var. *acidulus*. Pathotype 4 was highly compatible with all of the aforementioned hosts plus *Citrullus lanatus*. Pathotype 5 was highly compatible with all hosts mentioned above plus *Cucurbita* spp.

Additional key word: cucurbit downy mildew.

In 1974, Palti (16) summarized host range studies with *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow. on cucurbits and concluded that the reported divergencies mainly were caused by different physiological races in various countries. The lack of concurrency and uniformity of test hosts did not allow for race delimitations to be drawn from the pronounced divergencies in host range reactions reported both within and between countries. Rather this work, the work of Bains et al (1–4) in India, and the work of Iwata (8–13) in Japan pointed to the need for unified, international research to determine the relationships of host specificities for downy mildew on cucurbits. The work of Thomas (19) emphasized this need when he reported that among selected muskmelon genotypes, the level of resistance to downy mildew depended on the challenge isolate of the pathogen.

The following studies were conducted to determine more accurately the host specificities of *P. cubensis* in an effort to develop information that would be useful to the future characterization and identification of isolates.

MATERIALS AND METHODS

Controlled inoculations were conducted with isolates of *P. cubensis* collected in Israel, Japan, and the United States to determine host specificities. Each isolate was studied only in its country of origin. Inoculations were made to 26 cultivars representing 13 species within seven genera of the family Cucurbitaceae and were repeated at least once (Table 1). Plants were grown in the greenhouse to the two-expanded-leaf stage. For each isolate, the first and second leaves of 10–20 plants of each entry were sprayed to incipient runoff with a suspension of 5×10^3 sporangia per milliliter. Inoculated plants were then placed in dark moist chambers at 20 C for 20 hr. After this treatment, plants were placed on greenhouse benches at 20–27 C and observed daily for symptom development. Whenever symptom development appeared favorable for sporulation, usually at 5–8 days after inoculation, plants were returned to the moist chambers at 20 C for 20 hr. If no evidence of infection was present by 10 days after inoculation, plants were placed under high humidity during their dark periods through 14 days in an effort to stimulate sporulation. Symptom development and sporulation were observed both macroscopically and microscopically at magnifications of up to 50×. Inoculated plants were evaluated for the presence of lesions and presence and intensity of sporulation.

Two, two, and four isolates were used in inoculations in the United States, Israel, and Japan, respectively. Isolates in the United States were obtained from field-grown muskmelon, *Cucumis melo* var. *reticulatus* Naud., at Charleston, SC, designated "C," and from field-grown squash, *Cucurbita pepo* L., at Weslaco, TX, designated "T." Both of these isolates were maintained on *C. m.* var. *reticulatus* 'Perlita.' The isolate from squash could not be adequately maintained on *C. pepo* because of constant contamination problems from powdery mildew infection incited by *Sphaerotheca fuliginea* (Schlecht.:Fr.) Poll. Test inoculations were conducted with both isolates C and T after three and after 61 generations of maintenance. The two isolates studied in Israel were obtained from cucumber, *Cucumis sativus* L., one in 1983 and the other in 1985. These isolates were designated "83" and "85" and were maintained on *C. sativus* 'Bet Alpha' for 20 and 10 generations before testing, respectively. Of the four isolates studied in Japan, two were obtained from *C. sativus*, designated "C1" and "C2," and two were obtained from *C. m.* var. *reticulatus*, designated "M1" and "M2." Isolate C1 was collected in a vinyl greenhouse in 1983 and isolate C2 was collected in the field in 1984. Both of these isolates were maintained on *C. sativus* 'Sagamihanjiro' and were tested after 12 and after 16 generations. Isolates M1 and M2 were collected in vinyl houses in 1983 and 1984, respectively. These isolates were maintained on *C. m.* var. *reticulatus* 'Earl's Favourite' and tested after 12 and after 24 generations.

Sporangial length and width and sporangiophore length were measured to compare isolates. Sporangia and sporangiophores of isolates C1 and C2 were produced on leaves of *C. sativus*, and those of isolates M1, M2, C, and T were produced on *C. m.* var. *reticulatus* at 20 C; those of isolate 85 were produced on *C. sativus* at about 15 C. Two hundred sporangia and sporangiophores were measured for each isolate.

RESULTS

Inoculation studies in Israel, Japan, and the United States, especially observations of the intensity of sporulation, showed that

all hosts were not equally compatible with the isolates tested (Table 1). However, all plants within a host genotype reacted uniformly to each isolate. Based on lesion production and the intensity of sporulation within those lesions, *Cucumis sativus* 'Sagamihanjiro' and *C. m.* var. *reticulatus* 'Ananas Yokneam' were highly compatible with all *P. cubensis* isolates tested. All other *C. m.* var. *reticulatus* cultivars and most cultivars of *C. m.* var. *conomon* (Thunb.) Gre. were highly compatible with all isolates except C1 and C2. Cultivars Kuroto and Numame were highly compatible with isolate C2, but not C1. All other cultivars of *C. m.* var. *conomon* were very lowly compatible or lowly compatible with both C1 and C2. All *C. m.* var. *acidulus* Naud. cultivars were highly compatible with all isolates except C1 and C2.

Only the C and T isolates of *P. cubensis* tested in the United States demonstrated a highly compatible reaction with any of the other Cucurbitaceae genera included in these studies. Both U.S. isolates were highly compatible with *Citrullus lanatus* (Thunb.) Matsum. & Nakai 'Shinyamato-2'. Isolate T, originally isolated from *Cucurbita pepo*, was also highly compatible with all *Cucurbita* spp. when tested after three generations of maintenance on *C. m.* var. *reticulatus*. However, after 61 generations on *C. m.* var. *reticulatus* lesion development was poor and sporulation was very sparse when isolate T was used to challenge any *Cucurbita* spp.

Determinations of length and width of sporangia and length of sporangiophores indicated no significant differences in these dimensions among isolates. Means with standard deviations for sporangial length and width for individual isolates varied from 24.2 ± 3.5 to 25.8 ± 3.4 μm and 16.4 ± 2.1 to 18.8 ± 2.2 μm, respectively. Sporangiophore length varied from 316 ± 82 to 432 ± 103 μm.

Host specificity tests with the isolates studied confirmed the existence of distinct physiological forms of *P. cubensis* based on reactions with the most susceptible host genotypes encountered. Because these physiological forms can be distinguished based on highly compatible reactions at the genera, species, and subspecies level of the hosts, pathotype designations are proposed for them (Table 2).

DISCUSSION

These data substantiate the existence of distinct physiological forms of *P. cubensis* that may be delimited based on host genera, species, and subspecies specificities. Because each of these forms can be differentiated on this basis, it is tempting to assign *forma speciales* designations to them. To do so would probably be a misleading oversimplification (5). This is especially true when considered in light of the loss of virulence to the genus *Cucurbita* of isolate T obtained from *Cucurbita pepo* after many generations of maintenance away from the source host as occurred in the present studies.

The forms of *P. cubensis* delimited in this work are most conveniently and correctly designated as pathotypes. Such a designation is intended to indicate a dynamic situation in the pathogen population in which the major component conforms to certain host range characteristics, but allows for the presence of other components in varying frequencies (5).

It is proposed that a pathogen population that carries specific virulence factors constitutes a specific pathotype that is determined by the frequencies of those factors in the pathogen population. For example, pathotype 5, which is highly compatible with *Cucurbita* spp., carries a high frequency of virulence factors not only against *Cucurbita*, but also against *Cucumis sativus, C. m.* var. *reticulatus, C. m.* var. *conomon, C. m.* var. *acidulus*, and *Citrullus lanatus*. Likewise, pathotype 1, which is highly compatible with only *C.*

TABLE 1. Reaction of selected cultivars of representative cucurbit host species to *Pseudoperonospora cubensis* in controlled inoculations in Israel, Japan, and the United States

		Reaction to downy mildew[x]							
		Japan				Israel		USA	
Species	Cultivar	C1	C2	M1	M2	83	85	C	T
Cucumis sativus	Sagamihanjiro	+++	+++	+++	+++	+++	+++	+++	+++
C. melo var. *reticulatus*	Ananas Yokneam	+++	+++	+++	+++	+++	+++	+++	+++
	Earl's Favourite	+	+	+++	+++	+++	+++	+++	+++
	Fukamidori	+	+	+++	+++	+++	+++	+++	+++
	Green Pearl	+	+	+++	+++	+++	+++	+++	+++
	Sunrise	±	+	+++	+++	+++	+++	+++	+++
C. m. var. *conomon*	Aonagao	±	+	+++	+++	+++	+++	+++	+++
	Katsura	+	±	+++	+++	+++	+++	+++	+++
	Kuroto	+	+++	+++	+++	+++	+++	+++	+++
	Numame	±	+++	+++	+++	+++	+++	+++	+++
	Tokyo	+	+	+++	+++	+++	+	+++	+++
*C. m.*var. *acidulus*	Ginsen	+	+	+++	+++	+++	+++	+++	+++
	Houkou-new melon	+	+	+++	+++	+++	+++	+++	+++
	Kinpyo	±	±	+++	+++	+++	+++	+++	+++
	Ogatakiku-melon	+	+	+++	+++	+++	+++	+++	+++
Citrullus lanatus	Shinyamato-2	±	±	±	+	±	−	+++	+++
Cucurbita maxima	(Israel cultivar)	+	−	−	−	−	0	+	+++[y]
C. pepo	Soumen	−	−	−	−	0	nt[z]	+	+++
	Beiruti	−	−	−	−	−	0	0	+++
C. moschata	Shirokikuza	−	−	−	−	0	0	0	+++
Benincasa hispida	Naga-tougan	±	±	±	±	−	−	+	+
Luffa acutangula	(Israel cultivar)	+	±	−	−	−	0	−	+
	(USA cultivar)	+	−	−	±	−	0	−	±
L. cylindrica	Futo-hechima	−	−	−	−	±	nt	−	±
Mormordica charantia	Futo-reishi	±	±	±	±	−	−	±	±
Lagenaria siceraria	Oomaru-yuugao	±	±	±	+	−	−	nt	nt

[x] 0 = No evidence of lesions detected, classified incompatible. − = Suspect lesions present, but no evidence of sporulation; classified incompatible. ± = Sporulation limited to a very few sporangiophores requiring microscopic examination for detection, but sporangiophores present in only a few, not all lesions; sporulation in this class sometimes limited to one sporangiophore in one lesion on one leaf of one plant; classified as very lowly compatible. + = Sparse sporulation present (about 5×10^3 per square centimeter of lesion area), but for virtually all lesions; classified as lowly compatible. +++ = Abundant sporulation present (about 5×10^4 per square centimeter of lesion area) for all lesions; classified highly compatible.

[y] Ratings for isolate T on all listed *Cucurbita* spp. reflect reactions soon after isolate was originally obtained from *C. pepo* in the field. Reaction for all *Cucurbita* spp. would be rated as ± when challenged with isolate T after it had been maintained on *C. melo* var. *reticulatus* for 61 generations.

[z] nt = No test, poor seed germination.

sativus and *C. m.* var. *reticulatus*, carries a very high and probably concentrated frequency of virulence factors against these hosts, but a low frequency against those other host entities that were tested.

A comparative examination of the available literature demonstrates that some, though not all, of the host specificities reported for isolates of *P. cubensis* fit into the proposed pathotype designations even though challenged genotypes were not identical with those that we tested. In the United States, isolate H of Hughes and Van Haltern (6) would fit either pathotypes 1, 2, or 3, and their isolate V would fit pathotype 4, whereas the host specificities of the 1978 and 1979 isolates reported by Thomas (19) fit pathotypes 4 and 5, respectively. In Japan, the isolates studied by Iwata (8–13) and Kurosawa (14) were not tested against any *C. m.* var. *reticulatus*, which hinders any valid direct comparisons with our results. However, the recent report of Inaba et al (7), in which only Japanese cultivars were tested, can be directly compared with our results. Their results indicated the existence of three races in Japan, which were designated cucumber races 1 and 2 and muskmelon race. Cucumber race 1 was highly compatible with only the *C. sativus* cultivars Sagamihanjiro and Ochiai. In our tests, this same isolate of *P. cubensis* was also highly compatible with *C. m.* var. *reticulatus* 'Ananas Yokneam' and would thus fit pathotype 1. Cucumber race 2 was highly compatible with *C. sativus* 'Sagamihanjiro' and 'Ochiai' as well as *C. m.* var. *conomon* 'Kuroto' and 'Numame' in their report. Because in our tests this same isolate was also highly compatible with *C. m.* var. *reticulatus* 'Ananas Yokneam', cucumber race 2 would fit pathotype 2. On the basis of their report for Japanese cultivars and our study on additional cultivars, the two isolates that they designated muskmelon race fit our pathotype 3. Most reports from India (1,2,4) indicate pathotypes with more extensive host ranges than included in the present proposed scheme. These usually include *Luffa* spp. in addition to the hosts with which our pathotype 5 is highly compatible.

The designation of pathotypes based on host specificities at the genera, species, and subspecies levels leaves available the assignment of physiologic race designations within these pathotypes in situations where incompatible resistant genotypes are encountered. Also, because *C. melo* constitutes such a diverse, polymorphic species, all forms of which readily hybridize with each other (20), some of the reactions at the subspecies level noted in this work might better be considered genotypic responses at the species level.

Palti (16) stated that the divergencies in distribution of *P. cubensis* on its crop hosts in various countries could be attributed to physiologic races, resistance of some local cultivars, and the effects of local environment on infection. We agree with his premise, but feel that the "local environment" must be partitioned into two parts; namely, meteorological environment and specific host environment. When host and pathogen are present, meteorological conditions determine if infection can occur, but the virulence of the pathogen population to the specific host encountered determines if it will occur. Thus, selection pressures for virulence that are exerted by the host population are directly related to the relative frequencies of specific hosts in that population. This holds true both for races on different genotypes within a given species and for pathotypes on different species within a higher taxonomic group, such as the Cucurbitaceae.

Although oospores of *P. cubensis* have been observed in the U.S.S.R., Japan, China, and India (17), their occurrence is rare, and the lack of successful infection studies with them precludes determination of their importance to the genetic variability of this highly plastic pathogen. The prevalence of the highly infectious diploid asexual stage indicates that it is an important source of genetic variability in *P. cubensis*. The dual set of genes provides for a wider range of pathogen response to selection pressures exerted on field populations and may increase their adaptive ability to specific hosts (18).

There are two host systems in the disease cycle of downy mildew on cucurbits that can affect selection for virulence toward specific hosts. These are the hosts encountered during the seasonal cultivation of these crops in the field and the overwintering hosts.

TABLE 2. Proposed pathotype designations for *Pseudoperonospora cubensis* isolates studied in Israel, Japan, and the United States[y]

Host	Isolate (country)				
	C1 (Japan)	C2 (Japan)	M1, M2 (Japan) 83, 85 (Israel)	C (USA)	T (USA)
Cucumis sativus	+[z]	+	+	+	+
C. melo var. *reticulatus*	+	+	+	+	+
C. m. var. *conomon*	−	+	+	+	+
C. m. var. *acidulus*	−	−	+	+	+
Citrullus lanatus	−	−	−	+	+
Cucurbita spp.	−	−	−	−	+
Proposed pathotype designation	1	2	3	4	5

[y]Pathotypes are based on the presence of a highly compatible reaction between the isolates and the most susceptible host genotypes encountered at the genera, species, and subspecies levels.

[z]+ = Highly compatible host-pathogen interaction. − = Incompatible, very lowly, or lowly compatible host-pathogen interaction.

Both of these host situations in the disease cycle are primary environmental components that tend to stabilize selection for virulence genes in the pathogen population. A gene for virulence that is too high in the pathogen population will decrease in frequency, and a gene that is too low in the pathogen population will increase in frequency (15). Thus, part of the explanation for the occurrence of different pathotypes of *P. cubensis* in different geographic regions may be found through examinations of the selection pressures exerted by both the overwintering hosts and the seasonally cultivated hosts common to that particular region.

The pathotype concept discussed above for this pathosystem is not presented as the entire, definitive picture of physiological specialization in *P. cubensis*, but as a framework for continued study. Because it is based on the results of the most detailed examinations of host specificities within *P. cubensis* isolates yet conducted using identical host genotypes in each country, it should be a valid framework.

LITERATURE CITED

1. Bains, S. S., and Jhooty, J. S. 1976. Overwintering of *Pseudoperonospora cubensis* causing downy mildew of muskmelon. Indian Phytopathol. 29:201-202.
2. Bains, S. S., and Jhooty, J. S. 1976. Host-range and possibility of pathological races in *Pseudoperonospora cubensis*—cause of downy mildew of muskmelon. Indian Phytopathol. 29:214-216.
3. Bains, S. S., and Jhooty, J. S. 1979. Epidemiological studies on downy mildew of muskmelon caused by *Pseudoperonospora cubensis*. Indian Phytopathol. 31:42-46.
4. Bains, S. S., Sokhi, S. S., and Jhooty, J. S. 1977. *Melothria maderaspatana*—a new host of *Pseudoperonospora cubensis*. Indian J. Mycol. Plant Pathol. 7:86.
5. Crute, I. R. 1981. The host specificity of Peronosporaceous fungi and the genetics of the relationship between host and pathogen. Pages 237-253 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London. 636 pp.
6. Hughes, M. B., and Van Haltern, F. 1952. Two biological forms of *Pseudoperonospora cubensis*. Plant Dis. Rep. 36:365-367.
7. Inaba, T., Morinaka, T., and Hamaya, E. 1986. Physiological races of *Pseudoperonospora cubensis* isolated from cucumber and muskmelon in Japan. Bull. Natl. Inst. Agro-Environ. Sci. 2:35-43.
8. Iwata, Y. 1941. Specialisation in *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. I. Comparative studies on the pathogenicities on the fungi from *Cucumis sativus* L. and *Cucurbita moschata* Duch. Ann. Phytopathol. Soc. Jpn. 11:101-113.
9. Iwata, Y. 1942. Specialisation in *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. II. Comparative studies on the morphologies of the fungi from *Cucumis sativus* L. and *Cucurbita moschata* Duch. Ann. Phytopathol. Soc. Jpn. 11:172-185.
10. Iwata, Y. 1942. Host plants of *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. Ann. Phytopathol. Soc. Jpn. 12:48-50.
11. Iwata, Y. 1950. Specialisation in *Pseudoperonospora cubensis* (B. and C.) Rostow. IV. Studies on the fungus from Oriental Pickling Melon (*Cucumis melo* var.: *conomon* Makino). Bull. Fac. Agric. Mie Univ.

1:30-35.
12. Iwata, Y. 1951. Specialisation in *Pseudoperonospora cubensis* (B. and C.) Rostow. III. Studies on the fungus from white gourd (*Benincasa hispida* Cogn.) Forsch. Biebiet Pflkrank. 4:124-128.
13. Iwata, Y. 1953. Specialisation in *Pseudoperonospora cubensis* (B. and C.) Rostow. V. On the fungus from Calabash gourd *Lagenaria vulgaris* Ser. var. *clavata* Ser. Bull. Fac. Agric. Mie Univ. 6:32-36.
14. Kurosawa, E. 1927. Studies on *Plasmopara cubensis*. J. Natl. Hist. Soc. Formosa 17:1-18.
15. Leonard, K. J., and Czochor, R. J. 1980. Theory of genetic interactions among populations of plants and their pathogens. Annu. Rev. Phytopathol. 18:237-258.
16. Palti, J. 1974. The significance of pronounced divergencies in the distribution of *Pseudoperonospora cubensis* on its crop hosts. Phytoparasitica 2:109-115.
17. Palti, J., and Cohen, Y. 1980. Downy mildew of cucurbits (*Pseudoperonospora cubensis*): The fungus and its hosts, distribution, epidemiology and control. Phytoparasitica 8:109-147.
18. Tommerup, I. C. 1981. Cytology and genetics of downy mildews. Pages 121-142 in: The Downy Mildews. Academic Press, London. 636 pp.
19. Thomas, C. E. 1982. Resistance to downy mildew in *Cucumis melo* plant introductions and American cultivars. Plant Dis. 66:500-502.
20. Whitaker, T. W., and Davis, G. N. 1972. Cucurbits. Interscience Publishers, Inc., New York. 250 pp.