Effect of the Systemic Fungicide 4-n-butyl-1,2,4-triazole on the Development of Puccinia recondita f. sp. tritici in Wheat

J. E. Watkins, L. J. Littlefield, and G. D. Statler

Former Graduate Student (present address: Assistant Professor, Department of Plant Pathology, University of Nebraska, Lincoln, NB 68583); and Professor and Associate Professor, respectively, of Plant Pathology, North Dakota State University, Fargo, ND 58102.

Journal paper number 734, North Dakota Agricultural Experiment Station, Fargo.
Accepted for publication 4 March 1977.

ABSTRACT

WATKINS, J. E., L. J. LITTLEFIELD, and G. D. STATLER. 1977. Effect of the systemic fungicide 4-*n*-butyl-1,2,4-triazole on the development of Puccinia recondita f. sp. tritici in wheat. Phytopathology 67: 985-989.

The systemic fungicide 4-*n*-butyl-1,2,4-triazole (Indar; RH-124) applied as a seed treatment on wheat was taken up by the seeds and inhibited development of leaf rust caused by *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* Eriks. Treated seedlings of the near-isogenic wheat line *Lr*2 exhibited no macroscopic evidence of leaf rust 10 days after inoculation. Urediospore germination and subsequent stomatal penetration were not affected by the seed treatment. Plants from treated seed had fewer and shorter infection hyphae and fewer haustoria per infection site by 12 hr after inoculation than did the controls, but the differences were not significant until 36 hr after inoculation. The antifungal activity of Indar also was exhibited by swollen, distorted hyphae in leaves of treated plants. Beyond 36 hr, fungal development was almost completely inhibited. Indar appeared to restrict continued development of existing infection structures as well as the formation of new structures. The inhibitory effect of the test compound was not accompanied by necrosis in either the host or the pathogen, suggesting that the mechanism of this control differs from the hypersensitive form of rust resistance.

Additional key words: chemical control.

The compound, 4-*n*-butyl-1,2,4 -triazole (Indar; RH-124) is a systemic fungicide selective for the control of wheat leaf rust which is caused by *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* Eriks. (8, 11). In foliar, soil, and seed applications Indar was superior to benomyl, triamol, or oxycarboxin against leaf rust, although the duration of effectiveness within the wheat plant was relatively short (8). Field tests showed that seed treatment with Indar had a high potential for control of leaf rust on spring wheat (8). As a foliar spray or as a seed treatment, the chemical is rapidly absorbed and translocated to younger tissues (3, 12). Foliar sprays of Indar were not effective against bean rust, oat crown rust, and wheat stem rust (12). This narrow spectrum of control makes Indar a valuable research tool as well as an effective fungicide.

The mode of action of oxycarboxin, benomyl, and other systemic fungicides has been documented ultrastructurally and biochemically (2, 3, 4, 5, 6, 7, 10, 11). This study reports the histological effects of the systemic fungicide, Indar, on the development of the wheat leaf rust fungus. This information is contrasted with that from another study of the hypersensitive form of resistance (13, 14).

MATERIALS AND METHODS

Seed of the near-isogenic line *Lr*2 (1) of wheat *(Triticum aestivum* L.) were treated with 113.4 g active ingredient of a 25% wettable powder formulation of Indar (Rohm and Haas Company, Philadelphia, PA 19105) per 45.4 kg (4 oz/100 lb) of seed 1 day prior to planting. Test seedlings were grown in an autoclaved mixture of peat, sand, and clay soil in 12.7-cm-diameter clay pots. Three, replicate, randomized pots with approximately 50 seedlings each were used per treatment. Plants were kept in a growth chamber at 20-22 C with a 16-hr photoperiod (10,000 lux) prior to inoculation. Ten days after planting, test seedlings were inoculated by dusting with a 5% (w/w) mixture of urediospores of *P. recondita tritici* (isolate 71-112) in talc. The urediospores had been sealed in glass vials and stored in liquid nitrogen. Shortly before inoculation, the vials were removed from the liquid nitrogen and the urediospores were activated by heat shock in 44 C water for 1.5 min. The seedlings were inoculated in dew chambers maintained at 21 C, kept in those chambers for a 24-hr photoperiod (1,400 lux), and then returned to the growth chamber where they were kept for the duration of the experiment. The host-parasite combination used for this study gave a compatible infection type on the nontreated test seedlings.

Development of the fungus within leaf tissue was examined on treated and nontreated seedlings using a

whole-leaf clearing and staining method (9). Inoculated primary leaves were cut into two sections 3 cm in length and stored in alcoholic lactophenol-cotton blue (1 part lactophenol cotton blue to 2 parts 95% ethanol). Specimens were observed using a Leitz Dialux light microscope. Collection of data began 12 hr after inoculation. Only isolated urediospores, appressoria, or infection sites were examined.

Observations of urediospore germination and substomatal vesicle formation were made on 20 random urediospores and 10 random appressoria, respectively, on each of five leaves. Comparisons of the number of haustoria were made on 10 random infection sites on each of the three leaves at 12-hr intervals through 48 hr after inoculation. The number of infection hyphae longer than 5 μm and the number of haustoria per infection site were counted. Measurements of hyphal length were made with a calibrated eyepiece micrometer by measuring from the tip of the longest hypha to its origin at the substomatal vesicle. Observations of the effects of Indar on rust morphology and development were continued for 72 hr after inoculation.

The completely randomized design was used for all experiments of this investigation. Data were analyzed by a general analysis of variance.

RESULTS

Macroscopic effects of fungicide.—Treated and nontreated seedlings were examined 10 days after inoculation for macroscopic evidence of the effects of Indar on rust development. A compatible infection type developed on the nontreated seedlings, whereas the treated seedlings showed no visible evidence of infection.

Light-microscopic evidence of fungicide effects.—Germination and penetration were not affected by Indar. Urediospore germination at 12 hr after inoculation was 96% on the treated seedlings and 94% on the nontreated seedlings. Substomatal vesicle formation 12 hr after inoculation was 80 and 84%, respectively, on treated and nontreated seedlings.

The whole-leaf preparations made it possible to examine infection structures by focusing down through a penetrated stoma of a leaf. Usually, one, but occasionally two, major hyphae developed from the substomatal vesicle (Fig. 2-4). A hypha was classified as major when it originated from the substomatal vesicle. As the fungal thallus continued to develop, numerous lateral branches formed from each major hypha.

By 12 hr after inoculation, differences in fungal thallus development within infection sites on treated and nontreated seedlings began to appear. These differences became significant 36 hr after inoculation (Table 1).

Hyphal initiation and elongation within infection sites on the treated seedlings (Fig. 1-4) proceeded at a much slower rate than in the nontreated seedlings (Fig. 9, 10). Although major hyphae continued to grow and form new lateral branches within the treated seedlings, the degree of hyphal development failed to reach a level comparable with that in the nontreated seedlings. Cessation of hyphal development in the treated seedlings occurred at approximately 36 hr after inoculation (Table 1).

The most striking differences in fungal development in treated and nontreated seedlings were reflected in the formation of haustoria (Table 1). Haustoria first were observed in the infection sites on the nontreated seedlings at 12 hr after inoculation, and were found in all infection sites examined at 48 hr after inoculation. By comparison, haustoria were not observed until 24 hr after inoculation in the infection sites on the treated seedlings. Although haustorium formation increased slightly in the latter between 24 and 36 hr, the magnitude of this increase was considerably less than that observed for the nontreated

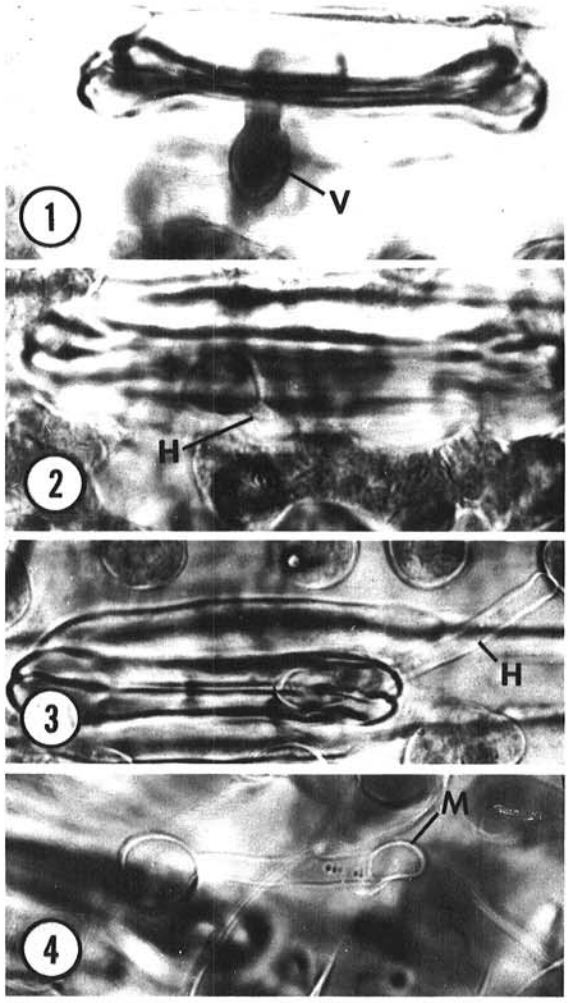

Fig. 1-4. Effect of seed treatment with 4-*n*-butyl-1,2,4-triazole on thallus development of *Puccinia recondita* f. sp. *tritici*, in leaves of the near-isogenic wheat line *Lr*2: **1)** 48 hr after inoculation with the substomatal vesicle (V) present (× 500); **2)** 48 hr after inoculation at an early stage in the formation of intercellular infection hypha (H) from the substomatal vesicle (× 500); **3)** 48 hr after inoculation the infection hypha (H) has extened into intercellular space and contacts a host cell but the haustorium mother cell has not yet become differentiated (× 500); **4)** 72 hr after inoculation showing the haustorium mother cell (M) present at distal end of infection hypha (× 500). Contrast this limited growth with the extensive development of the pathogen in nontreated plants in Fig. 9, 10.

seedlings (Table 1). Hyphae in the treated seedlings often formed haustorial mother cells but these usually failed to form haustoria (Fig. 4,7). Necrosis occurred in neither the host nor the pathogen in the treated plants.

After 48 hr most of the hyphae within the infection sites on the treated seedlings failed to take up the lactophenol-cotton blue stain, and appeared as colorless outlines (Fig. 2, 3, 4, 7). In the nontreated seedlings the dark-blue stain concentrated in hyphal tips (Fig. 8, 9, 10) and haustoria. The older, less metabolically-active structures in nontreated hosts appeared colorless, similar to all fungal structures in later infection stages (48 and 72 hr) of the treated host (Fig. 7).

Morphogenic effects of fungicide on hyphae.—The fungicide also affected morphology of the hyphae. Many appeared abnormally distorted and/or swollen (Fig. 5, 6), and some were constricted or shriveled at points along their length. The gnarled, distorted hyphae were typical of those infection sites in which mycelium developed beyond a single hypha. However, the more frequent response was the failure of thallus development beyond the first hypha.

DISCUSSION

Indar applied as a seed treatment was an effective systemic protectant against wheat leaf rust. It readily entered the germinating seed of the near-isogenic wheat line *Lr*2, was translocated to the foliar tissue, and there arrested infection. Activity of Indar was demonstrated macroscopically by the absence of flecking or pustule development on treated seedlings. This mechanism of leaf rust control produced macroscopic symptoms very similar to the natural immune reaction. It differed microscopically, however, from the hypersensitive reaction (13) in that inhibition of fungal development was not accompanied by host cell necrosis. Furthermore, Indar appeared to arrest development of the invading pathogen within 36 hr after inoculation. A similar histological study showed that the hypersensitive reaction

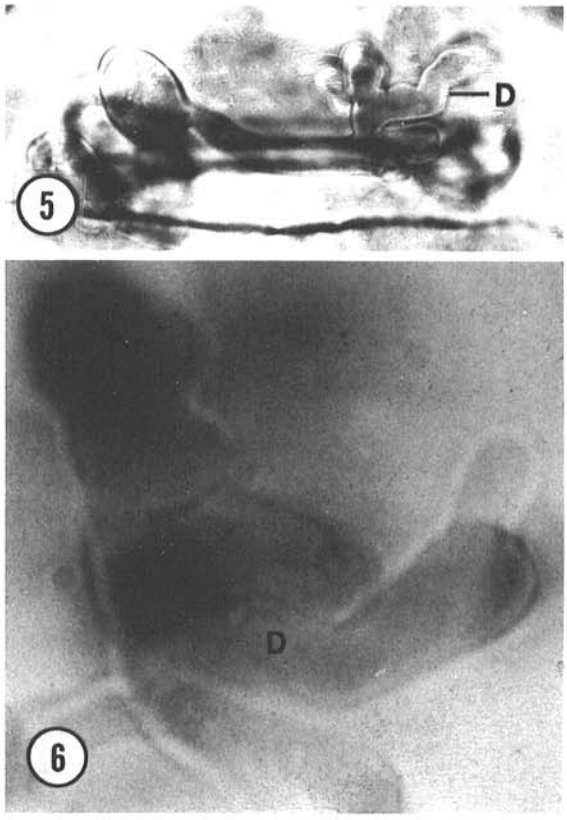

Fig. 5, 6. Distortion of hyphae of *Puccinia recondita* f. sp. *tritici* caused by 4-*n*-butyl-1,2,4-triazole applied as a seed treatment to the near-isogenic wheat line *Lr*2 72 hr after inoculation: **5)** distorted, gnarled hyphae (D) at the distal end of an infection hypha (× 500). **6)** A cluster of distorted hyphae (D) typical of thalli that develop beyond the infection hypha. Note the lack of septae and the swollen appearance of the hyphae (× 1,300).

TABLE 1. Effect of 4-*n*-butyl-1,2,4-triazole (Indar) applied as a seed treatment[a] to the near-isogenic wheat line *Lr*2 on hyphae and haustoria of *P. recondita tritici* isolate 71-112[b] at various hours after inoculation

Fungicide treatment	No. and length of hyphae[c] and no. of haustoria formed per hour following inoculation after			
	12 hr	24 hr	36 hr	48 hr
	No. of hyphae per infection site			
Nontreated	0.9	1.5	6.0	12.5
Treated	0.3	0.8	41.4*[d]	1.5*
	Length (μm) of longest hypha per infection site			
Nontreated	23.4	35.5	81.7	121.9
Treated	4.9	25.5	45.9*	45.5*
	No. of haustoria per infection site			
Nontreated	0.26	0.43	1.27	3.57
Treated	0.00	0.07	0.13*	0.03*

[a]Seed treatment at 113.4 g active ingredient/45.5 kg (4 oz/100 lb) seed.
[b]Inoculation of the nontreated control resulted in a susceptible reaction.
[c]All hyphae longer than 5 μm were counted.
[d]Asterisks (*) indicate a significant difference between means for the control and 4-*n*-butyl-1,2,4-triazole treatments by LSD test, $P = 0.05$.

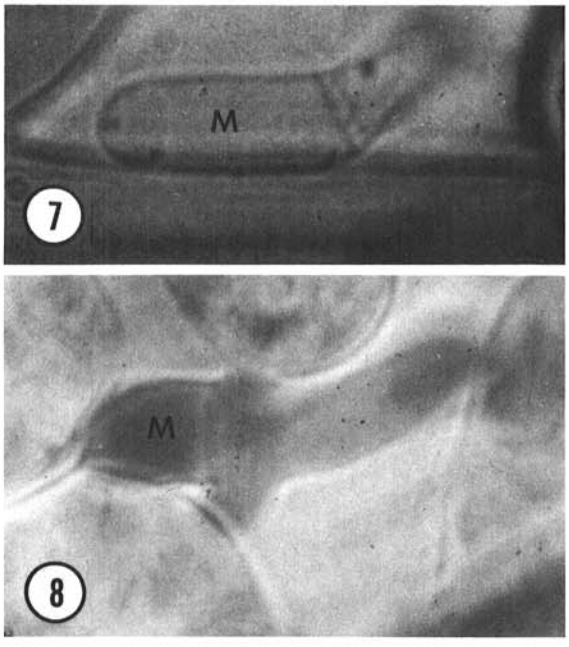

Fig. 7, 8. Effect of seed-applied 4-*n*-butyl-1,2,4-triazole on stain affinity (lactophenol-cotton blue) of *Puccinia recondita* f. sp. *tritici* in the near isogenic wheat line *Lr*2 72 hr after inoculation: **7)** little or no cytoplasmic staining occurs in the hypha or the haustorial mother cell (M) of the pathogen in treated plants (× 1,300). **8)** Cytoplasm of haustorial mother cell (M) and hypha stain darkly in the nontreated plants (× 1,300).

conditioned by this near-isogenic wheat line failed to completely inhibit pathogen development, since the fungus was observed in several of the 36- and 48-hr necrotic infection sites to have grown beyond the necrotic area and produced haustoria in healthy host cells (13).

The lack of inhibitory activity against urediospore germination and subsequent penetration suggests that as a seed treatment, Indar is not effective until after formation of the substomatal vesicle. Indar had no effect on frequency or rate of germination of *P. recondita tritici* urediospores (8). Although superior to oxycarboxin as a protectant, Indar was not effective when applied as an eradicant spray or soil drench, apparently due to absence of fungitoxicity against urediospores (8).

Indar, as a seed treatment, limited hyphal formation, elongation, branching, and haustorium initiation. Failure of some substomatal vesicles to form intercellular hyphae suggested that Indar was effective soon after penetration. For example, 10% of the substomatal vesicles examined at 48 and 72 hr in the treated seedlings failed to form a hypha; variation at other sites ranged from the development of one unbranched hypha to sites that contained several hyphae and one or more haustoria. This degree of variation was not observed in the corresponding infection sites on the nontreated seedlings. It is difficult to explain the cause of this variation, although mobilization of the test compound may not have been uniform throughout the leaf.

The most obvious effect of Indar on fungal development was the prevention of haustorium initiation. This is in contrast to the hypersensitive reaction which limits hyphal elongation and branching but does not appear to affect initiation of haustoria (13, 14).

Indar also induced morphological abnormalities in the hyphae, the most striking of which was distortion and swelling of some hypal branches. Other morphological distortions observed in *P. recondita tritici* were constrictions at points along a hypal segment, deformed hyphal tips, and gnarled groups of hyphae at older infection sites. Swelling of fungus structures is a common effect produced by certain systemic fungicides (2, 7, 10). Exposure of germinating spores of *Neurospora crassa* (2) and conidia of *Botrytis fabae* (7) to benomyl limited germ tube expansion and produced swollen, highly branched, and distorted germ tubes. This swelling is not restricted to gross morphology, but is also reflected in the morphology of cell organelles (7, 10). Haustoria of *Puccinia coronata* exposed to oxycarboxin and benomyl were greatly enlarged as were mitochondria and other cell organelles (10). The effects of other systemic and nonsystemic fungicides on morphology of fungi have been reviewed (6).

Pathogen development in the treated host virtually ceased by 36 hr, as ascertained histologically. Apparently the invading fungus either was killed or forced into a static state at most sites during the first 36 hr. The conclusion that cell death of the pathogen occurred is

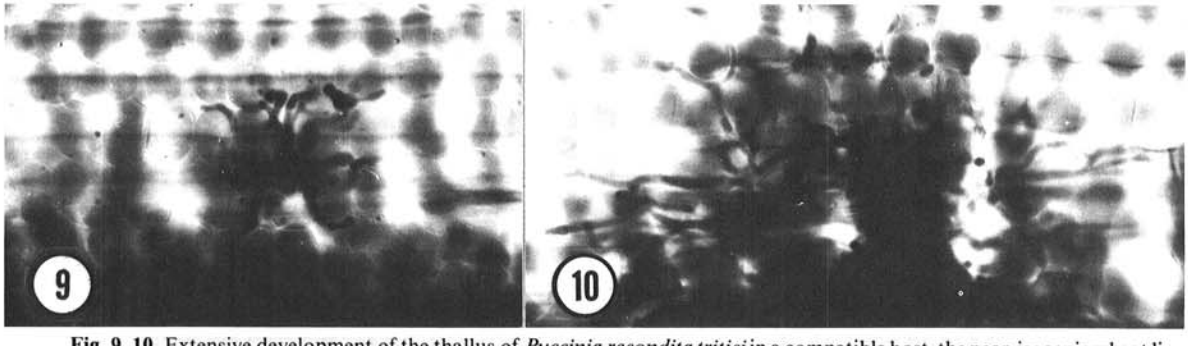

Fig. 9, 10. Extensive development of the thallus of *Puccinia recondita tritici* in a compatible host, the near-isogenic wheat line, *Lr*2, that was not treated with 4-*n*-butyl-1,2,4-triazole. **9)** 48 hr after inoculation (× 105); **10)** 72 hr after inoculation (× 105). Note also the intensity of the stain reaction.

supported by the presence of nonstaining hyphae at many of the infection sites in the treated seedlings 48 and 72 hr after inoculation. In contrast, corresponding infection structures in the nontreated seedlings stained dark blue. Cytoplasm of young, actively growing hyphae is generally concentrated toward the tip. As a result, this region, as well as young haustoria, stain much darker than the older, less active portions of the thallus. A lack of stain concentrations in these infection structures exposed to Indar strongly suggests a reduction or inhibition of metabolic activity in the developing hyphae and haustoria.

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