Production of Ethylene by Oats Resistant and Susceptible to Victorin

88

Louis Shain and Harry Wheeler

Assistant Professor and Professor, respectively, Department of Plant Pathology, University of Kentucky, Lexington, 40506. Journal Series Paper No. 74-11-102, Kentucky Agricultural Experiment Station.

The authors gratefully acknowledge the technical assistance of Ellen Elbel.

ABSTRACT

Ethylene production by detached first leaves of oats susceptible (Vg 48-93) and resistant (C. I. 7418) to victorin, the pathotoxin produced by Helminthosporium victoriae, was determined 2 hours after a 4-hour uptake period in aqueous dilutions of victorin. Susceptible tissues were treated with victorin solutions diluted 10⁴- to 10⁸-fold; resistant ones with solutions diluted 20- to 10³-fold. The lowest concentration of victorin which caused a significant increase in ethylene evolution was a dilution of 10⁷ for susceptible, and 10² for resistant, tissues. At these concentrations, ethylene production was ca. 25 nl/gdry weight per hour for both susceptible and resistant tissues. With susceptible tissues, ethylene production increased with increased concentrations of victorin until saturation was reached at ca. 550 nl/g dry weight per hour with victorin diluted 105-fold. In contrast, resistant leaves produced ca. 70 nl/g dry weight per hour with the highest concentration used (victorin diluted 20fold). The victorin used inhibited root growth by 50% when diluted 5×10^7 -fold and 20-fold for susceptible and resistant plants, respectively. These results indicate that increased ethylene production, a common physiological response of plants to infection, may provide a sensitive and rapid method for assaying toxicants such as victorin.

Phytopathology 65:88-89

Ethylene production is a general host response to injury or infection. Early increases in production of this gas have been associated with resistance in some diseases (6, 8, 10) and with susceptibility in others (2). Ethylene production by host and pathogen are sometimes difficult to separate because this gas is produced by some pathogenic fungi (5) and bacteria (4).

Progeny of Victoria oats are highly susceptible to *Helminthosporium victoriae* Meehan & Murphy and to its pathotoxin, victorin. The victorin-oats model therefore has the advantage of eliminating any contribution of ethylene produced by the causal fungus during pathogenesis.

The objectives of this study were to gain information on (i) ethylene production by resistant and susceptible oats in response to victorin, and (ii) the feasibility of using ethylene production as a bioassay for victorin. A preliminary report on these studies was presented earlier (9).

MATERIALS AND METHODS.—Oat cultivars resistant (C. 1. 7418) and susceptible (Vg 48-93) to victorin and *H. victoriae* were grown under controlled conditions as described previously (12). Three first leaves of 8- to 13-day-old plants were detached and their cut ends placed in flasks containing aqueous dilutions of victorin. The range of victorin dilutions tested was from 10⁴-10⁸ for susceptible tissues, and from 20-10³ for resistant tissues.

Results with leaves which had taken up distilled H_2O were not significantly different from those obtained with solutions of detoxified victorin which served as additional controls. The victorin used in these studies inhibited root growth by 50% when diluted 5×10^7 -fold and 20-fold for susceptible and resistant plants, respectively.

After a 4-hour uptake period in the above-mentioned solutions, leaves were placed into containers which then were sealed with septa. Ethylene production was measured 2 hours later by withdrawing a 2.0-ml sample from each container and injecting this into a gas chromatograph (Varian 2100) as described elsewhere (8). At least six determinations were made for each dilution of victorin tested. The identity of ethylene was based upon its retention time, its adsorbance by mercuric perchlorate, and its nonadsorbance by potassium hydroxide (1).

RESULTS AND DISCUSSION.—Ethylene production by susceptible tissues increased with increasing concentration of victorin until saturation was reached at ca. 550 nl/g dry weight per hour after treatment with victorin diluted 10⁵-fold (Fig. 1). Saturation, however, was not reached in tests with resistant tissues which produced only ca. 70 nl/g dry weight per hour after treatment with the highest concentration of victorin tested (a 20-fold dilution).

A series of *t*-tests revealed that the lowest concentration of victorin which caused a significant increase (P=0.01) in ethylene production over controls was a dilution of 10^7 for susceptible and 10^2 for resistant tissues. Expressed on the basis of dry weight of total solids in the victorin preparation, the threshold for a significant effect was $0.0002~\mu g/ml$ for susceptible and $20.0~\mu g/ml$ for resistant tissues. These results are in keeping with others which also indicate that responses of resistant and susceptible oats to

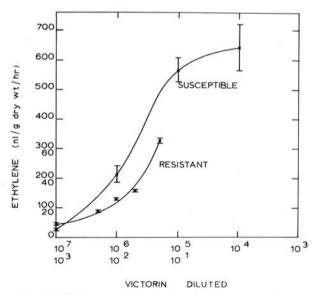


Fig. 1. Ethylene production by detached first leaves of oats resistant (C. I. 7418) and susceptible (Vg 48-93) to victorin. The upper figures on both axes refer to the curve for susceptible tissues; the lower figures to those for resistant tissues. Each point represents the mean of at least six determinations. Horizontal bars denote \pm the standard error of the mean.

victorin are similar qualitatively, but differ substantially in magnitude (12).

Our results further suggest that ethylene production may provide a sensitive and rapid means of bioassaying toxicants such as victorin. Ethylene evolution is at least as sensitive a test for victorin activity as root growth inhibition or electrolyte loss which have been used as bioassays in the past (3, 7, 11).

LITERATURE CITED

- BURG, S. P. 1962. The physiology of ethylene formation. Annu. Rev. Plant Physiol. 13:265-302.
- DALY, J. M., P. LUDDEN, and P. SEEVERS. 1971. Biochemical comparisons of resistance to wheat stem rust disease controlled by the Sr6 or Sr11 alleles. Physiol. Plant Pathol. 1:397-407.
- DAMANN, K. E., JR., J. M. GARDNER, and R. P. SCHEFFER. 1974. An assay for Helminthosporium victoriae toxin based on induced leakage of electrolytes from oat tissue. Phytopathology 64:652-654.
- FREEBAIRN, H. T., and I. W. BUDDENHAGEN. 1964. Ethylene production by Pseudomonas solanacearum. Nature 202:313-314.

- ILAG, L. and R. W. CURTIS. 1968. Production of ethylene by fungi. Science 159:1357-1358.
- JAWORSKI, J. G., J. KUĆ, and E. B. WILLIAMS. 1973.
 Effect of ethrel and Ceratocystis fimbriata on the accumulation of chlorogenic acid and 6-methoxy mellein in carrot root. Phytopathology 63:408-413.
- LUKE, H. H., and H. E. WHEELER. 1955. Toxin production by Helminthosporium victoriae. Phytopathology 45:453-458.
- SHAIN, L., and W. E. HILLIS. 1972. Ethylene production in Pinus radiata in response to Sirex-Amylostereum attack. Phytopathology 62:1407-1409.
- SHAIN, L., and H. WHEELER. 1975. Victorin-induced production of ethylene. Annu. Proc. Am. Phytopathol. Soc. for 1974. 1: (In press).
- STAHMANN, M. A., B. G. CLARE, and W. WOODBURY. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by black rot infected sweet potato tissue. Plant Physiol. 41:1505-1512.
- WHEELER, H., and H. S. BLACK. 1963. Effects of Helminthosporium victoriae and victorin uponpermeability. Am. J. Bot. 50:686-693.
- WHEELER, H., and B. DOUPNIK, JR. 1969. Physiological changes in victorin-treated, resistant oat tissues. Phytopathology 59:1460-1463.