Identification of the Germination Self-inhibitor from Uredospores of Puccinia striiformis

V. Macko, E. J. Trione, and S. A. Young

First author, Boyce Thompson Institute, Yonkers, NY 10701; second author, Agricultural Research Service, USDA; and second and third authors, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

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ABSTRACT

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The endogenous germination inhibitor from uredospores of the stripe rust fungus, *Puccinia striiformis*, was identified as methyl *cis*-3,4-dimethoxycinnamate. The inhibitor was extracted from field-collected spores with water, partitioned into ether, and purified by thin-layer and gas chromatography. The inhibitor activity was monitored with a germination assay employing fresh, water-washed, stripe rust uredospores. The chemical structure was confirmed by chromatography in several systems and by mass spectrometry. The ED_{50} of the *cis*-form of the inhibitor was 4 ng/ml, whereas the *trans*-isomer had little or no activity as a germination inhibitor.

Additional key words: wheat.

Stripe rust, which is caused by *Puccinia striiformis* West., is a serious disease of wheat in many parts of the world. In the United States it is common in the Pacific intermountain areas and occasionally causes severe losses; e.g., on wheat cultivar Pitic 62 in Northern California in 1974 and on Yamhill wheat in Western Washington in 1975.

An important aspect in understanding the development of *Puccinia striiformis* is the characterization of regulatory mechanisms of dormancy and activation processes of uredospore germination (3). The presence of an endogenous germination inhibitor has been reported in these spores, but the active principle was not identified (9). Recent studies have emphasized the specific roles of germination inhibitors in control of fungal spore germination (1, 6). With a view toward defining these control mechanisms in *P. striiformis*, we have studied the chemical nature and biological activity of the self-inhibitor from uredospores of this fungus.

MATERIALS AND METHODS

About 180 g of fresh uredospores were collected from stripe rust lesions on field-infected wheat in June 1975 by a vacuum-trap method. The spores were stored in liquid N_2 for 2 mo. In a preliminary experiment, the germination inhibitor was extracted from the uredospores by stirring them in water (8 g/100 ml) at 22 C. After the spores were removed by filtration, they were extracted with water two more times. The filtrates were pooled and extracted three times with an equal volume of diethyl ether. The crude ether extract was evaporated to near dryness with a rotary vacuum evaporator at 22 C. The residue was taken up in a small volume of diethyl ether (1 mg equivalent of spores per 2 μliters ether), spotted on silica gel thin-layer chromatography plates, and developed in benzene:ether (8:2, v/v). The silica gel plate was air-dried and then divided into 10 equal zones that were scraped off separately, eluted with methanol, and tested for inhibitory activity.

For the bioassay, a measured volume of the solution to be tested was placed in a small test tube. The solvent was evaporated with the aid of an air stream at room temperature. Water (1 ml) was added to each tube and stirred vigorously. Aliquots (0.5 ml) of this aqueous extract were placed into each of two small vials and about 500 stripe rust uredospores were floated on the surface of the solution in each vial. The uredospores previously had been stirred in double-distilled water for 15 min before they were transferred to the bioassay vials. The spores were incubated in the dark on the test solutions at 10 C for 16 hr before the number of germinating spores was determined microscopically. Experimental data were collected only if the germination of the control spores was greater than 90%.

Analytical techniques used, including gas chromatography, thin-layer chromatography, and mass

spectrometry, were as described in detail previously (5).

RESULTS

In the preliminary extraction experiment, the zone containing inhibitor coincided with a UV fluorescent band at R_f 0.6 on the silica gel plates. The crude ether extract from 4 mg of spores inhibited germination by 50% in this assay. Sixty g of spores were then extracted as described above, except that the concentrated crude ether extract was dried and sublimed in a short-path sublimation apparatus with the condenser set at −75 C. The sublimate then was chromatographed on silica gel plates, as described above. A zone 1.5 cm wide, with an R_f of 0.6 at the center, was scraped from the plates, extracted with ether, and the concentrated ether extract separated by gas chromatography (GLC) on 1% FFAP (free fatty acid phase). A GLC peak observed at 3.2 min was identical to the retention time of synthetic methyl *cis*-3,4-dimethoxycinnamate. The *trans*-isomer of this compound was not detected. The effluent from the peak at 3.2 min in the gas chromatograph was collected, analyzed in a mass spectrometer, and found to be identical to synthetic methyl *cis*-3,4-dimethoxycinnamate (5).

This compound has been shown previously to be the major self-inhibitor in five other species of rust fungi (6). This inhibitor was synthesized (5), and the resulting mixture of the *cis*- and *trans*-isomers was separated by high-pressure liquid chromatography (microporasil column, Waters Assoc., eluted with hexane:acetone, 92:8, v/v). The isomers were bioassayed separately with fresh stripe rust spores. The ED_{50} of the *cis*-isomer was 4 ng/ml, whereas the *trans*-isomer had little or no activity as a germination inhibitor at concentrations up to 3,000 ng/ml.

DISCUSSION

The present studies, together with those reported previously (cf. 6), show that uredospores in the family Pucciniaceae contain cinnamate esters as germination self-inhibitors. The stripe rust spores are not so sensitive to their natural inhibitor as some of the other rust species (1, 5, 6). There are several reports in the literature (8, 12) which suggest that self-stimulators of germination also may be present in stripe rust spores. For example, when spores were crowded together on an agar surface, they germinated better than when they were widely dispersed. Schröder and Hassebrauk (9), however, demonstrated water-soluble germination inhibitors in stripe rust uredospores. We have not made extensive population density studies on these spores, but have noted that the germination patterns of the spores are extremely variable, and both self-inhibition and stimulation are obtained in different experiments. We collected experimental data only when the germination of the control spores was in the 90-100% range. The great variation that several authors have noted in the germination characteristics of stripe rust uredospores may be related to the age and maturity of the spores, environmental and host conditions during spore formation, concentration and type of air ions, and the ratio and levels of endogenous stimulators and inhibitors of germination (3, 9, 10, 11).

The information now developing on self-inhibitors is being used in experimental analysis of the germination process; however, it may also aid in studies of rust pathogenesis. For example, in susceptible plants the cinnamate inhibitor was detected early after inoculation and, as the time of sporulation approached, its concentration greatly increased (2, 6). In contrast, in resistant plants, in the absence of sporulation, only trace amounts of the inhibitor were present (2). In another system it has been shown that peroxidase activity increased in resistant plants (7), and the cinnamate inhibitor may very well be a natural substrate (hydrogen donor) for this enzyme (4). Thus, studies should be directed toward the following possibilities: the importance of cinnamates in sporulation and the interaction of peroxidases with their natural substrates during pathogenesis.

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