Ecological Races of Helminthosporium maydis Race T

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ABSTRACT

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A cool-environment population and a warm-environment population of *Helminthosporium maydis* race T were evaluated for virulence, infection efficiency, and sporulation efficiency under cool and warm temperature regimes. Each population was superior to the other in an environmental regime comparable to its native habitat. Infection and sporulation efficiencies were greater for the cool-environment population under the cool regime and greater for the warm-environment population under the warm regime. Infection and sporulation efficiencies of the two populations were similar after continued exposure to a cool or a warm temperature regime, apparently due to selection for subpopulations that were better able to persist under those regimes.

Additional key words: southern corn leaf blight, epidemiology.

Ecological races are populations of an organism that are better adapted to particular ecological niches because they possess certain unique genetic characteristics. The occurrence of ecological races within species of higher plants and animals is well documented (3, 4, 6). Their possible existence within plant-pathogenic organisms has been suggested by several investigators (1, 2, 5).

The current view is that ecological races exist because of selection within populations for subpopulations better suited to a particular ecological or environmental niche. This offers a philosophical basis for speculation as to why southern corn leaf blight (SCLB), which is incited by race T of *Helminthosporium maydis* Nisikado & Miyake *(Cochliobolus heterostrophus* Dreschler), failed to reach epidemic levels in 1971 in the midwestern "Corn Belt" of the United States. It generally is assumed that the bulk of the inoculum for the 1970 Corn Belt epidemic was introduced from the southern United States and that the inoculum for 1971 overwintered on corn debris in the Corn Belt. If the concept of ecological races is valid, it seems reasonable to assume that the southern population of *H. maydis* had been selected for fitness to persist in the warm southern environment and would be less fit to function efficiently in the cooler environment of the northern states.

During the period of 1955-1966, the junior author collected a large number of *H. maydis* isolates from cool- and warm-environment areas throughout the world. Many of these isolates were subsequently identified as race T (7). The objectives of this study were to determine if ecological races existed within race T of *H. maydis* and to use this information for a partial explanation of the absence of a SCLB epidemic in 1971.

MATERIALS AND METHODS

Two populations of 11 isolates each of *H. maydis* race T were used. One population, which was obtained from leafspots on nine gramineous species in England, Netherlands, Scotland, and Switzerland, was designated as the cool-environment population (CEP). The other population, which was obtained from five gramineous species located in the tropical areas of Africa, Argentina, Brazil, El Salvador, Guinea, Mexico, and Spain, was designated the warm-environment population (WEP). The isolates were obtained as single spores from the original preserved leaf material to avoid any changes in their behavior that might have developed during in vitro culturing.

Seeds of corn (*Zea mays* L.) hybrid P-A-G 15029T (W. R. Grace and Co., Carrollton, Mo. 64633) were planted in 10-cm diameter plastic pots (four seeds per pot) and grown in the greenhouse. A sterilized potting mixture of soil, peat, and perlite (1:1:1, v/v) was utilized. Plants were inoculated at the five-leaf stage.

Inoculum was obtained by incubating sections of diseased leaf material in petri dishes lined with moistened filter paper at 21 C for 96 hours to induce sporulation. Spores were obtained by washing sporulating leaf material with 0.05% water agar. Inocula were calibrated to 100 spores per 0.1 ml in 0.05% water agar and sprayed (with an atomizer) on plants at the rate of 0.6 ml per plant.

Isco Growth Chambers, Models E2 and E-3A (Instrumentation Specialties Co., Lincoln, NB 68507) and Percival Dew Chambers, Model DC 20 (Percival Refrigeration and Mfg., Co., Boone, IA 50036) were utilized in all studies.

The lesion size (virulence), infection, and sporulation

efficiencies of the two populations were compared under cool and warm temperature regimes. Lesion size was assessed by measuring the length and width of 15 lesions on the third leaf, to ensure as much uniformity as possible. The leaves were numbered consecutively starting with lowest leaf and progressing to the top of the plant. Starting from the base of the leaf and progressing to the leaf tip, all lesions were measured until 15 were recorded. If less than 15 were present, lesions on the third leaf of another plant in the same pot were measured to complete the set of 15 lesions. A table had been assembled previously by measuring the area of several hundred lesions with a planimeter and averaging the area for a specific length and width for 0.5 mm units (8). Length and width measurements were converted to area in square millimeters by reference to the table.

The number of lesions resulting from a known amount of inoculum constituted a measure of infection efficiency. Lesions were counted on leaves two through five.

Sporulation efficiency was determined by collecting conidia from 15 lesions on the third leaves of four plants per pot. Lesions were selected at random, labeled with a permanent marker pen, and measured prior to sporulation induction in the dew chamber. The large opening of a glass eyedropper was plugged with a piece of filter paper and attached to a rubber hose, which in turn was attached to a vacuum source. Conidia, as well as some conidiophores and mycelial fragments, were sucked from lesion surfaces on the upper and lower sides of the leaf and trapped in the filter paper. The filter paper containing the conidia was placed in a test tube containing 5 ml of 0.05% water agar with 0.5% copper sulfate to inhibit germination. The contents were agitated to remove the conidia from the filter paper and the filter paper was removed. Following a second agitation to disperse the conidia evenly within the solution, a 0.1-ml aliquot of the spore suspension was spread evenly on a strip of water agar placed on a glass slide and the spores were counted. The total number of spores collected per test tube was calculated and the data were converted to number of spores per square millimeter of lesion surface. The converted data constituted sporulation efficiency.

Temperatures for the warm-regime studies were 28 ± 1 C for infection and sporulation in the dew chamber, and 31 ± 1 C for colonization in the growth chamber. Inoculated plants were incubated in a dew chamber for 6 hours and transferred to a growth chamber. Lesion number was determined 2 days after inoculation and lesion size was measured 1 day later. Sporulation studies were initiated 4 days after inoculation by placing plants in the dew chamber for 16 hours.

Temperatures for the cool-regime studies were 16 ± 1 C for infection and sporulation, and 20 ± 1 C for colonization. The dew period for infection was 10 hours. Lesions were counted 3 days after inoculation and lesion size was determined 1 day later. Sporulation studies were initiated 5 days after inoculation. The slower growth of the fungus under cooler temperatures required the use of longer time periods.

All studies dealt with populations. Ten milliliters of calibrated spore suspension (100 spores/0.1 ml) of each of the 11 isolates were mixed to form WEP and CEP populations.

The two populations were compared initially under cool and warm regimes. Subsequently, a study was designed to determine if selection could affect the populations after six generations under a particular regime. A generation consisted of infection, colonization, and sporulation. Inoculum was obtained by inducing spore production on diseased leaf material from the previous generation, which required 6 days under the cool regime at 16 C and 3 days under the warm regime at 28 C to obtain a sufficient quantity of spores. The first generation was started by inoculating plants with the original mixture of 10 ml of each calibrated inoculum.

The infection efficiency, lesion size, and sporulation efficiency of the populations were measured each generation to evaluate the rate, if any, at which significant changes in the attributes occurred. The CEP and WEP were evaluated concurrently for each generation under each temperature regime. In a final study, the first and sixth generations of each population were evaluated concurrently under each regime.

TABLE 1. Infection efficiency, lesion size, and sporulation efficiency of the CEP[a] and WEP[a] under the cool (16 C, 20 C) and warm (28 C, 31 C) temperature regimes

	Cool regime		Warm regime	
	CEP	WEP	CEP	WEP
Avg. no. lesions per plant	84	*[b] 70	68	* 117
Avg. lesion size (mm^2)	12.9	15.9	15.8	18.4
Spores per mm^2	51	37	97	* 192

[a] *Helminthosporium maydis*: CEP = cool-environment population, and WEP = warm-environment population.

[b] Values on either side of the asterisks are significantly different, $P = 0.05$, according to the Behrens-Fisher unpaired *t*-test.

RESULTS

The differences between the two populations under cool and warm regimes are evident (Table 1). According to the Behrens-Fisher unpaired *t*-test, the CEP had a significantly greater infection efficiency than the WEP under the cool regime, whereas the WEP was significantly greater than the CEP under the warm regime. No significant differences were detected for lesion size for either population under either regime. The sporulation efficiency of the WEP was significantly superior under the warm regime, but no significant differences in sporulation were detected under the cool regime.

The attributes of both populations after each of the six serial generations under the cool and warm regimes are summarized in Table 2. The populations were compared by the Behrens-Fisher unpaired *t*-test. The infection efficiency and the sporulation efficiency of the two populations were comparable by the third generation under the cool regime. The significantly greater infection efficiency of the WEP after two generations under the cool regime perhaps can be attributed to variation in the experimental techniques. No differences in virulence were detected at any generation.

The infection efficiency of the two populations under the warm regime was comparable by the fifth generation, although the results of the first generations were variable. Sporulation efficiency was comparable by the second generation. No differences in lesion size were observed.

In a final study, both populations were again maintained for six generations under both temperature regimes, after which the first and sixth generations were compared concurrently under each regime. The data are summarized in Table 3. Analysis of variance and Duncan's modified (Bayesian) least significant difference tests were used to analyze the data. The two populations again were comparable in all attributes after six generations under the cool regime. Selection for improved infection efficiency and sporulation efficiency within the WEP under the cool regime is evident by the significant differences in those attributes between the first and the sixth generations of the WEP. Lesion size of the two populations was comparable at all times.

Under the warm temperature regime, the first and sixth generations of the WEP remained comparable for infection efficiency and sporulation efficiency, whereas selection for superior ability for those attributes occurred within the CEP from generation one to six. The lesion size of the first and sixth generations of the CEP and the sixth generation of the WEP were comparable. The lesion size of the first generation of the WEP was superior to both generations of the CEP.

DISCUSSION

The results presented herein provide substantial evidence for the existence of ecological or environmental races within race T of *H. maydis*. The apparent selection within populations for subpopulations better adapted for a particular ecological or environmental niche is demonstrated in that each population studied herein is superior to the other when placed under an environmental regime comparable to its native habitat. That fact is further supported by the series of generation studies in which the two populations become comparable after relatively few generations under both regimes.

TABLE 2. Infection efficiency, lesion size, and sporulation efficiency of the CEP[a] and WEP[a] for each of six serial generations under the cool (16 C, 20 C) and warm (28 C, 31 C) temperature regimes

Temperature regime and generation number	Avg. no. lesions per plant			Avg. lesion size (mm^2)		Spores per mm^2		
	CEP		WEP	CEP	WEP	CEP		WEP
Cool regime:								
1	81	*[b]	65	7.7	8.8	29	*[b]	17
2	42	*	53	8.4	8.3	10	*	6
3	56		62	5.6	6.2	13		11
4	78		73	5.8	6.3	6		6
5	73		81	7.1	7.0	22		27
6	86		78	7.7	7.0	13		15
Warm regime:								
1	64	*	87	9.6	8.9	91	*	167
2	47	*	56	10.9	11.2	64		101
3	71		72	8.7	8.8	74		95
4	66	*	87	9.7	10.4	98		112
5	50		51	7.9	8.6	47		47
6	58		64	8.1	8.1	68		76

[a] *Helminthosporium maydis*: CEP = cool-environment population, and WEP = warm-environment population.
[b] Values on either side of the asterisks are significantly different, $P = 0.05$, according to the Behrens-Fisher unpaired t-test.

TABLE 3. Infection efficiency, lesion size, and sporulation efficiency of first and sixth generations of CEP[a] and WEP[a] compared concurrently under cool (16 C, 20 C) and warm (28 C, 31 C) temperatures regimes

	CEP1[b]	WEP1	CEP6[b]	WEP6
Cool regime:				
Avg. no. of lesions per plant	71 A[c]	51	65 A	66 A
Avg. lesion size (mm^2)	6.9 B	6.9 B	6.8 B	6.4 B
Spores per mm^2	10 C	7	9 C	10 C
Warm regime:				
Avg. no. of lesions per plant	64	88 D	81 D	85 D
Avg. lesion size (mm^2)	7.4 F	9.2 E	6.7 F	7.9 EF
Spores per mm^2	75	154 G	142 G	144 G

[a] *Helminthosporium maydis:* CEP = cool-environment population, and WEP = warm-environment population.
[b] CEP1 and WEP1 = first generations; CEP6 and WEP6 = sixth generations.
[c] Means followed by same letter are not significantly different, $P = 0.05$, according to Duncan's modified (Bayesian) least significant difference test.

Differences in lesion size between the two populations were not evident. Lesion-size (virulence) has been the main, and often the only, criterion utilized to monitor population changes. Our studies suggest that infection and sporulation efficiencies may be important factors to evaluate in monitoring population changes. Increased efficiency for either of these attributes could result in an increase in epidemic-inciting capacity comparable to that resulting from increased virulence within a population.

The generation studies may provide a possible explanation for the failure of SCLB to reach epidemic levels in 1971. Even with abundant initial inoculum in 1971, early spread of the disease was negligible and should have been, in view of the reduced efficiencies of both populations under the cool regime. In addition, normally cooler temperatures early in the season in the Corn Belt may have resulted in a selection within race T populations for genotypes more efficient under a cool environment. As temperatures increased, selection should have shifted to genotypes more effective under a warm environment. Shifts within the population and the time required for preferred genotypes to increase in frequency may have delayed the progress of the disease enough to prevent an epidemic. The massive inoculum carried to the Corn Belt from the South in 1970 probably was comprised of genotypes most effective under a warm environment. Since the inoculum arrived during warm weather, the potential for rapid disease increase was realized. The planting in the South in 1971 of substantial acreages of corn hybrids with normal cytoplasm eliminated this source of inoculum.

Lack of data concerning overwintering effects on the populations and the effects of other selection pressures (e.g., host genotype, host nutrition, light quality, light quantity, etc.) limit interpretation of these studies in explaining field behavior. These factors may influence the net effect of temperature selection in nature. They should be studied before a complete hypothesis concerning natural selection of *H. maydis* race T under natural conditions can be formulated.

The techniques outlined herein may be applicable for studying population behavior of other foliar pathogens. Many crops are cultivated over a wide ecological area and it may be advisable to study pathogens over the entire environmental range of their host(s). A pathogen that causes little damage in one environment may cause a serious problem in a different environment. Detection of ecological races of a plant pathogen indicates the variability of that pathogen. In view of the recent history of *H. maydis,* the discovery of ecological races in a pathogen may warrant close monitoring of the population for changes of pathological consequence.

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