Host Effect on Selection of Virulence Factors Affecting Sporulation by *Pseudoperonospora cubensis*

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ABSTRACT

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Subpopulations of an isolate of *Pseudoperonospora cubensis* pathotype 5, which is virulent to both squash (*Cucurbita pepo*) and muskmelon (*Cucumis melo*), were maintained on each host for 18 generations. After each generation, each subpopulation was inoculated to both squash and muskmelon, and the subsequent sporulation rate on each host was monitored as an indicator of virulence. The subpopulation maintained on muskmelon showed a 99.7% reduction in sporulation rate on squash and a 226.1% increase in sporulation rate on muskmelon from generations five through 18. Sporulation on squash in this subpopulation returned to the original level after two generations of continuous culture on that host. This study illustrates the important effects of cucurbit host species in the selection of virulence factors that affect sporulation by this pathogen and provides insight into the occurrence of different pathotypes in cucurbit production areas from year to year.

Downy mildew, incited by *Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovzev, is an important disease of cucurbits in humid areas of the world (7). Wide divergencies in host range within the Cucurbitaceae have been reported for *P. cubensis* both within and among countries. Palti (3) summarized these reports and concluded that the divergencies could be attributed to the occurrence of different physiologic races. Thomas et al (6) studied isolates of *P. cubensis* in Israel, Japan, and the United States and designated five pathotypes of the pathogen that could be distinguished based on the occurrence of highly compatible reactions with specific cucurbit hosts. In those studies, they found that an isolate of pathotype 5 of the pathogen lost virulence toward *Cucurbita pepo* L., the original source host, when it had been maintained on *Cucumis melo* L. for 61 generations. They stated that an important environmental component contributing to the divergencies in distribution of the pathogen on its crop hosts in various countries was the frequency of occurrence of that host.

These studies were conducted to further investigate the loss of virulence of *P. cubensis* pathotype 5 against *C. pepo* as evidenced by sporulation rate and to better determine the effects of selection pressures for virulence that are exerted by the host population.

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MATERIALS AND METHODS

A fresh field sample of *P. cubensis* on *C. pepo* leaves was sent to us in 1986 by E. L. Cox, Texas A&M Agricultural Experiment Station, Weslaco, TX. This isolate was given the designation "S." It was maintained on cotyledons of squash, *C. pepo* 'Early Prolific Straightneck', in a 20-C growth chamber with a 12-hr photoperiod as described previously (5). Host specificity tests performed with this isolate as described by Thomas et al (6) demonstrated that it represented pathotype 5 because it was highly compatible with *Cucumis sativus* L., *C. melo* var. *reticulatus* Naudin, *C. m. conomon* (Thunb.) Gre., *C. m. acidulus* Naudin, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, and *C. pepo*.

Initial tests. Two subpopulations were derived from the S isolate. One subpopulation designated "S1" was continuously maintained on cotyledons of Early Prolific Straightneck squash. The second subpopulation was continuously maintained on cotyledons of the muskmelon (*C. m. reticulatus*) cultivar Ananas Yokneam and designated

"M1." S1 and M1 were inoculated to and maintained on their respective hosts for 32 generations through the following procedure. Seeds of squash and muskmelon were sown to separate flats of Jiffy Mix (Jiffy Products, West Chicago, IL) and placed in a glasshouse and watered as needed. Seedlings were inoculated 7 days after planting when cotyledons had expanded. Inocula were prepared by collecting infected cotyledons from each host and placing them in separate 250-ml flasks to which 50 ml of distilled water was added. Flasks were capped and shaken to dislodge the sporangia, and the resulting sporangial suspensions were filtered through one layer of cheesecloth.

The concentration of each suspension was determined with a hemacytometer and adjusted to 1.5×10^3. The sporangial suspensions were then sprayed onto the adaxial surfaces of cotyledons of their respective hosts with a Paasche Type-H airbrush (Paasche Airbrush Co., Chicago, IL) at 275 kPa to incipient runoff. After inoculation, plants were placed in separate dark dew chambers at 20 C for 18 hr. When removed from the dew chamber, plants were placed in a growth chamber at 20 C with a 12-hr photoperiod (631 $\mu E \cdot m^{-2} \cdot s^{-1}$). On the sixth day after inoculation, plants were returned to separate dew chambers at 20 C for 18 hr to stimulate sporulation. Upon removal from the dew chambers, cotyledons from each host were used as the source of inoculum to initiate the next generation on that respective host.

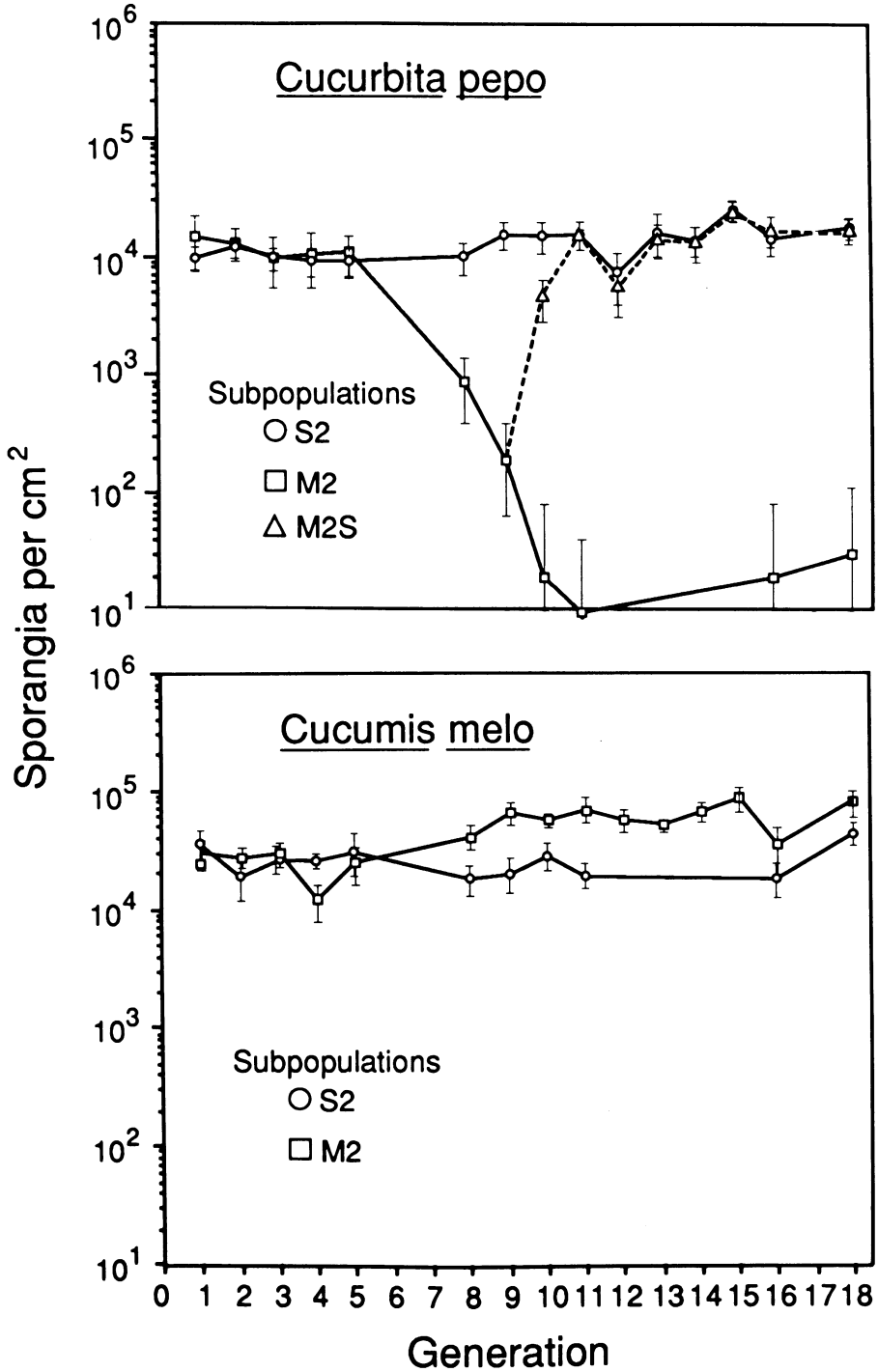

Fig. 1. Sporulation rates for subpopulations of an isolate of *Pseudoperonospora cubensis* pathotype 5 maintained on *Cucurbita pepo* (S2) or *Cucumis melo* (M2) for 18 generations and inoculated to *C. pepo* and *C. melo* after each generation. After subpopulation M2 showed a marked decrease in virulence against *C. pepo*, subpopulation M2S was derived from M2 and continuously inoculated to *C. pepo*.

After generations 11, 14, 26, and 31, tests were conducted to detect any changes in virulence of S1 and M1 due to maintenance on their respective hosts. For these tests, the cotyledons of two sets of 10, six-plant replicates of squash and muskmelon were inoculated. Two sets of squash cotyledons and two sets of muskmelon cotyledons were inoculated separately with either S1 or M1. Seven days after these inoculations, plants were removed from the dew chamber and the 12 cotyledons were harvested from each of the 10 replications of each treatment. These sets of cotyledons then were used to prepare sporangial suspensions for each replication as described above for inoculum, except that the final volume of each was standardized to 50 ml. The concentration of sporangia in each suspension was determined based on 10 counts with a hemacytometer.

After counting, suspensions of 1.5×10^3 sporangia per milliliter were made from the S1 and M1 subpopulations and were used to inoculate the next generation on their respective hosts. Cotyledon areas for each of the 10 replicates in each treatment were determined with a Li-Cor LI-3000 (Li-Cor, Lincoln, NE) area meter. Production of sporangia per square centimeter of cotyledon area then was calculated. All determinations of sporulation rate in these and the detailed studies reported below were made at the same time interval, 7 days after inoculation.

Detailed tests. Because the tests described above were not performed at intervals sufficient to detect at which generation the loss of virulence against squash occurred in the M1 subpopulation, the study was repeated with more frequent determinations of subpopulation sporulation rates. Two subpopulations were again derived from the S isolate that had been maintained on squash. These two subpopulations were derived and maintained as described above and were designated "S2" and "M2" to denote their maintenance on squash and muskmelon, respectively.

After the initial generation of subpopulations S2 and M2 on their respective hosts and after each succeeding generation, the cotyledons of two sets of 10, six-plant replicates were

inoculated as described above. Except where noted, after each generation, determinations of production of sporangia per square centimeter of cotyledon area were made as described above. These tests were conducted through 18 generations. Determinations of sporulation rate were made after generations one through five. For generations six and seven, the sets of 10, six-plant replicates for each subpopulation were inoculated as described above but were observed only for any qualitative changes in the appearance of lesion development. When lesions visually decreased in severity in one subpopulation during generation eight, determinations of sporulation rate were resumed and continued through the remainder of the study.

In these tests, when a marked loss of virulence against squash was detected in the M2 subpopulation, as evidenced by a sharp decrease in the sporulation rate of this subpopulation on this host, those few sporangia that were produced were used to establish a secondary subpopulation designated M2S. These sporangia were inoculated to and subsequently maintained on 10, six-plant replicates of squash for each generation for the remainder of the study. The sporulation rate of M2S was determined after each generation and the sporangia produced by each generation were used to perpetuate this secondary subpopulation.

RESULTS

Initial tests. A marked reduction in virulence of the M1 subpopulation against *C. pepo* was evidenced by the sporulation rate of generation 12, which was two sporangia per square centimeter. The sporulation rate of this M1 subpopulation on *C. pepo* remained low at generations 15, 27, and 32. The sporulation rates of S1 on *C. pepo*, S1 on *C. melo*, and M1 on *C. melo* remained high at generations 12, 15, 27, and 32 so that no decreases in virulences of these subpopulations against their respective hosts were indicated. In generation 32, the sporulation rate of S1 on *C. pepo* was 15,200 ± 4,300 and on *C. melo* was 32,800 ± 6,300 sporangia per square centimeter. The sporulation rate for M1 on *C. pepo* was 80 ± 100 and on *C. melo* was 73,200 ± 18,300 sporangia per square centimeter at generation 32.

Detailed tests. There were no pronounced changes in the sporulation rates of either the S2 or M2 subpopulations on either *C. pepo* or *C. melo* through generation five (Fig. 1). Lesion appearance at generations six and seven did not indicate any change in virulence of either subpopulation against either host and sporulation was general over the surface of the cotyledons. However, at generation eight, nonsporulating necrotic lesion centers that were smaller than normal were produced when *C. pepo* was inoculated with subpopulation M2, and general sporulation on the surface of the cotyledons appeared to be much less. Lesion development and accompanying general sporulation appeared normal for the other subpopulation-host combinations.

Determinations of sporulation rate, which were resumed at generation eight, revealed a substantial decrease in virulence of M2 against *C. pepo*. No such decrease in virulence of either subpopulation toward their other hosts was indicated by their sporulation rates, which remained high. The sporulation rate of M2 on *C. pepo* continued to decrease in generations nine through 11, reaching a low of 10 sporangia per square centimeter (99.7% decrease) in generation 11 and remained low for the remainder of the study. No such decreases were evidenced in the sporulation rates for the other subpopulation host combinations. Through the 18th generation, the sporulation rates of S2 on *C. pepo* and *C. melo* remained relatively constant, whereas the sporulation rate of M2 on *C. melo* increased 226.1% from generations five through 18.

When sporangia produced by the M2 subpopulation on *C. pepo* were used to establish the M2S subpopulation by inoculation to *C. pepo*, the sporulation rate increased. The sporulation rate of this subpopulation returned to and remained at the level of the S2 subpopulation on *C. pepo* at generations 10–18.

DISCUSSION

These studies illustrate the important effects of cucurbit host species in selection of virulence factors that affect sporulation in natural populations of *P. cubensis*. This information should be considered when conducting host range studies with isolates of this pathogen, because maintenance on a particular host may exert selection pressure for these virulence factors. This selection could substantially alter the original virulence spectrum of a particular isolate within a few generations. Host range studies should be conducted to determine the pathotype of an isolate within a few generations after it is collected. Before these studies, an isolate should be maintained on the host of origin. Once the pathotype is determined, the maintenance host for any particular isolate should be the species or subspecies that represents the widest divergence in host range that is characteristic of that pathotype. For instance, pathotype 4 should be maintained on *C. lanatus* and pathotype 5 should be maintained on *C. pepo* (6). If maintenance of an isolate is for the purpose of evaluating disease resistance in a particular host species, then the isolate should be maintained on that species.

It often has been observed that in some years all cucurbit crops in a particular production area are infected by *P. cubensis*, whereas in other years only certain species are infected. Consideration of the selection pressure that host species can exert on populations of this pathogen helps to explain this phenomenon. There are two host species systems in the disease cycle of downy mildew on cucurbits that can affect selection for virulence toward specific hosts. These are the overwintering hosts and the hosts encountered during the seasonal cultivation of cucurbit crops in the field (6). Both of these host situations in the disease cycle are important environmental components that could tend to stabilize selection for virulence genes in the pathogen population (4). A gene for virulence that is too high in the pathogen population would be expected to decrease in frequency, and a gene that is too low in the pathogen population would be expected to increase in frequency (1). The seasonally cultivated host system can be further broken down into several host species components.

In the United States, downy mildew of cucurbits occurs mainly in production areas near the Atlantic seaboard and the Gulf of Mexico (7). The overwintering hosts of downy mildew in each of these areas are *C. pepo* and *C. lanatus* in southern Florida (2) and various cucurbits, often *Cucurbita* spp., in southern Mexico (C. E. Thomas, *unpublished*). Primary inoculum from these overwintering hosts is blown northward as the growing season progresses and various combinations of host populations are encountered in commercial fields including *C. pepo, C. melo, C. sativus*, and *C. lanatus*. All of these hosts may exert selection pressure for virulence factors that affect sporulation by the pathogen population so that inocula blowing northward from these fields may carry different concentrations of these virulence factors than did the primary inoculum that originally infected them.

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