European Journal of Plant Pathology **105:** 825–829, 1999.

Short communication

Endogenous ethane and ethylene of *Poa pratensis* leaf blades and leaf chlorosis in response to biologically active products of *Bipolaris sorokiniana*[*]

Clinton F. Hodges[1] and Douglas A. Campbell
Department of Horticulture, Iowa State University, Ames, IA 50011, USA (Fax: +1-515-294-0730; E-mail: hodges@iastate.edu)

Accepted 9 August 1999

Key words: Drechslera, Helminthosporium sativum, Kentucky bluegrass, pathogenesis, phytotoxin

Abstract

Infection of *Poa pratensis* leaf blades and callus tissue by *Bipolaris sorokiniana* increases the production of ethylene and ethane. The ethylene is responsible for most of the chlorosis that occurs during pathogenesis. The nonselective toxin(s) produced by *B. sorokiniana* is known to disrupt membranes and to damage chlorophyll, but it is not known whether it can induce an increase in ethylene or ethane. Research was initiated to determine the effect of a biologically-active extract of *B. sorokiniana* on the endogenous ethylene and ethane of intact *P. pratensis* leaf blades and on subsequent development of chlorosis. The extract did not increase endogenous ethylene of treated leaves, but it was associated with an increase in endogenous ethane between 24 and 96 h after treatment. Chlorophyll loss occurred 96 h after treatment and persisted for the duration of the study (168 h). The chlorophyll content of treated leaf blades ranged from 72% to 80% of control leaf blades. The observations suggest that the extract of *B. sorokiniana* can induce chlorophyll loss from treated leaf blades independent of an increase in endogenous ethylene by directly damaging chloroplasts with a concurrent release of ethane. The ethane is believed to be a by-product of pathogenesis.

Results and discussion

The increase in endogenous ethylene in leaves of *Poa pratensis* L. infected by *Bipolaris sorokiniana* (Sacc.) Shoemaker is the primary cause of chlorosis during pathogenesis (Hodges, 1990; Hodges and Coleman, 1984). Inoculation of *P. pratensis* callus tissue with *B. sorokiniana* also induces ethylene evolution and a substantial release of ethane (Hodges et al., 1999). Ethane is associated with cell decompartmentalization and membrane disruption (Dumelin and Tappel, 1977; John and Curtis, 1977; Elstner and Konze, 1976). Altered cell permeability during pathogenesis has long been recognized (Thatcher, 1939, 1942), but a concurrent release of ethane has not been reported.

A biologically active extract of *B. sorokiniana* has been shown to induce chlorosis of *P. pratensis* leaf blades (Hodges and Campbell, 1996). *Bipolaris sorokiniana* produces a number of nonselective toxic sesquiterpenes that damage membranes (Liljeroth et al., 1994; Carlson et al., 1991; White and Taniguchi, 1972) and induce chlorosis (Pena-Rodriguez et al., 1988; Harbone, 1983; Ludwig, 1957). Also, some fungal and bacterial toxins are known to induce ethylene synthesis during pathogenesis (Moussatos et al., 1994; Kenyon and Turner, 1992; Ferguson and Mitchell, 1985), but it is not known if the extract of *B. sorokiniana* can similarly induce ethylene evolution. In that the toxic products of *B. sorokiniana* damage membranes, the potential also exists for ethane evolution during pathogenesis. The research presented was initiated to determine the ability of a biologically-active extract of *B. sorokiniana* to induce ethylene and ethane

[*]Journal Paper No. J-18104 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50011. Project 3193.

evolution, and chlorophyll loss from intact leaf blades of *P. pratensis*.

Plants and intact leaf blades of *P. pratensis* were grown and prepared for treatment, and the biologically active extract of *B. sorokiniana* was produced (Hodges and Campbell, 1996). Nondiluted, 10^{-1} and 10^{-2} dilutions of the extract were deposited on the surface of water agar (3%) for treatment of leaf blades. Four agar cylinders (4 mm) were placed about 1 cm apart on each leaf blade; the surface of the cylinder with the extract was placed against the surface of each leaf, and 25 µl of sterile distilled water was placed at the base of the agar cylinder to form an aqueous continuum between the agar cylinders and the leaf-blade surface. Ten-centimeter lengths of the four treated leaf blades of individual plants were collected and combined at 24, 48, 72, 96, 120, 144, and 168 h and analyzed for endogenous ethylene and ethane, and for chlorophyll content (Hodges et al., 1999; Hodges and Campbell, 1996). The study was conducted in a randomized complete block design consisting of five replicate plants for each extract dilution and their respective controls. An ANOVA and LSD ($P_{0.05}$) were calculated between and among the various extract dilution treatments for endogenous ethane and ethylene, and for chlorophyll content at each 24 h sampling period through 168 h.

Endogenous ethylene production by control and treated leaf blades did not differ within the 24, 48, and 72 h sampling periods in response to the extract, however, ethylene decreased in response to the 10^{-1} and 10^{-2} dilutions of the extract at 96, 120, 144 and 168 h (Figure 1). Endogenous ethylene production by control leaf blades and leaf blades treated with the nondiluted extract did not differ between the 24 h sampling periods for the 168 h duration of the study (Figure 1). Ethylene concentrations in response to the 10^{-1} and 10^{-2} dilutions did not differ between the 24, 48, and 72 h sampling periods, but the concentrations associated with each dilution at 96, 120, 144, and 168 h decreased relative to the 72 h values.

Endogenous ethane production by leaf blades treated with the nondiluted extract was greater than that of the controls within the 24, 48, 72, and 96 h sampling periods, but did not differ from controls within the 120, 144, and 168 h sampling periods (Figure 2). The 10^{-1} and 10^{-2} dilutions of the extract had no effect on the endogenous ethane production by the leaf blades within the 24 h sampling periods. Ethane values increased in response to the nondiluted extract between the 24, 48, 72, and 96 h sampling periods and then decreased at

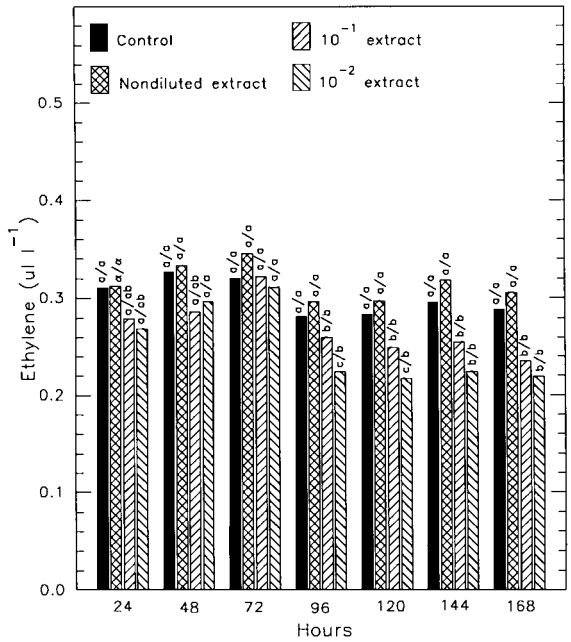

Figure 1. Endogenous ethylene of intact leaf blades of *P. pratensis* in response to the extract from *Bipolaris sorokiniana*. Ethylene concentrations were determined at 24 h intervals for a period of 168 h. Within the 24 h sampling periods, values followed by the same letter (a /) were not significantly different ($P_{0.05}$). Between the 24 h sampling periods, values for the control and individual dilutions followed by the same letter (/a) were not significantly different ($P_{0.05}$).

120, 144, and 168 h. Ethane values between the 24 h sampling periods did not differ between 24, 48, 72, 96, and 120 h in response to the 10^{-1} dilution, but at 144 and 168 h ethane was less than that at 24 h. Ethane values did not differ between the 24, 48, and 72 h sampling periods in response to the 10^{-2} dilution, but the values at 96, 120, 144, and 168 h were lower than that at 24 h.

Mean chlorophyll content of control leaf blades at each 24 h sampling period (24–168 h) was 12.8, 13.4, 12.7, 12.9, 13.0, 13.0, and 14.4 µg mg^{-1} dry tissue weight, respectively. The chlorophyll content of leaf blades treated with all dilutions of the extract did not differ from controls within the 24, 48, and 72 h sampling periods (Figure 3). Within the 96, 120, 144, and 168 h sampling periods, chlorophyll content of leaf blades treated with the nondiluted extract decreased relative to controls; leaf blades treated with the 10^{-1} and 10^{-2} dilutions did not differ from the controls. The chlorophyll content of leaf blades treated with the nondiluted extract did not differ between the 24, 48, 72,

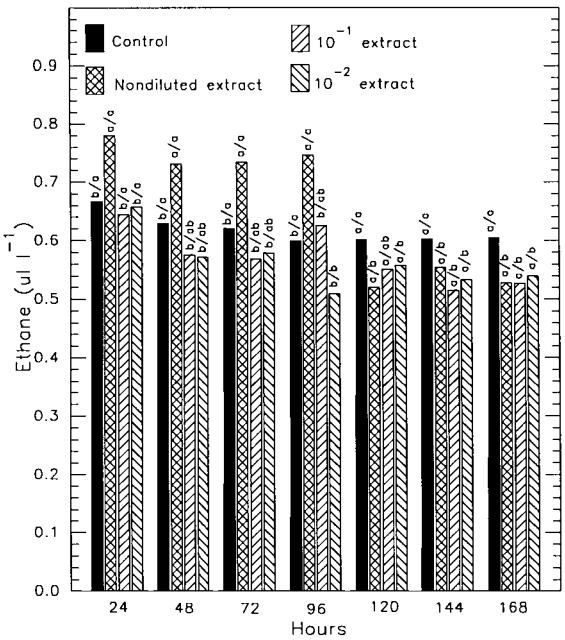

Figure 2. Endogenous ethane of intact leaf blades of *P. pratensis* in response to the extract from *B. sorokiniana*. Ethane concentrations were determined at 24 h intervals for a period of 168 h. Within the 24 h sampling periods, values followed by the same letter (a /) were not significantly different ($P_{0.05}$). Between the 24 h sampling periods, values for the control and individual dilutions followed by the same letter (/a) were not significantly different ($P_{0.05}$).

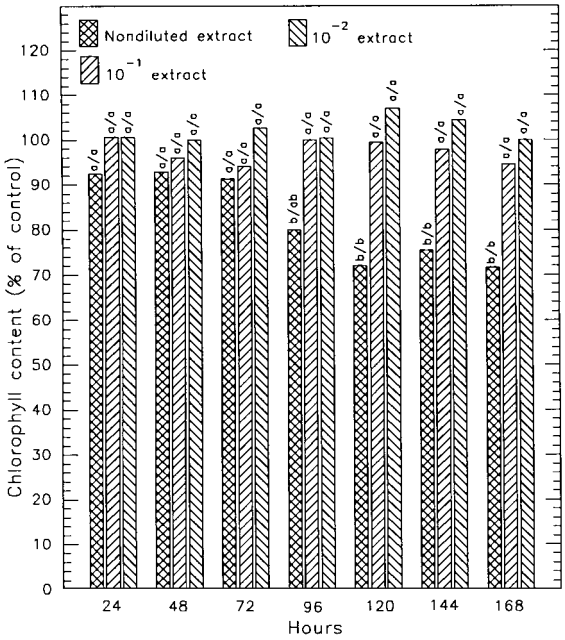

Figure 3. Chlorophyll content of intact leaf blades of *P. pratensis* in response to the extract from *B. sorokiniana*. Chlorophyll content was determined at 24 h intervals for a period of 168 h. Within the 24 h sampling periods, values followed by the same letter (a /) were not significantly different ($P_{0.05}$). Between the 24 h sampling periods, values for the control and individual dilutions followed by the same letter (/a) were not significantly different ($P_{0.05}$).

and 96 h; however, at 120, 144, and 168 h the chlorophyll content decreased (Figure 3). The 10^{-1} and 10^{-2} dilutions of the extract did not affect the leaf blade chlorophyll between the 24 h sampling periods.

The results of this study support three general conclusions: (1) the biologically active extract of *B. sorokiniana* does not increase endogenous ethylene in leaf blades of *P. pratensis*; (2) the extract is associated with an increase in endogenous ethane; and (3) the loss of chlorophyll from leaf blades associated with the extract is not due to ethylene. In the present study, the nondiluted extract induced a 20–28% loss of chlorophyll between 120 and 168 h (Figure 3), but it did not increase the endogenous ethylene. This response shows that the nondiluted extract did not increase ethylene and that it is capable of inducing chlorosis independent of a surge in ethylene. The cause of the decrease in ethylene level in response to the 10^{-1} and 10^{-2} dilutions of the extract between 96 and 168 h is unknown. The response is suggestive of an inhibition of ethylene synthesis associated with dilution of the extract, but the data presented and that of other research do not provide an explanation for the response.

Ethane is the product of α-linolenic acid oxidation catalyzed by lipoxygenases (Bressan et al., 1979; Konze and Elstner, 1978) resulting from membrane disruption and cell death (Cassells and Tamma, 1986; Dumelin and Tappel, 1977; Elstner and Konze, 1976). The presence of lipoxygenase and lipid peroxidation during pathogenesis has been reported in several host–pathogen interactions (Croft et al., 1993; Gönner and Schlösser, 1993; Lupu et al., 1980). The nonselective phytotoxin produced by *B. sorokiniana* is capable of membrane disruption, inhibition of mitochondrial electron transport and oxidative phosphorylation, general degeneration of nuclei and mitochondria, and interference with electron transport (Åkesson et al., 1996; Olbe et al., 1995; Liljeroth et al., 1994; Fadeev et al., 1987; White and Taniguchi, 1972; Taniguchi and White, 1967); more important, it can induce chlorosis (Pena-Rodriguez et al., 1988; Harbone, 1983; Ludwig, 1957).

It is probable that the *B. sorokiniana* extract contains components that directly damage chloroplasts with a subsequent release of ethane.

The observations of this study suggest that chlorosis of *P. pratensis* leaf blades infected by *B. sorokiniana* is the result of at least two different pathways. The greater portion of the chlorosis is associated with a surge in endogenous ethylene in response to *B. sorokiniana* infection that can be controlled by blocking the biosynthesis and/or mode of action of the ethylene (Hodges and Campbell, 1993, 1994; Hodges, 1990; Hodges and Coleman, 1984). A smaller portion of the chlorosis seems associated with membrane attacking components of the extract that directly damage chloroplasts with a release of ethane. Although an increase in endogenous ethylene does not seem necessary for chlorosis induced by the extract, the increase in ethylene resulting from natural infection by *B. sorokiniana* might enhance membrane damage by the extract (Pegg, 1981). The evolution of ethane in response to the extract is believed to be a byproduct of membrane damage as opposed to being an active participant in pathogenesis or subsequent chlorosis.

References

Åkesson H, Carlemalm E, Everitt E, Gunnarsson T, Odham G and Jansson H-B (1996) Immunocytochemical localization of phytotoxins in *Bipolaris sorokiniana*. Fungal Genetics and Biology 20: 205–216

Bressan RA, LeCureux L, Wilson LG and Filner P (1979) Emission of ethylene and ethane by leaf tissue exposed to injurious concentrations of sulfur dioxide or bisulfite ion. Plant Physiology 63: 924–930

Carlson H, Nilsson P, Jansson H-B and Odham G (1991) Characterization and determination of prehelminthosporal, a toxin from the plant pathogenic fungus *Bipolaris sorokiniana*, using liquid chromatography/mass spectrometry. Journal of Microbiological Methods 13: 259–269

Cassells AC and Tamma L (1986) Ethylene and ethane release during tobacco protoplast isolation and protoplast survival potential *in vitro*. Physiologia Plantarum 66: 303–308

Croft KPC, Jüttner F and Slusarenko AJ (1993) Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv *phaseolicola*. Plant Physiology 101: 13–24

Dumelin EE and Tappel AL (1977) Hydrocarbon gases produced during *in vitro* peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxidases. Lipids 12: 894–900

Elstner EF and Konze JR (1976) Effect of point freezing on ethylene and ethane production by sugar beet leaf disks. Nature 263: 351–352

Fadeev YN, Adeishvili TS, Simonyan GG, Koshkin EA, Tarabrin GA and Tishenkov AD (1987) Effect of *Bipolaris sorokiniana* Sch. fungus toxins on functioning of the electron transport chain of wheat chloroplasts. Soviet Agricultural Sciences 8: 9–13

Ferguson IB and Mitchell RE (1985) Stimulation of ethylene production in bean leaf discs by the pseudomonad phytotoxin coronatine. Plant Physiology 77: 969–973

Gönner von M, and Schlösser E (1993) Oxidative stress in interactions between *Avena sativa* L. and *Drechslera* spp. Physiological and Molecular Plant Pathology 42: 221–234

Harbone JB (1983) Toxins of plant fungal interactions. In: Keeler RF and Tu AT (eds) Handbook of Natural Toxins, Vol 1, Plant and Fungal Toxins (pp 743–782) Marcel Dekker, New York

Hodges CF (1990) Endogenous ethylene response and chlorophyll loss in sequentially older leaves of *Poa pratensis* infected by *Bipolaris sorokiniana*. Journal of Plant Physiology 136: 670–674

Hodges CF and Campbell DA (1993) Inhibition of ethylene biosynthesis and the endogenous ethylene and chlorophyll content of *Poa pratensis* leaves infected by *Bipolaris sorokiniana*. Journal of Plant Physiology 142: 699–703

Hodges CF and Campbell DA (1994) Inhibition of endogenous ethylene biosynthesis by L-canaline in leaves of *Poa pratensis* infected by *Bipolaris sorokiniana*. Physiological and Molecular Plant Pathology 44: 301–309

Hodges CF and Campbell DA (1996) Chlorosis of sequentially older leaves of *Poa pratensis* induced by biologically active products of *Bipolaris sorokiniana*. Journal of Phytopathology 144: 565–569

Hodges CF and Coleman LW (1984) Ethylene-induced chlorosis in the pathogenesis of *Bipolaris sorokiniana* leaf spot of *Poa pratensis*. Plant Physiology 75: 462–465

Hodges CF, Stephens LC and Campbell DA (1999) Ethylene and ethane from *Poa pratensis* callus and from leaf blades of regenerated and seed-derived plants inoculated with *Bipolaris sorokiniana*. Journal of Plant Physiology 154: 113–118

John WW and Curtis RW (1977) Isolation and identification of the precursor of ethane in *Phaseolus vulgaris* L. Plant Physiology 59: 521–522

Kenyon JS and Turner JG (1992) The stimulation of ethylene synthesis in *Nicotiana tabacum* leaves by the phytotoxin coronatine. Plant Physiology 100: 219–224

Konze JR and Elstner EF (1978) Ethane and ethylene formation by mitochondria as indication of aerobic lipid degradation in response to wounding of plant tissue. Biochimica et Biophysica Acta 528: 213–221

Liljeroth E, Franzon-Almgren I and Gustafsson M (1994) Effect of prehelminthosporal, a phytotoxin produced by *Bipolaris sorokiniana*, on barley roots. Canadian Journal of Botany 72: 558–563

Ludwig RA (1957) Toxin production by *Helminthosporium sativum* P.K. & B. and its significance in disease development. Canadian Journal of Botany 35: 291–303

Lupu R, Grossman S and Cohen Y (1980) The involvement of lipoxygenase and antioxidants in pathogenesis of powdery mildew on tobacco plants. Physiological. Plant Pathology 16: 241–248

Moussatos VV, Yang SF, Ward B and Gilchrist DG (1994) AAL-toxin induced physiological changes in *Lycopersican esculentum* Mill: roles for ethylene and pyrimidine intermediates in necrosis. Physiological and Molecular Plant Pathology 44: 455–468

Olbe M, Sommarin M, Gustafsson M and Lundborg T (1995) Effect of the fungal pathogen *Bipolaris sorokiniana* toxin prehelminthosporal on barley root plasma membrane vesicles. Plant Pathology 44: 625–635

Pegg GF (1981) The role of nonspecific toxins and hormone changes in disease severity. In: Staples RC and Toenniessen GH (eds) Plant Disease Control (pp 13–31) John Wiley & Sons, New York

Pena-Rodriguez LM, Armingeon NA and Chilton WS (1988) Toxins from weed pathogens, I. Phytotoxins from a *Bipolaris* pathogen of Johnson grass. Journal of Natural Products 51: 821–828

Thatcher FS (1939) Osmotic and permeability relations in the nutrition of fungus parasites. American Journal of Botany 26: 449–458

Thatcher FS (1942) Further studies of osmotic and permeability relations in parasitism. Canadian Journal of Research, Section C. 20: 283–311

White GA and Taniguchi E (1972) The mode of action of Helminthosporal. II. Effect on the permeability of plant cell membranes. Canadian Journal of Botany 50: 1415–1420

Taniguchi E and White GA (1967) Site of action of the phytotoxin, helminthosporal. Biochemical and Biophyical Research Communications 28: 879–885