Effect of High Temperature Stress on Peroxidase Activity and Electrolyte Leakage in Maize in Relation to Sporulation of *Bipolaris maydis* Race T

M. O. Garraway, M. Akhtar, and E. C. W. Wokoma

First and second authors: professor and post-doctoral researcher, respectively, Department of Plant Pathology, Ohio Agricultural Research and Development Center and The Ohio State University, Columbus 43210. Present address of second author: Institute for Microbial and Biochemical Technology, USDA Forest Service, Forest Products Laboratory, Madison, WI 53705. Third author: assistant lecturer, Faculty of Biological Sciences, University of Port Harcourt, Rivers State, Nigeria.

We thank Dr. C. W. Ellett and Dr. P. E. Lipps for reviewing the manuscript. Salaries and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Submitted as Journal Article 305-88 from the Ohio Agricultural Research and Development Center. Other support provided by a Government of India post-doctoral fellowship to M. A.

Accepted for publication 13 February 1989.

ABSTRACT

Garraway, M. O., Akhtar, M., and Wokoma, E. C. W. 1989. Effect of high temperature stress on peroxidase activity and electrolyte leakage in maize in relation to sporulation of *Bipolaris maydis* race T. Phytopathology 79:800-805.

Exposure of maize (*Zea mays* L.) leaves to high temperature stress, i.e., 42 C for 6 hr before inoculation with *Bipolaris maydis* race T followed by incubation in the dark at 28 C for 24 hr, resulted in a significant decrease in peroxidase activity in both resistant and susceptible isolines compared with the control (leaves not exposed to high temperature stress before inoculation). Also at 48 hr of incubation, high temperature stress before inoculation decreased peroxidase activity compared with the control in the resistant but not in the susceptible isoline. Moreover, the level of peroxidase activity in high temperature stress-treated and control leaves was significantly lower in the susceptible than in the resistant isoline 48 hr after inoculation. Exposure to high temperature stress resulted in a significant increase in electrolyte leakage as well as in sporulation in both isolines. Maize leaf extracts containing peroxidase activity as well as leachates from leaves of both isolines exposed to high temperature stress caused an increase in sporulation in vitro. Whereas increased sporulation on maize leaves in response to high temperature stress appeared to be related to increased electrolyte leakage, such a relationship was not found with high temperature stress-induced changes in maize peroxidase.

Additional keywords: N cytoplasm isoline, teleomorph *Cochliobolus heterostrophus*, Tms cytoplasm isoline.

High temperature stress when applied before inoculation can affect host-pathogen interactions (7,9,24,34,37,42). High temperature stress-induced changes in phenolic compounds, including phytoalexins (3,9,10,13), heat shock proteins and mRNA (21), and peroxidase (37) have been studied in attempts to identify mechanisms to explain these interactions. But these physiological parameters showed no consistent response to high temperature stress or to the effect of high temperature stress on the severity of infection and disease. For example, in-depth studies with peroxidase showed that this enzyme has been implicated in several physiological processes (19), including disease resistance (23,27,40). Other studies showed that either peroxidase is not an important factor in disease resistance (36) or that increases in peroxidase activities are not directly involved in induced resistance (31). Moreover, high temperature stress-induced resistance of cucumber to *Cladosporium cucumerinum* was accompanied by enhanced peroxidase activity (37), but high temperature stress-induced susceptibility of tobacco to *Phytophthora parasitica* was not correlated with a decrease in peroxidase activity (34).

An alternative mechanism by which high temperature stress might predispose plants to disease could be through its effect on membrane permeability. For example, Lin et al (26), working with soybean seedlings, have shown that high temperature stress affects electrolyte leakage through its effect on membrane permeability. Because electrolyte leakage represents the availability of nutrients needed for the growth and development of pathogens, one would assume that high temperature stress might function like other abiotic (33) or biotic (38) stresses to predispose plants to diseases.

Based on the above and our preliminary studies, which indicated that exposure of maize leaves to high temperature stress before inoculation with *Bipolaris maydis* race T appeared to increase the severity of infection and disease (2), we sought to determine the relationship, if any, of peroxidase activity and electrolyte leakage to the above phenomenon. Therefore, we undertook a study to determine: 1) the effect of exposure of leaves to high temperature stress on peroxidase activity and electrolyte leakage in *B. maydis* race T-infected maize leaves; and 2) the relationship of high temperature stress-induced changes in peroxidase activity and electrolyte leakage to increases in sporulation of *B. maydis* race T on infected maize leaves.

MATERIALS AND METHODS

Host. Two isolines of the maize (*Zea mays* L.) inbred W64A, with normal (N) cytoplasm (resistant) and Texas male sterile (Tms) cytoplasm (susceptible), were grown in the greenhouse at 28 C as previously described (4,5,15). The fungal pathogen used in this study was *Bipolaris maydis* (Nisikado) Shoemaker (syn. *Helminthosporium maydis* Nisikado and Miyake, teleomorph stage *Cochliobolus heterostrophus* Drechlser) race T. Leaf samples of comparable age from 3- to 4-wk-old plants were detached from each isoline, washed with double distilled water, cut into pieces about 5 × 2 cm in size and then placed on a sheet of Whatman No. 3 filter paper, which was kept moist with double distilled water. Such leaves were exposed for 6 hr in the dark either at 42 (high temperature stress) or 28 C (control) as this regime of high temperature stress proved optimum for predisposing maize leaves to disease (2,41). Moreover, this regime did not affect the viability of cells, as indicated by cyclosis and as tested against Evans blue (Fisher Scientific Company, St. Louis), a pigment to detect dead cells in intact tissues (14). High temperature stress-treated and control leaves were then inoculated with a *B. maydis* race T spore suspension (10,000–15,000 conidia/ml) in double distilled water containing Tween 20 (50 μl/100 ml) as a surfactant. Then both high temperature stress-treated

and control leaves were incubated either for 24 or 48 hr in the dark at 28 C. The spore suspension used as inoculum was prepared from cultures grown on a glucose-L-asparagine agar medium for 7 days at 28 C (16).

Pathogen. A single spore isolate of *B. maydis* race T recovered from an ear of maize from a field in Franklin County, OH, in 1970 (ATCC 36180) was grown on a glucose-L-asparagine agar medium in the dark at 28 C for 7 days as previously described (16). Results obtained with this isolate in our previous preliminary studies were similar to those with other isolates of race T.

Determination of peroxidase activity. Procedures related to peroxidase (donor: H_2O_2 oxidoreductase: E.C.1.11.1.7) extraction were the same as those used previously (4,5,15). The tissue was homogenized in 5 ml of 10 mM sodium phosphate buffer (pH 6) using a Brinkmann polytron homogenizer. The resulting homogenate was centrifuged at 20,000 *g* for 5 min at 4 C. The supernatant was assayed for the buffer-extractable, or soluble peroxidase activity. To recover salt-extractable, or iconically bound peroxidase activity, the unwashed pellet was resuspended in 5 ml of 1 M NaCl in 10 mM phosphate buffer (pH 6), stirred at 4 C for 1 hr, centrifuged at 20,000 *g* for 5 min at 4 C, and then the supernatant was assayed. Our data are based on the soluble and ionically bound peroxidase activity because these two fractions constitute about 90% of the total peroxidase activity in maize and could be recovered with ease and speed. Cadena-Gomez and Nicholson (8), using the same method for peroxidase extraction in maize, have also recovered about 93% of the total peroxidase activity. The reaction mixture for the peroxidase assay included 200 μl of enzyme solution, 1,300 μl of sodium phosphate buffer (10 mM, pH 6), 500 μl of 50 mM guaiacol, and 100 μl of 100 mM H_2O_2. One unit of peroxidase activity is defined as the change of 1.0 absorbance unit at 470 nm per minute per gram fresh weight of leaf tissue.

Determination of electrolyte leakage. Detached leaves of both isolines were exposed for 6 hr in the dark either at 42 or 28 C, inoculated with *B. maydis* race T as described above, then incubated in the dark for 24 hr at 28 C. These leaves were cut into 3-cm sections, then washed with double distilled water. Each leaf section was immersed in 25 ml of sterile double distilled water in screw cap vials (40-ml capacity). They were further incubated in the dark for 24 hr at 28 C. To determine the quantity of electrolyte leakage, the change in conductance of the double distilled water with immersed leaf sections was measured at 0 and 24 hr with a conductivity bridge (model 4959) and a conductivity cell ($k = 0.01$, Leeds and Northrup Co., Philadelphia, PA). The amount of electrolyte leakage is expressed as micromhos (μmhos) per milligram dry weight of leaf tissue.

Determination of sporulation on infected maize leaves. Detached leaves of both isolines were exposed for 6 hr in the dark either at 42 or 28 C and inoculated with *B. maydis* race T as described above. Such leaves were then incubated in the dark in a water-vapor saturated incubator for 48 hr at 28 C, as reported previously (4,41). At the end of the incubation period, infected leaf sections were placed in screw cap vials (15-ml capacity) containing 3 ml of preservative solution (5% commercial bleach, 20% ethanol, and 2% NaOH) to inactivate the conidia. These vials were then agitated to dislodge conidia from the leaf surface. Conidium concentrations were determined with a hemacytometer as described previously (16). Results are expressed as the number of *B. maydis* race T conidia produced per milligram dry weight of leaf tissue.

Determination of sporulation in vitro. To study the effect of maize extracts containing peroxidase activity or of commercial horseradish peroxidase (95 units/mg solid, Sigma) on *B. maydis* race T sporulation, sterile cellulose analytical filter paper disks (12.7 mm diameter, Carl Schleicher & Schuell, Keene, NH) were used. Detached leaves from either the N cytoplasm or Tms cytoplasm isoline were homogenized in 5 ml of 10 mM sodium phosphate buffer (pH 6) with a Brinkmann polytron homogenizer. The resulting homogenate was centrifuged at 20,000 *g* for 5 min at 4 C. The supernatant solution was diluted to give a peroxidase activity equal to 200 absorbance units at 470 nm per min/g fresh weight of leaf tissue. This was equivalent to 0.1 μg of commercial peroxidase solution per milliliter, which was purified as described below. Half of the diluted supernatant was boiled to inactivate the enzyme (control) and the other half was unboiled. To each cellulose disk was added 25 μl of boiled or unboiled supernatant. A conidial suspension was prepared as above and adjusted to 30,000–40,000 conidia per milliliter, then 25 μl of this conidial suspension was added to each disk. Such disks were placed in petri dishes and incubated in the dark for 7 days at 28 C. Sporulation was measured as previously described (16). To determine the extent to which peroxidase in extracts from maize leaves contribute to *B. maydis* race T sporulation, sporulation on cellulose disks containing different concentrations of commercial peroxidase (0, 0.01, 0.1, and 1.0 μg/ml) was also measured. Such disks were also inoculated and incubated as described above. The commercial peroxidase (salt free 95 units/mg solid, Sigma) used above was further purified using an ammonium sulfate precipitation method. One gram of this enzyme preparation was added to a solution containing 55% of ammonium sulfate, stirred, and allowed to stand overnight in the refrigerator at 4 C then centrifuged at 10,000 *g* for 10 min. To the supernatant was added enough ammonium sulfate to yield 83% saturation. This preparation was allowed to stand at 4 C then centrifuged again at 10,000 *g*. The resulting precipitate was redissolved in 10 ml of double distilled water placed in a dialysis bag, which was immersed in a beaker containing 200 ml of double distilled water, then incubated for 24 hr at 4 C. During this period, the double distilled water was replaced three times. After dialysis, the dialyzates were further centrifuged to remove traces of particulate material.

To study the effect of leachates on *B. maydis* race T sporulation, detached leaves of both isolines were exposed for 6 hr in the dark either at 42 or 28 C, inoculated with *B. maydis* race T as described above and then incubated in the dark for 24 hr at 28 C as described previously (15). Such leaves were cut into 3-cm sections, rinsed, and immersed in 50 ml of double distilled water in a beaker. After 12 hr of immersion, double distilled water containing leachates was autoclaved at 15 lb $in.^{-2}$ (1 lb $in.^{-2} = 703.1$ kg m^2) for 20 min and then used to constitute 20 ml of 2% agar media. This experimental media either nonamended (water agar) or amended with double distilled water containing leachates was seeded with 0.5 ml of a sterile *B. maydis* race T spore suspension (30,000–40,000 conidia/ml). The conidia were uniformly distributed on the surface of each plate with a sterile glass spreader. Seeded plates were incubated in the dark at 28 C for 7 days. Conidia were collected from the culture by scraping and washing with 1 ml of preservative solution, and the number per milliliter was determined as previously described (16). Sporulation was expressed as conidia per milligram dry weight of fungus.

All experiments involving peroxidase, electrolyte leakage, sporulation in vivo and in vitro were repeated three times with five replicates in each. Data presented are the means of five replicates and are representative of three separate replicated experiments.

RESULTS

Effect of high temperature stress on peroxidase activity. For the N cytoplasm as well as for the Tms cytoplasm isolines, high temperature stress before inoculation and subsequent incubation for 24 hr in the dark at 28 C significantly ($P = 0.05$) decreased buffer-extractable peroxidase activity, salt-extractable peroxidase activity, and total peroxidase activity compared with the control (Fig. 1A). Also, at 48 hr of incubation, high temperature stress before inoculation significantly ($P = 0.05$) decreased peroxidase activity compared with the control in the N cytoplasm isoline but not in the Tms cytoplasm isoline (Fig. 1B). Moreover, the level of peroxidase activity in the high temperature stress-treated and control leaves was significantly ($P = 0.05$) lower in the Tms cytoplasm isoline than in the N cytoplasm isoline (Fig. 1B). Comparable changes in individual peroxidase isozymes were seen

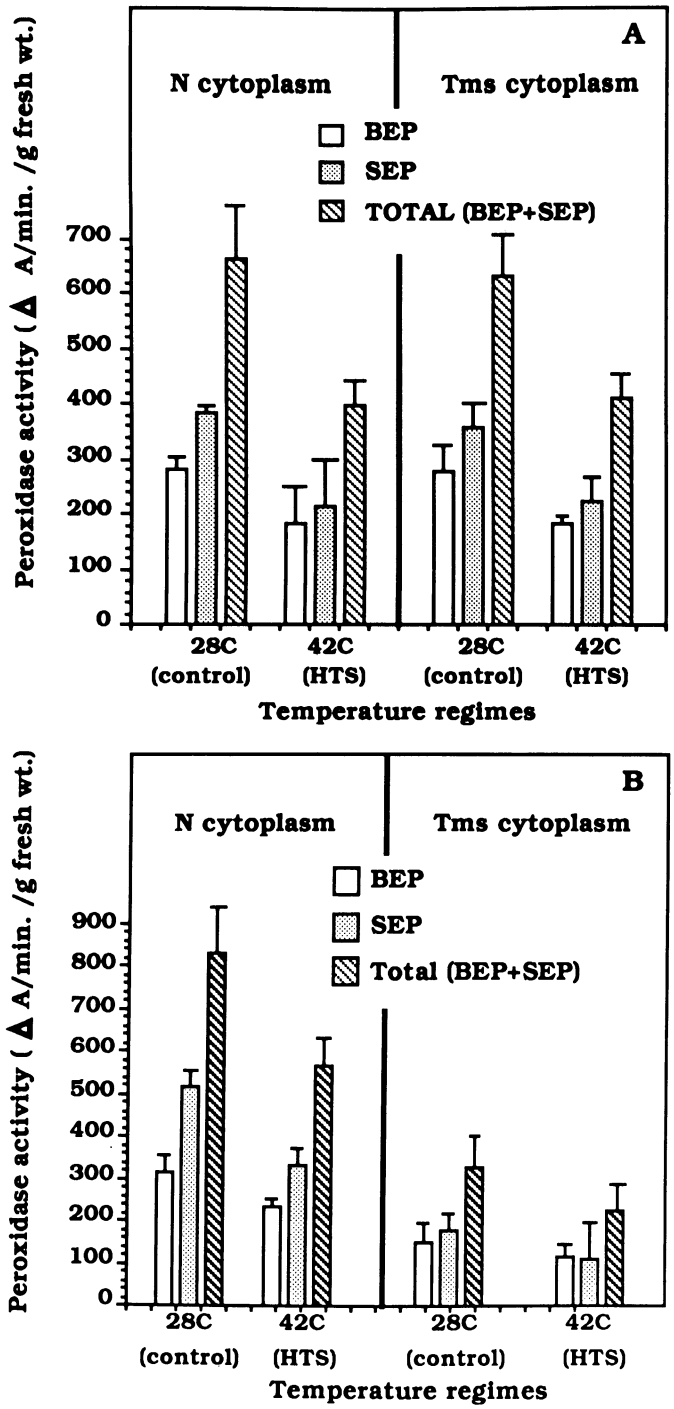

Fig. 1. Peroxidase activity in leaves of normal (N) cytoplasm and Texas male sterile (Tms) cytoplasm maize isolines exposed for 6 hr in the dark either at 42 C (HTS) or 28 C (control), inoculated with 10,000–15,000 conidia of *Bipolaris maydis* race T per milliliter, then incubated for 24 hr in the dark at 28 C (**A**) or 48 hr in the dark at 28 C (**B**). BEP (buffer extractable peroxidase). SEP (salt extractable peroxidase). Total peroxidase activity in leaves of the N and Tms cytoplasm isolines immediately after detachment (0 hr) was 500 ± 42(SE) and 497 ± 30(SE), respectively. At 24 hr of incubation in the dark at 28 C, total peroxidase activity in the control and in the HTS-treated leaves (uninoculated) from the N cytoplasm isoline was 497 ± 62(SE) and 490 ± 36(SE), respectively, and from the Tms cytoplasm isoline was 530 ± 43(SE) and 600 ± 61(SE), respectively. At 48 hr of incubation in the dark at 28 C, peroxidase activity in the control and in the HTS-treated leaves (uninoculated) from the N cytoplasm isoline was 501 ± 68(SE) and 504 ± 28(SE), respectively, and from the Tms cytoplasm isoline was 548 ± 35(SE) and 590 ± 28(SE), respectively. Peroxidase activity is expressed as change in absorbance at 470 nm/min g fresh weight of leaf with guaiacol as the hydrogen donor. N cytoplasm and Tms cytoplasm maize isolines are resistant and susceptible respectively, to *B. maydis* race T. The vertical lines represent standard error of the mean.

when the above fractions were separated by starch gel electrophoresis (1,5).

Effect of high temperature stress on electrolyte leakage. In addition to the effect of high temperature stress on peroxidase activity, we also studied the effect of high temperature stress on electrolyte leakage from both isolines of maize. High temperature stress before inoculation significantly ($P = 0.05$) increased electrolyte leakage from leaves of both isolines compared with the control (Fig. 2). Also, electrolyte leakage was greater from the Tms cytoplasm isoline than from the N cytoplasm isoline (Fig. 2).

Effect of high temperature stress on sporulation of *B. maydis* race T in vivo. The effect of high temperature stress on the level of sporulation on infected leaves of both isolines was also examined. This information was needed to critically evaluate the relationship, if any, of high temperature stress-induced peroxidase activity and electrolyte leakage to sporulation in vivo. Sporulation on leaves of either the N or Tms cytoplasm isoline exposed to high temperature stress before inoculation was significantly ($P = 0.05$) higher than that on the leaves of either isoline not exposed to high temperature stress (Fig. 3). For each temperature regime sporulation on the Tms cytoplasm isoline was higher than on the N cytoplasm isoline.

Effect of maize extracts containing peroxidase activity on sporulation of *B. maydis* race T in vitro. To determine the relationship between increased sporulation and changes in peroxidase activity in response to high temperature stress before inoculation, in vitro studies were undertaken. Maize extracts from the N cytoplasm isoline containing peroxidase activity significantly ($P = 0.05$) increased sporulation in vitro compared with the boiled control (Fig. 4). Similar results were obtained when maize extracts from the Tms cytoplasm isoline containing

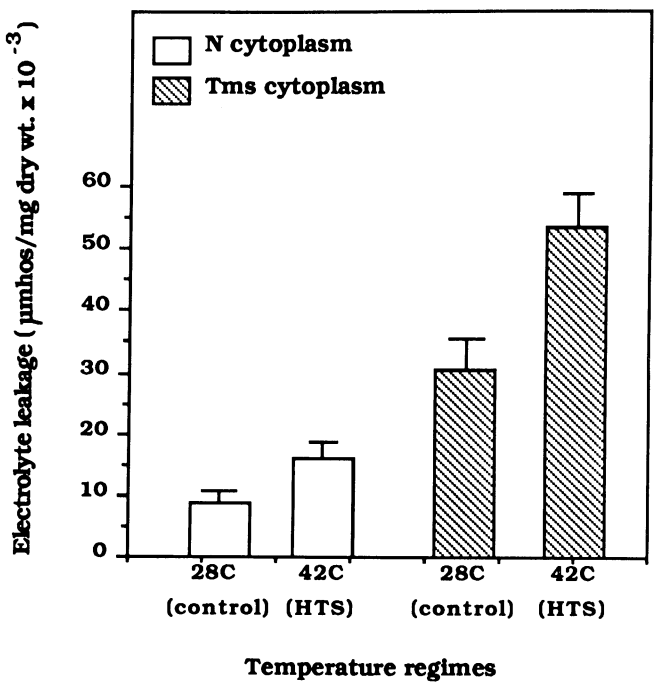

Fig. 2. Electrolyte leakage from leaves of normal (N) and Texas male sterile (Tms) cytoplasm maize isolines exposed for 6 hr in the dark either at 42 C (HTS) or 28 C (control), inoculated with 10,000–15,000 conidia of *Bipolaris maydis* race T per milliliter, then incubated for 24 hr in the dark at 28 C. Such leaves were cut into 3-cm sections and then floated on 25 ml of double distilled water for 24 hr in the dark at 28 C. Electrolyte leakage values for the control and for the HTS-treated leaves (uninoculated) from the N cytoplasm isoline were 4.75 ± 1.3(SE) and 10.69 ± 2.3(SE) μmhos/mg dry wt. × 10^{-3}, respectively, and from the Tms cytoplasm isoline were 4.00 ± 0.9 (SE) and 16.28 ± 3.1(SE) μmhos/mg dry wt. × 10^{-3}, respectively. N cytoplasm and Tms cytoplasm maize isolines are resistant and susceptible respectively, to *B. maydis* race T. The vertical lines represent standard error of the mean.

peroxidase activity were used (data not shown). Because maize extracts containing peroxidase activity may also have other constituents, the effect of peroxidase in these extracts on sporulation was compared with that of three concentrations (0.01, 0.1, 1 μg/ml) of commercial peroxidase prepared as described previously. Of these concentrations, 0.1 μg/ml of commercial peroxidase which had enzyme activity equal to that in maize leaf extracts, also increased sporulation significantly ($P = 0.05$) as compared with the control (Fig. 4).

Effect of leachates on sporulation of *B. maydis* race T in vitro. In the present investigation, there appeared to be a relationship between electrolyte leakage and sporulation in vivo in response to high temperature stress. Because electrolytes contain constituents needed by *B. maydis* race T for its growth and development, we also examined their effect on sporulation in vitro. Sterilized leachates from high temperature stress-treated tissues of either isoline significantly ($P = 0.05$) increased sporulation in vitro compared with the control (Fig. 5). The magnitude of increase in electrolyte leakage in response to high temperature stress appeared to be comparable to the magnitude of increase in sporulation on media amended with leachates from high temperature stress-treated tissues.

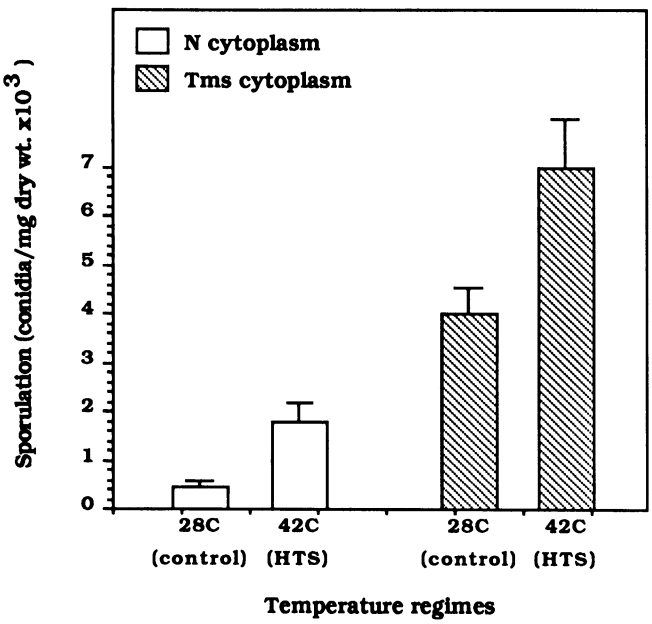

Fig. 3. Sporulation of *Bipolaris maydis* race T on leaves of the normal (N) and Texas male sterile (Tms) cytoplasm maize isolines exposed for 6 hr in the dark either at 42 C (HTS) or 28 C (control), inoculated with 10,000–15,000 conidia of *B. maydis* race T per milliliter, then incubated for 48 hr in the dark at 28 C. N cytoplasm and Tms cytoplasm maize isolines are resistant and susceptible, respectively, to *B. maydis* race T. The vertical lines represent standard error of the mean.

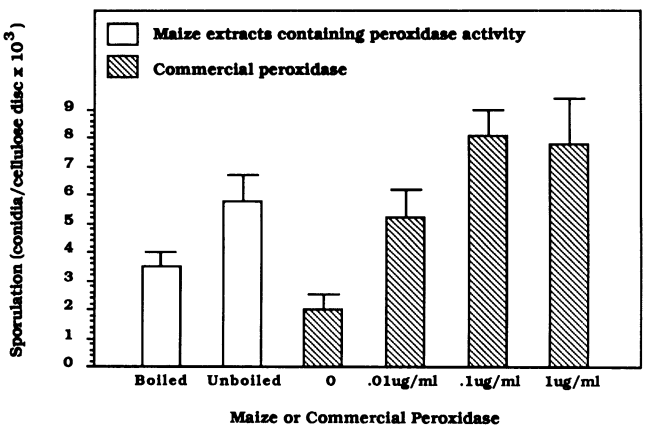

Fig. 4. Sporulation of *Bipolaris maydis* race T after 7 days of incubation in the dark at 28 C on cellulose disks containing either boiled (control) or unboiled extracts containing peroxidase activity from the N cytoplasm isoline or commercial peroxidase. An inoculum suspension of 30,000–40,000 conidia of *B. maydis* race T per milliliter was used to seed the disks. Similar results were obtained when extracts containing peroxidase activity from the Tms cytoplasm isoline was used. The vertical lines represent standard error of the mean.

DISCUSSION

When detached leaves of two isolines of W64A maize are exposed to high temperature stress before inoculation and are incubated in the dark at 28 C for 48 hr, peroxidase activity in the leaves of the N cytoplasm isoline decreased but sporulation increased. On the other hand, no differences in peroxidase activity occurred in the stressed leaves of the Tms cytoplasm isoline but sporulation increased. Thus, there appeared to be no relationship between sporulation increases in response to a prior exposure of maize leaves to high temperature stress and peroxidase activity. These findings are consistent with our previous studies, which indicated that the N and Tms cytoplasm isolines of maize which differ in their susceptibility to *B. maydis* race T contained comparable peroxidase isozymes, both in the buffer-extractable peroxidase as well as in the salt extractable peroxidase (4). Our findings also support other studies with wheat (36), cotton (28), and tobacco (31).

Peroxidase containing extracts appeared to increase sporulation in vitro. Addition of commercial peroxidase having enzyme activity equal to that present in maize leaf extracts also increased sporulation in vitro. This supports the observation that maize extracts containing peroxidase activity per se could contribute to increased sporulation in vitro.

It is generally recognized that a temperature regime such as the one we used in this study causes changes in cell membrane properties (6,39), including an increase in permeability to metabolites. This could lead to increased leakage of cell constituents such as carbohydrates, inorganic electrolytes, and vitamins, which may be used by an invading pathogen as a source

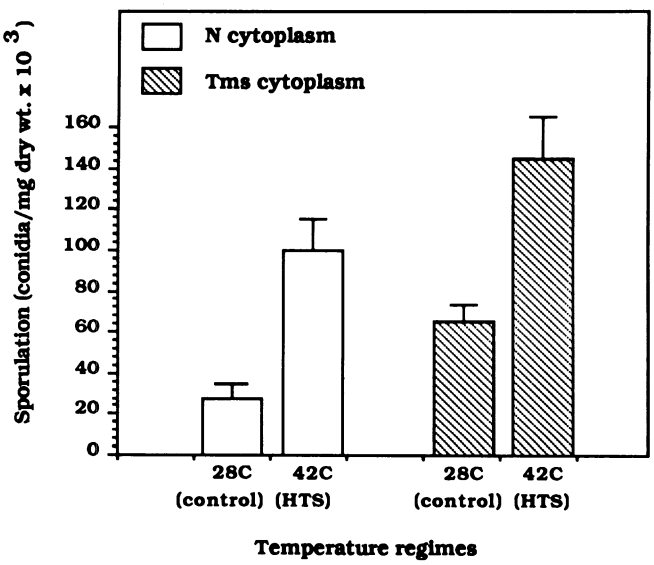

Fig. 5. Sporulation of *Bipolaris maydis* race T after 7 days of incubation in the dark at 28 C on a medium either nonamended (water agar) or amended with leachates from normal (N) and Texas male sterile (Tms) cytoplasm maize isolines exposed for 6 hr in the dark either at 42 C (HTS) or 28 C (control), inoculated with 30,000–40,000 conidia of *B. maydis* race T per milliliter, then incubated for 24 hr in the dark at 28 C. Sporulation on water agar medium was 21 ± 2(SE) conidia/mg dry wt. × 10^3. Leachates were prepared as described in the Materials and Methods. N cytoplasm and Tms cytoplasm maize isolines are resistant and susceptible, respectively, to *B. maydis* race T. The vertical lines represent standard error of the mean.

of nutrients (15,17,18). In the present investigation, high temperature stress before inoculation caused a significant amount of electrolyte leakage from both isolines. Moreover, the magnitude of electrolyte leakage appeared to be comparable to the magnitude of sporulation in vivo. The observation that leachates from infected high temperature stress-treated tissues produce comparable increases in sporulation in vitro to those seen in vivo supports the idea that the increased sporulation on high temperature stress-treated leaves may be due to an increase in availability of nutrients to the pathogen.

The above findings are consistent with those of others who established the relationship of electrolyte leakage with other parameters such as the number of germ tubes and appressoria formation (38) or rate of lesion development (33) on infected plants exposed to abiotic or biotic stresses. This is consistent with the idea that induced electrolyte leakage may be a part of a more general response to stress and that nutrients in leachates may play a key role in the growth and development of pathogens in vivo. Thus, these findings have significance for the influence of a prior exposure to high temperature stress on the establishment of *B. maydis* on maize, because it is a necrotroph (25) and its growth and development depend on metabolites released from host cells.

The effect of high temperature stress on membrane permeability as measured by electrolyte leakage could be comparable to the mechanism of action of host-selective pathotoxins proposed for plant pathogenic fungi (32,35), including *B. maydis* race T (20). This mechanism could help to explain some of our results. For example, it is known that HmT-toxin produced by *B. maydis* race T causes substantial leakage of cell constituents (22) from the Tms cytoplasm isoline (susceptible) compared with the N cytoplasm isoline (resistant) of maize. Because membranes of the Tms cytoplasm isolines are sensitive to this toxin (29,30), increased electrolyte leakage may be attributed to increased membrane permeability. In the present study, high temperature stress before inoculation caused an increase in electrolyte leakage from the N cytoplasm isoline as well. Because membranes of this isoline are relatively insensitive to HmT-toxin, it may be assumed that increased electrolyte leakage in response to high temperature stress may also be attributed to altered membrane permeability.

The actual mechanism by which high temperature stress nonspecifically alters membrane permeability is not known. However, constituents of host or pathogen origin or their interaction, similar to the elicitors produced in the *Cladosporium fulvum*-tomato interaction (12), might be produced in infected heat-stressed tissues. If so, the possibility exists that polypeptide(s) or related products formed in maize in response to high temperature stress (11) might nonspecifically alter membrane permeability of either isoline, but by a mechanism similar to that seen for cyclic peptides produced by *Helminthosporium carbonum* (35).

LITERATURE CITED

1. Akhtar, M., and Garraway, M. O. 1987. Changes in maize peroxidase associated with variation in susceptibility to *Bipolaris maydis* race T. (Abstr.) Phytopathology 77:1739.
2. Bassey, E. O., and Garraway, M. O. 1984. Sporulation of *Bipolaris maydis* race T *in vitro* is increased by leachates from corn leaves exposed to high temperature stress prior to inoculation. (Abstr.) Phytopathology 74:1268.
3. Bell, A. A., and Presley, J. T. 1969. Temperature effects upon resistance and phytoalexin synthesis in cotton inoculated with *Verticillium albo-atrum*. Phytopathology 59:1141-1146.
4. Birecka, H., Catalfamo, J. L., and Garraway, M. O. 1975. Cell wall and protoplast isoperoxidases of corn leaves in relation to cut injury and infection with *Helminthosporium maydis*. Plant Physiol. 55:607-610.
5. Birecka, H., and Garraway, M. O. 1978. Corn leaf isoperoxidase reaction to mechanical injury and infection with *Helminthosporium maydis*. Effects of cycloheximide. Plant Physiol. 61:561-566.
6. Briggs, S. P., Scheffer, R. P., and Haug, A. R. 1984. Selective toxin of *Helminthosporium victoriae:* Thermal relationships in effects on oat tissues and protoplasts. Phytopathology 74:768-773.
7. Byther, R. S., and Steiner, G. W. 1975. Heat-induced resistance of sugarcane to *Helminthosporium sacchari* and helminthosporoside. Plant Physiol. 56:415-419.
8. Cadena-Gomez, G., and Nicholson, R. L. 1987. Papilla formation and associated peroxidase activity: A non-specific response to attempted fungal penetration of maize. Physiol. Mol. Plant Pathol. 31:51-67.
9. Chamberlain, D. W. 1972. Heat-induced susceptibility to non-pathogens and cross-protection against *Phytophthora megasperma* var. *sojae* in soybean. Phytopathology 62:645-646.
10. Chamberlain, D. W., and Gerdemann, J. W. 1966. Heat-induced susceptibility of soybeans to *Phytophthora megasperma* var. *sojae, Phytophthora cactorum,* and *Helminthosporium sativum*. Phytopathology 56:70-73.
11. Cooper, P., and Ho, T. D. 1983. Heat shock proteins in maize. Plant Physiol. 71:215-222.
12. De Wit, P. J. G. M., Buurlage, M. B., and Hammond, K. E. 1986. The occurrence of host, pathogen and interaction-specific proteins in the apoplast of *Cladosporium fulvum* (syn. *Fulvia fulva*) infected tomato leaves. Physiol. Plant Pathol. 29:159-172.
13. Ferraris, L., Gentile, I. A., and Matta, A. 1987. Variation of phenol concentrations as a consequence of stresses that induce resistance to *Fusarium* wilt of tomato. Z. Pflanzenkr. Pflanzensch. 94:624-629.
14. Gaff, D. F., and Okong'o-ogola, O. 1971. The use of non-permeating pigments for testing the survival of cells. J. Exp. Bot. 22:756-758.
15. Garraway, M. O. 1973. Electrolyte and peroxidase leakage as indicators of susceptibility of various maize inbreds to *Helminthosporium maydis* races O and T. Plant Dis. Rep. 57:518-522.
16. Garraway, M. O. 1973. Sporulation in *Helminthosporium maydis*. Inhibition by thiamine. Phytopathology 63:900-902.
17. Garraway, M. O., and Evans, R. C. 1977. Sporulation and peroxidase in *Bipolaris maydis:* Effects of xylose and thiamine. Can. J. Bot. 55:1996-2000.
18. Garraway, M. O., and Evans, R. C. 1984. Fungal Nutrition and Physiology. John Wiley & Sons, New York. 401 pp.
19. Gaspar, T., Penel, C., Thorpe, T., and Greppin, H. 1982. Peroxidases 1970-1980. A Survey of Their Biochemical and Physiological Roles in Higher Plants. University of Geneva Press, Geneva, Switzerland. 324 pp.
20. Gregory, P., Earle, E. D., and Gracen, V. E. 1980. Effects of purified *Helminthosporium maydis* race T toxin on the structure and function of corn mitochondria and protoplasts. Plant Physiol. 66:477-481.
21. Hadwiger, L. A., and Wagoner, W. 1983. Effect of heat shock on the mRNA-directed disease resistance response of peas. Plant Physiol. 72:553-556.
22. Halloin, J. M., Comstock, J. C., Martinson, C. A., and Tipton, C. L. 1973. Leakage from corn tissues induced by *Helminthosporium maydis* race T toxin. Phytopathology 63:640-642.
23. Hammerschmidt, R., Nuckles, E. M., and Kuć, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20:73-82.
24. Heath, M. C. 1979. Effects of heat shock, actinomycin D, cycloheximide and blasticidin S on nonhost interactions with rust fungi. Physiol. Plant Pathol. 15:211-218.
25. Heath, M. C. 1987. Evolution of plant resistance and susceptibility to fungal invaders. Can. J. Plant Pathol. 9:389-397.
26. Lin, C. Y., Chen, Y. M., and Key, J. L. 1985. Solute leakage in soybean seedlings under various heat shock regimes. Plant Cell Physiol. 26:1493-1498.
27. Lovrekovich, L., Lovrekovich, H., and Stahmann, M. A. 1968. The importance of peroxidase in the wildfire disease. Phytopathology 58:193-198.
28. Mellon, J. E., and Lee, S. L. 1985. Elicitation of cotton isoperoxidases by *Aspergillus flavus* and other fungi pathogenic to cotton. Physiol. Plant Pathol. 27:281-288.
29. Mertz, S. M., and Arntzen, C. J. 1977. Selective inhibition of K^+, Na^+, Cl^- and PO_4^{3-} uptake in *Zea mays* L. by *Bipolaris* (*Helminthosporium*) *maydis* race T pathotoxin. Evidence for a plasmalemma target site? Plant Physiol. 60:363-369.
30. Miller, R. J., and Koeppe, D. E. 1971. Southern corn leaf blight: Susceptible and resistant mitochondria. Science 173:67-69.
31. Nadolny, L., and Sequeira, L. 1980. Increases in peroxidase activities are not directly involved in induced resistance in tobacco. Physiol. Plant Pathol. 16:1-8.
32. Novacky, A. 1983. The effects of disease on the structure and activity of membranes. Pages 347-366 in: Biochemical Plant Pathology. J. A. Callow, ed. John Wiley & Sons, New York.
33. Rist, D. L., and Lorbeer, J. W. 1984. Ozone-enhanced leaching of

onion leaves in relation to lesion production by *Botrytis cinerea.* Phytopathology 74:1217-1220.
34. Sanden, G. E., and Moore, L. D. 1978. Effect of heat-induced susceptibility of tobacco to black shank on protein content and on activity of peroxidases. Phytopathology 68:1164-1167.
35. Scheffer, R. P., and Livingston, R. S. 1984. Host-selective toxins and their role in plant disease. Science 223:17-21.
36. Seevers, P. M., and Daly, J. M. 1970. Studies on wheat stem rust resistance controlled at the Sr6 locus. II. Peroxidase activities. Phytopathology 60:1642-1647.
37. Stermer, B. A., and Hammerschmidt, R. 1984. Heat shock induces resistance to *Cladosporium cucumerinum* and enhances peroxidase activity in cucumbers. Physiol. Plant Pathol. 25:239-249.
38. Stevens, C., and Gudauskas, R. T. 1982. Relation of maize dwarf mosaic virus infection to increased susceptibility of corn to *Helminthosporium maydis* race O. Phytopathology 72:1500-1502.
39. Thebud, R., and Santarius, K. A. 1982. Effects of high-temperature stress on various biomembranes of leaf cells *in situ* and *in vitro.* Plant Physiol. 70:200-205.
40. Urs, N. V. R., and Dunleavy, J. M. 1974. Function of peroxidase in resistance of soybean to bacterial pustule. Crop Sci. 14:740-744.
41. Wokoma, E. C. W. 1986. The effects of high temperature stress and nutrient release from maize (*Zea mays* L.) leaves on susceptibility to *Bipolaris maydis* (Nisikado) Shoemaker. Ph.D. thesis. The Ohio State University, Columbus. 236 pp.
42. Yarwood, C. E. 1956. HTS-induced susceptibility of beans to some viruses and fungi. Phytopathology 46:523-525.