Virulence, Temperature Optima, and Competitive Abilities of Isolines of Races T and O of Bipolaris maydis

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ABSTRACT

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Isolines of *Bipolaris maydis* races T and O and mating types *A* and *a* were compared with respect to (i) growth in vitro at 20, 24, 28, 32, and 36 C, (ii) capacity to induce disease lesions on corn plants with *cms-T* and normal (*N*) cytoplasm, and (iii) selection in mixed populations on plants with *cms-T* or *N* cytoplasm. All isolines grew fastest at 28 C. At 28 and 32 C race T isolines grew slightly faster than did race O isolines. Growth rates of isolines of mating types *A* and *a* were similiar at all temperatures. There were no significant differences in numbers of lesions induced on *cms-T* and *N* plants by race T and race O isolines, respectively. Race T lesions were 45% longer than race O lesions on *cms-T* plants but were 10% shorter than race O lesions on *N* plants. Isolines of mating type *A* induced significantly more lesions on both *cms-T* and *N* plants than did isolines of mating type *a*, and the lesions of *A* isolines were slightly longer than those of *a* isolines. However, in mixtures of isolines, there was little or no change in the frequencies of the two mating types over five conidial generations. In mixtures of isolines, race T was selected on *cms-T* plants (fitness of race T was 43% greater than fitness of race O), but on *N* plants race O was selected (fitness of race O was 12% greater than fitness of race T). The lower fitness of race T on *N* plants may account for much of the decline of race T in the southern U. S. and northern Queensland. The greater persistence of race T in the northern U. S. and southern Queensland may indicate that the original race T population was better adapted to cool than warm temperatures. Race T conidial isolates collected from the southern USA in 1972 grew faster in vitro at 36 C than did race T conidial isolates collected from the southern or midwestern U. S. in 1970, but at 20, 24, and 28 C the isolates collected in 1970 grew faster than those collected in 1972.

Additional key words: *Cochliobolus heterostrophus*, *Zea mays*, maize, selection.

Changes in race and mating type frequencies that occurred in populations of *Bipolaris maydis* (Nisikado) Shoemaker after race T epidemics were similiar in the USA (3, 7, 8, 9, 13, 14, 15) and Australia (1). In each country, the race T population at the beginning of the epidemic consisted entirely, or almost entirely, of mating type *A*. After the first year of the epidemic, there was a rapid increase in mating type *a* in the southern U.S. and northern Queensland (both are warm areas). In the midwestern and northeastern United States and southern Queensland (cooler areas) there was little change, and mating type *A* continued to predominate in the race T population. Also, in the southern USA and northern Queensland, the proportion of race O in the population of *B. maydis* increased rapidly after the corn with Texas male sterile cytoplasm (*cms-T*) was replaced by corn with normal (*N*) cytoplasm. In the midwestern and northeastern USA and in southern Queensland, little or no race O was found and race T continued to be the predominant race for at least a few years even on corn with *N* cytoplasm. This agrees with the suggestion made by Hooker et al.(5) when race T was first identified, that race O was favored in warm areas and race T in cool areas. These observations suggest either that the genes for race T virulence (10) and *A* mating type (12) reduce the fitness of *B. maydis* in warm climates, or that the original race T populations of both the USA and Australia consisted of mating type *A* genotypes with better adaptation to cool than to warm climates. The studies described below were undertaken to test for stabilizing selection with respect to race T virulence and for possible pleiotrophic effects of genes for virulence and mating type on temperature adaptation.

MATERIALS AND METHODS

Isolines of *B. maydis* used in these studies were derived from a series of 11 or 12 generations of backcrossing race O mating type *A* progeny to the race T, mating type *a* conidial isolate Ch92 (ATCC 24773, IMI 175438, CBS 574.73) obtained in 1970 from a diseased corn leaf from Cleveland County, North Carolina. In the backcrossing program there was no selection with respect to degree of virulence on corn with *N* cytoplasm. In this paper, virulence is defined as the capacity of a pathogen to induce disease in a host. It may be general in its effects on host genotypes as race O virulence is, or it may be specific as race T virulence is specific for corn with *cms-T*. The probability that a particular race O ascospore isolate would be a parent in the next backcross generation was determined only by its ability to form fertile perithecia in combination with isolate Ch92, the recurrent parent.

In studies of growth in vitro and in comparisons of

virulence, 35 ascospore isolates from the 11th backcross generation were tested individually. Of these, 16 were race T and 19 were race O; 18 were mating type *A* and 17 were mating type *a*. In the selection experiment, a mixed population composed of 30 race O and 30 race T ascospore isolates from the 12th backcross generation was studied.

In vitro growth rates.—Growth rates of the 35 ascospore isolates from the 11th backcross generation were tested on Czapek's agar at five constant temperatures. Mycelial plugs (5 mm diameter) were punched from the margins of 7-day-old cultures of the isolates on potato-dextrose agar (PDA) and transferred to Czapek's agar in dam tubes (20 cm long × 2.2 cm inside diameter). An indentation of the glass wall near the open end of the tube served as a dam that allowed the tubes to be laid horizontally while the agar solidified. The tubes with the mycelial plugs were incubated in a horizontal position in the dark at 20, 24, 28, 32, and 36 C for 11 days after which the length of mycelial growth from the plug was measured.

The effects of temperature on in vitro growth of race T isolates collected from the southern and midwestern U.S. were also compared by the procedures described above. Of the 30 field isolates tested, 11 were collected from the South in 1970, 10 were collected from the Midwest in 1970, and nine were collected from the South in 1972.

Measurements of virulence.—Each of the 35 isogenic ascospore isolates from the 11th backcross generation described above were tested for virulence on corn plants of inbred lines B37 and B37 *cms-T*. The plants were inoculated in the five-leaf stage by spraying them with an aqueous suspension of conidia (5,000 conidia/ml) until the droplets began to coalesce and run off the leaves. Each

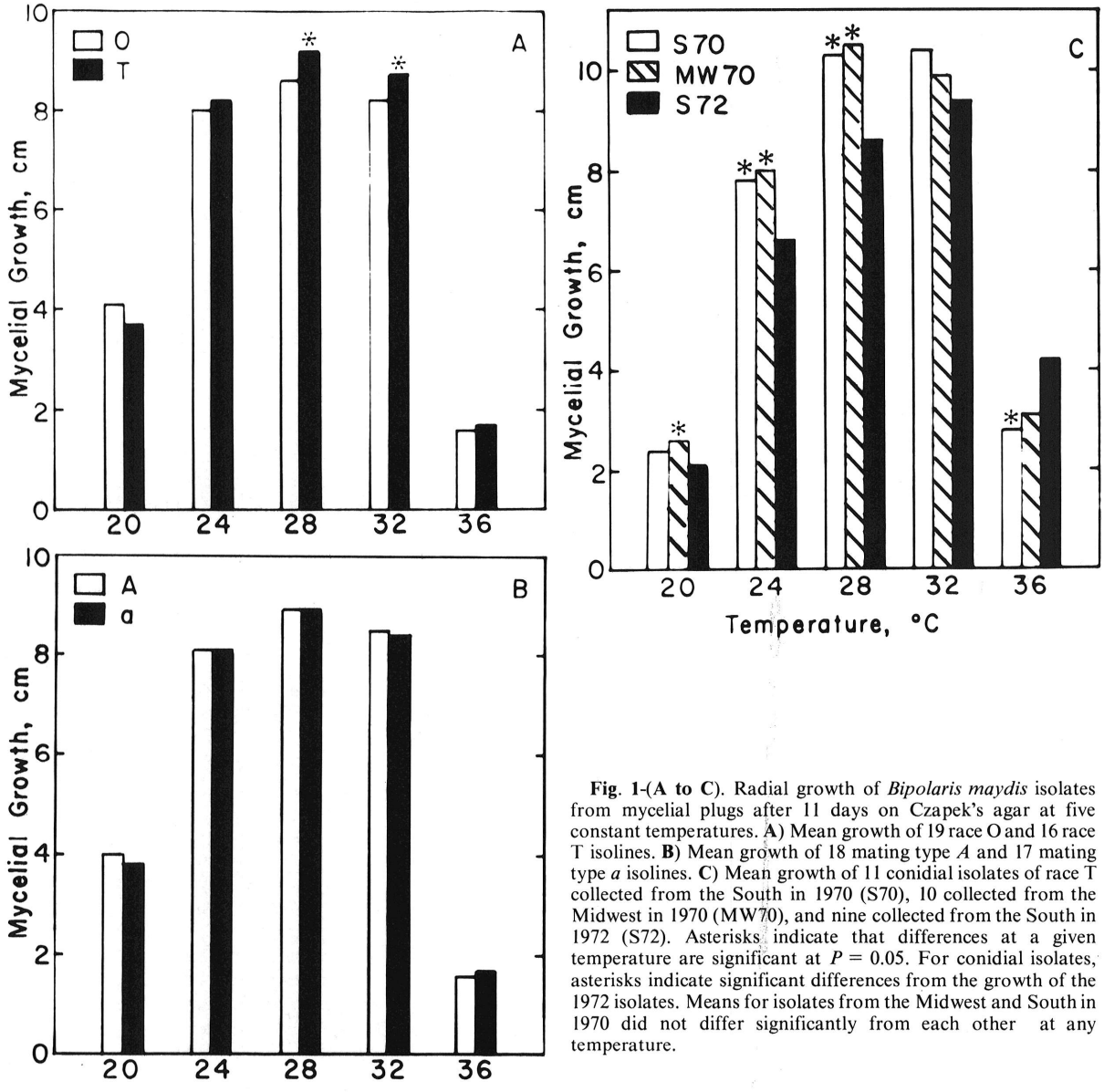

Fig. 1-(A to C). Radial growth of *Bipolaris maydis* isolates from mycelial plugs after 11 days on Czapek's agar at five constant temperatures. **A)** Mean growth of 19 race O and 16 race T isolines. **B)** Mean growth of 18 mating type *A* and 17 mating type *a* isolines. **C)** Mean growth of 11 conidial isolates of race T collected from the South in 1970 (S70), 10 collected from the Midwest in 1970 (MW70), and nine collected from the South in 1972 (S72). Asterisks indicate that differences at a given temperature are significant at $P = 0.05$. For conidial isolates, asterisks indicate significant differences from the growth of the 1972 isolates. Means for isolates from the Midwest and South in 1970 did not differ significantly from each other at any temperature.

isolate was tested on three pots of plants (two to five plants per pot) each of B37 and B37 *cms-T* placed in a randomized arrangement during inoculation. Inoculated plants were incubated 18 hr in a moist chamber, and then were returned to the greenhouse bench. Six days after inoculation, the lesions on the fourth leaf (from the bottom) of each plant were counted, and the lengths of the five largest lesions on the fourth leaf of each plant were measured.

Effects of selection.—Thirty race O and 30 race T ascospore isolates from the 12th backcross generation were grown on PDA. Conidia were scraped from 7-day-old cultures, suspended in water, filtered through cheesecloth to remove mycelial fragments, and adjusted to 4,000 conidia/ml. Equal parts of the race O and race T conidial suspensions were mixed and sprayed onto 6-wk-old corn plants of both B37 and B37 *cms-T*. Nine plants of each genotype were inoculated, incubated 18 hr in a moist chamber, and returned to the greenhouse bench.

Twelve days after inoculation, the infected plants were placed in a moist chamber for 2 days to induce sporulation. The plants were removed from the moist chamber, allowed to dry, and the conidia were then collected from the lesions with a cyclone spore collector attached to a vacuum pump.

A sample of the conidia from each corn genotype was dusted onto water agar in a petri dish, and about 70 single conidia were transferred to PDA slants in test tubes. The remainder of the conidia were suspended in water and adjusted to 4,000 conidia/ml. The conidia from B37

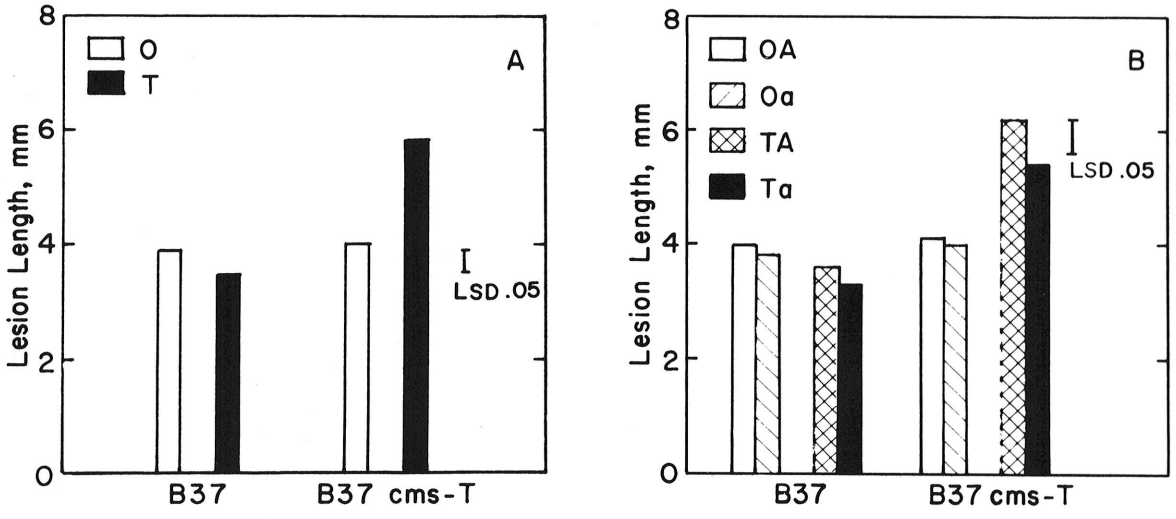

Fig. 2-(A, B). Mean length of the five longest lesions on the fourth leaves of B37 and B37 *cms-T* corn plants 6 days after inoculation with isolines of *Bipolaris maydis*. **A)** Mean lesion lengths of 19 race O and 16 race T isolines. **B)** Mean lesion lengths of nine race O, mating type *A*; 10 race O, mating type *a*; nine race T, mating type *A*; and seven race T, mating type *a* isolines.

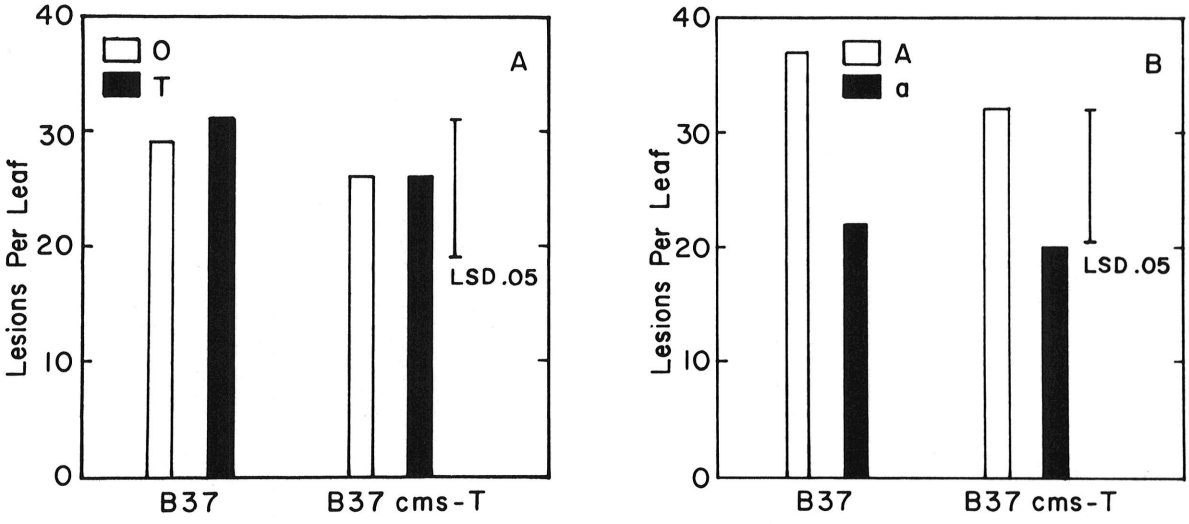

Fig. 3-(A, B). Mean numbers of lesions on the fourth leaves of B37 and B37 *cms-T* corn plants inoculated with isolines of *Bipolaris maydis*. **A)** Mean lesion numbers for 19 race O and 16 race T isolines. **B)** Mean lesion numbers for 18 mating type *A* and 17 mating type *a* isolines.

plants were used to inoculate another set of 6-wk-old B37 plants, and those from B37 *cms-T* plants were used to inoculate another set of B37 *cms-T* plants. The experiment was continued in this way through five generations. Single conidial isolates were collected at each generation and their race and mating type were determined.

The race identification of single conidial isolates was determined by growing them on PDA in petri dishes, scraping conidia from the culture with a clean, flat toothpick, and pressing them onto a leaf of B37 and a leaf of B37 *cms-T* corn plants. Inoculum for each isolate was applied in five spots about 5 mm apart on the leaf blade so that two or three isolates could be tested on each leaf. A

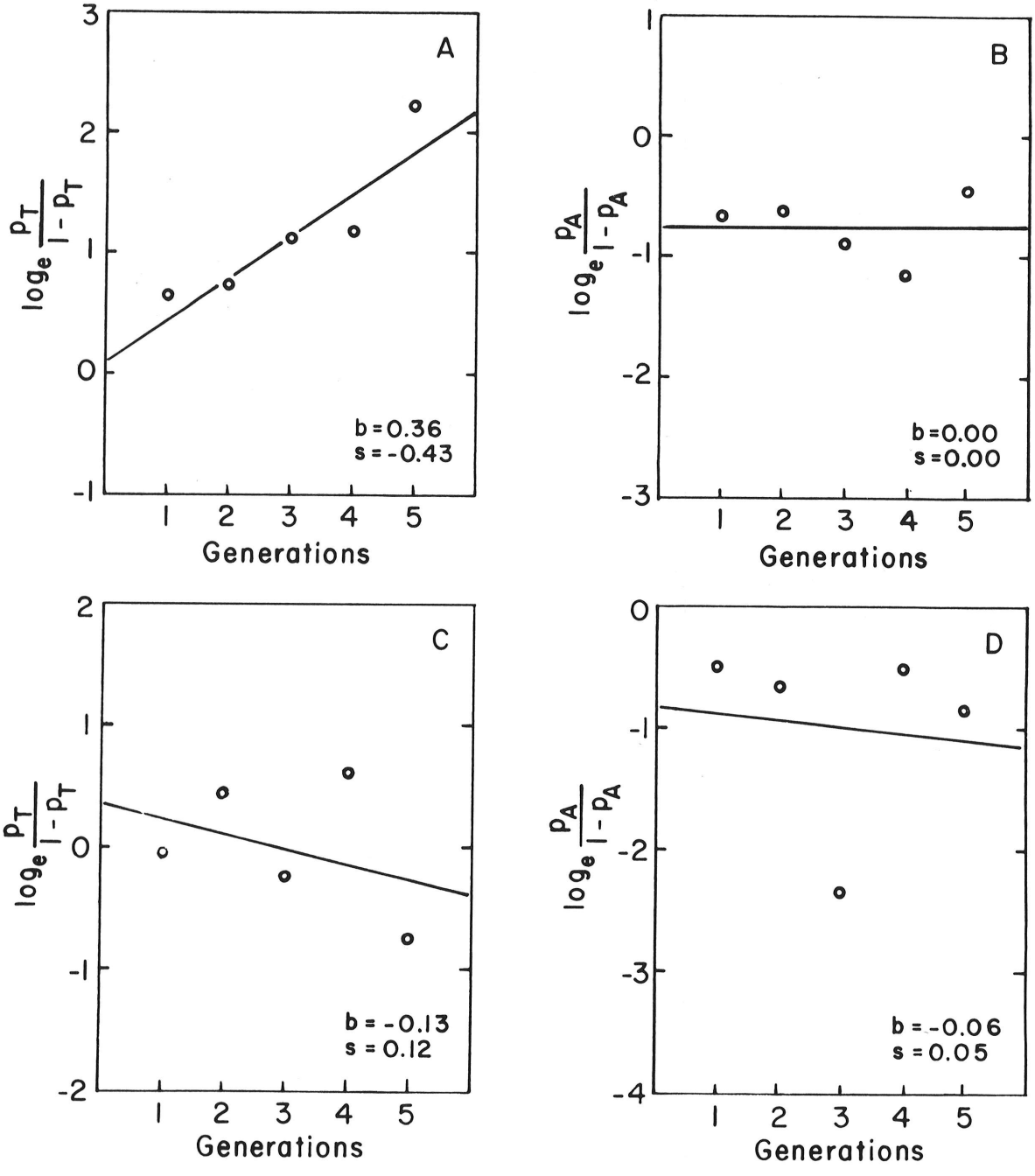

Fig. 4-(A to D). Rates of change in frequencies of races and mating types in mixed populations of isolines of *Bipolaris maydis* races O and T and mating types *A* and *a* on B37 *cms-T* and B37 corn plants. Data are plotted as logits of frequencies of race T (p_T) or mating type *A* (p_A) vs. number of generations of selection; *b* = slope determined by linear regression, and *s* = selection coefficient calculated from $s = 1 - e^b$. **A)** Change in frequency of race T on B37 *cms-T*. **B)** Mating type *A* on B37 *cms-T*. **C)** Race T on B37. **D)** Mating type *A* on B37.

separate toothpick was used for each isolate. Only the two youngest leaves of each plant were inoculated. Inoculated plants were incubated overnight in a moist chamber and returned to the greenhouse bench. Infected plants were examined daily from the third through the sixth day after inoculation. Lesions of race O and race T isolates on B37 plants were confined to the area around the inoculated spots, but on B37 *cms-T* plants lesions of race T isolates extended rapidly up and down the leaf from the inoculated area. If the reaction type could not be scored with certainty, the inoculation with that isolate was repeated.

Mating type was determined by pairing single conidial isolates with tester isolates of *A* and *a* mating types on opposite sides of corn leaf disks on Sachs' agar so that perithecia formed in the zone of contact between compatible isolates (7, 8, 9).

The logit of the proportion of each race and mating type in the population in each generation was calculated, and a linear regression analysis was performed on logits of gene frequency vs. generations of selection. Selection coefficients were determined from the relationship expressed in the equation:

$$\frac{p_n}{1-p_n} = (1-s)^n \frac{p_o}{1-p_o}$$

where p_n is the proportion of the race or mating type in question after n generations of selection, p_o is the initial proportion, and s is the selection coefficient. Plotting $\log_e [p_n/(1-p_n)]$ vs. n should give a straight line with a slope of $b = \log_e (1-s)$, so that $s = 1 - e^b$. The theoretical basis for this has been described (6).

To simplify the identification of races during selection in a mixed population, an albino mutant of isolate Ch92 (race T) was paired with a race O isoline from the 12th backcross generation to Ch92. Nine, 6-wk-old B37 plants were inoculated with a suspension of 5,000 conidia/ml made of equal parts of the albino race T and the wild-type race O isolines. Three wk after inoculation, conidia were collected from the inoculated plants and single conidial isolates were grown until they could be identified either as wild-type or albino. The remaining conidia were used to inoculate a second set of plants. Another collection of conidia was made from the first set of inoculated plants at 4 wk after inoculation, single conidia were isolated, and the remaining conidia were used to inoculate another set of B37 plants. Thus, in the second generation of selection there were two subpopulations: one from B37 plants at 3 wk after inoculation, and one from B37 plants at 4 wk after inoculation. Each subpopulation was sampled and continued for one more generation.

RESULTS

In vitro growth rates.—Mean growth rates for race T isolines were slightly greater than those of race O isolines at 28 and 32 C. There were no significant differences in mean growth rates at 20, 24, or 36 C (Fig. 1-A). Mating type alleles had no significant effects on growth rates at any of the temperatures (Fig. 1-B). Mean growth rates for race T conidial isolates collected in 1970 from the South and Midwest did not differ significantly at any of the five temperatures, but there was a tendency toward a slightly higher optimum temperature for the southern isolates (Fig. 1-C). Isolates collected from the South in 1972 appeared to have the highest optimum temperature for growth in vitro. They grew more slowly than did the 1970 isolates at 20, 24, and 28 C, but more rapidly at 36 C.

Measurements of virulence.—Race T isolines induced lesions on B37 *cms-T* plants that averaged 45% longer than race O lesions on either B37 *cms-T* or B37 plants (Fig. 2-A). On B37 plants the mean lesion length of race O isolines was 10% greater than that of race T isolines. There was a tendency for lesions of isolines of mating type *A* to be slightly longer than those of isolines of mating type *a*, but the difference was statistically significant only with race T on B37 *cms-T* plants (Fig. 2-B).

There were no significant differences in mean numbers of lesions per leaf induced by race O and race T isolines on either B37 or B37 *cms-T* (Fig. 3-A). Mating type did appear to affect the ability of isolines to infect the host. On both B37 and B37 *cms-T*, the isolines of mating type *A* induced significantly more lesions per leaf than did isolines of mating type *a* (Fig. 3-B).

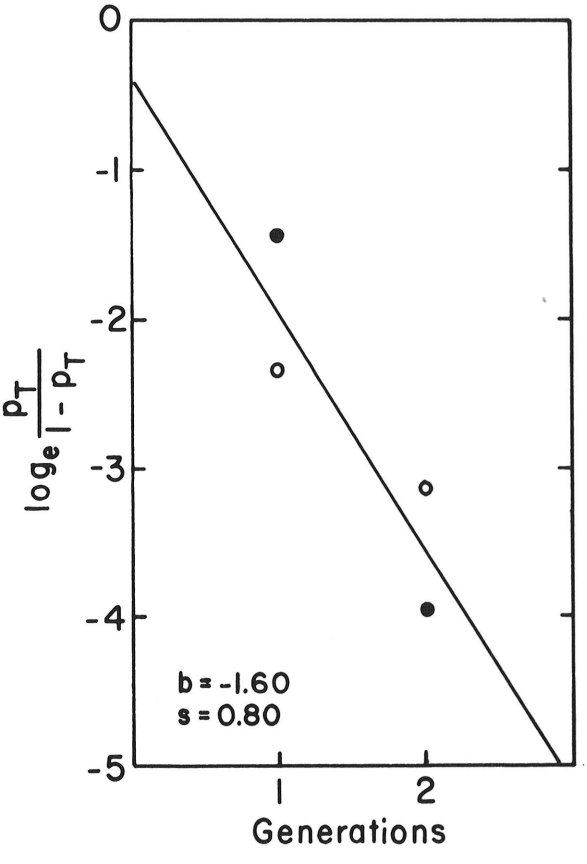

Fig. 5. Rate of change in frequency of an albino race T isoline of *Bipolaris maydis* in a mixture with a wild-type race O isoline on B37 corn plants. Data are plotted as logits of race T frequency vs. number of generations of selection; b = slope determined by linear regression, and s = selection coefficient calculated from $s = 1 - e^b$. Solid dots represent data from a subpopulation in which spores were collected 3 wk after inoculation, and open circles represent data from a subpopulation in which spores were collected at 4 wk after inoculation.

Effects of selection.—In the mixed population of race O and race T isolines on B37 *cms-T* plants, the frequency of race T increased at a rate equivalent to a selection coefficient of 0.43, or an amount of reproduction per generation 43% greater than that of race O (Fig. 4-A). In this population there was no significant change in the frequencies of mating types (Fig. 4-B). On B37 plants, the frequency of race T appeared to decline, although the selection coefficient of 0.12 was not significantly greater than 0 at $P = 0.05$ (Fig. 4-C). The frequency of mating type *A* in this population may have declined slightly, but the change was not statistically significant (Fig. 4-D). The experimental error was much greater for the population on B37 than for the population on B37 *cms-T*. This probably occurred because of the poor sporulation in most of the lesions on B37 plants. The few lesions on senescing leaves of B37 plants contributed an inordinately high proportion of the total spores from those plants.

The albino race T isoline declined rapidly in the mixture with the wild-type race O isoline (Fig. 5). The selection coefficient of 0.80 indicates that the amount of reproduction per generation by the albino was only 20% of that of the wild-type isoline.

DISCUSSION

The decline of race T on corn with *N* cytoplasm in the southern U.S. (7, 8, 9) and northern Queensland (1) could have been caused by (i) an inherent loss in fitness associated with the gene for race T virulence (i. e. stabilizing selection *sensu* Van der Plank), (ii) poor adaptation of the genotypes in the original race T population, or (iii) a combination of these two factors. Comparisons of sizes of lesions induced by isolines of *B. maydis* and the results of selection among isolines on B37 corn plants indicate that stabilizing selection can reduce fitness of *B. maydis* by 10-12%. If this level of stabilizing selection is representative of that in the natural populations of *B. maydis*, it could account for much of the decline of race T in the southern U.S. and northern Queensland.

Comparisons of in vitro growth rates of isolines of *B. maydis* do not support the hypothesis that genes for race T virulence or mating type *A* are necessarily associated with adaptation to cool temperatures. The prevalence of race T, mating type *A* isolates in the northern U.S. from 1970 to 1972 (3, 8, 9, 13, 14) and southern Queensland from 1971 to 1974 (1) probably resulted from the effects of other genes in the race T population. Fukuki and Aragaki (4) showed that race T isolates collected in Hawaii in 1970 were of limited genetic diversity and sporulated better at cool temperatures than at warm temperatures that were optimal for sporulation of race O isolates. In vitro growth data for the race T isolates collected in southern and midwestern U.S. in 1970 and 1972 indicate that in 1970 the temperature adaptation of the population in the South was similiar to that of the population in the Midwest. By 1972 the population in the South had shifted to a higher optimum temperature for growth.

The proportion of mating type *A* in the race T population declined from 80% to 33% from 1970 to 1972 in the southern U.S. (7, 8, 9) and from 100% to 60% from 1971 to 1974 in northern Queensland (1). Comparison of in vitro growth of isolines and selection among isolines on B37 *cms-T or B37 N* corn plants suggest that the mating type alleles in *B. maydis* are selectively neutral. However, mating type *A* isolines induced more and larger lesions on B37 *cms-T* and B37 *N* plants than did mating type *a* isolines. These results appear to be contradictory, but the experiments did not necessarily deal with exactly the same attributes of the isolines. It may be that mating type *a* isolines possess other advantages that compensate for their reduced virulence.

In natural populations the two mating types are probably maintained at nearly equal frequency by one or more forms of frequency dependent selection. The sexual stage of the fungus would tend to favor the least frequent mating type, but it may occur too rarely in nature to have a large effect. Caten (2) showed that heterokaryon incompatibility in *Aspergillus* restricts the spread of harmful cell inclusions such as viruses or mutant suppressive mitochondria from one mycelium to another. Lindberg (11) found that of the race T isolates he collected in Louisiana in 1970, most were diseased as evidenced by poor growth in culture. I found the same or a similiar disease evident in many of the isolates I collected. The causal agent of this disease, which reduced virulence on corn with *cms-T* or *N* cytoplasm as well as growth on artificial media, can be transmitted readily by hyphal anastomosis between *B. maydis* isolates of the same mating type, but is not transmitted between isolates of unlike mating type (Leonard, *unpublished*). This disease would tend to reduce the fitness of whichever mating type was most frequent in a population, because the disease agent would spread faster among isolates of that mating type.

Use of the albino marker to identify *B. maydis* races in selection experiments does not appear to be feasible. The decline in frequency of the albino race T isoline was many times more rapid than that of the wild-type race T isolines, and was probably due mostly to deleterious effects of the albino gene.

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