Effects of Triphenylphosphite on Bean Rust Development

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ABSTRACT

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Triphenylphosphite (TPP) at 125 μg/ml, when sprayed onto bean leaves 2 days before inoculation with *Uromyces phaseoli*, prevented formation of rust pustules. Spore germination, growth of germ tubes on the leaf surface, formation of appressoria, penetration through stomata, formation of substomatal vesicles, development of infection hyphae, and subsequent formation of haustorial mother cells proceeded normally in TPP-treated leaves. However, no haustoria were formed. Both α-aminooxyacetate (an inhibitor of the phenylpropanoid pathway) and cycloheximide (an inhibitor of protein synthesis) largely suppressed the inhibitory effect of TPP when applied to TPP-treated bean leaves 1 day before inoculation. Apparently, TPP triggered a resistance mechanism that prevented formation of haustoria in treated bean leaves inoculated with *U. phaseoli*.

In the screening of phosphorous-containing agents active against fungal plant pathogens, triphenylphosphite (TPP), when used as a foliar spray, inhibited the production of pustules in bean leaves subsequently inoculated with *Uromyces phaseoli* (Pers.) Wint. (8).

In preliminary experiments, TPP (1,000 μg/ml) sprayed on leaves 24 hr before inoculation sharply reduced the number of pustules induced by *U. phaseoli* in bean, by *Uromyces viciae-fabae* (Pers.) Schroet. in broadbean, by *Puccinia pelargonii-zonale* Doidge in *Pelargonium*, and by *Puccinia recondita* f. sp. *tritici* Rob. ex Desm. in wheat. However, no effect of such treatment was observed with the following host-pathogen combinations: *Fulvia fulva* (Cooke) Ciferri in tomato, *Colletotrichum lindemuthianum* (Sacc. et Magn.) Bri. et Cav. in bean, *Mycosphaerella pinodes* (Berk. et Blox.) Vestergr. in pea, *Ascochyta fabae* (Speg.) in broadbean, *Phytophthora infestans* (Mont.) de Bary in potato, *Erysiphe graminis* DC. in barley, and *E. cichoracearum* DC. in cucumber (*unpublished*).

With *U. phaseoli* in bean, TPP was still very active (more than 90% reduction in the number of rust lesions) when sprayed at 125 μg/ml. When tested in vitro, bean rust uredospores germinated normally in 125 μg/ml TPP. Uredospores of *U. phaseoli* also germinated normally on leaf surfaces that had been sprayed previously with TPP, even on leaves in which the final number of rust pustules was reduced by 97%. The TPP treatments were still effective when applied 2 wk before inoculation with *U. phaseoli* or within 2 days of inoculation. The inhibitory effect decreased sharply when TPP was applied 3 or 4 days after inoculation (8).

This paper presents results of experiments performed to study rust infection in TPP-treated leaves and to characterize the infection stage at which inhibition occurred.

MATERIALS AND METHODS

Seedlings of bean (*Phaseolus vulgaris* L. 'Prelude') or of broadbean (*Vicia faba* L. 'Maxime') were grown in a greenhouse and inoculated with an uredospore suspension of *U. phaseoli* or *U. fabae*, respectively. The original inoculum of *U. phaseoli* (isolate R6A) was obtained from Dr. Heitefuss (University of Göttingen, Germany). P. Meeus (Station de Phytopharmacie, Gembloux, Belgium) provided us with the isolate of *U. fabae*.

Eight days after inoculation, spores were collected on leaves bearing numerous pustules and stored at −20 C. The inoculum was prepared by suspending stored spores at a concentration of about 150,000 spores per milliliter in an aqueous solution containing a wetting agent. Inoculation was performed by spraying the inoculum on primary leaves of bean seedlings or on the four first expanded leaves of broadbean seedlings. Inoculated plants were placed in a humid chamber in the dark for 1 day. Plants were then placed in a greenhouse; numerous pustules formed on inoculated leaves within 6 days of inoculation.

Detergents and glycerol are known to enhance the effect of metabolically active molecules in plants. Investigations were conducted accordingly to select a suitable formulation that would not interfere with the specific effect of TPP.

In preliminary experiments, TPP (Fluka A.G., Buchs, Switzerland) was suspended in a mixture of 0.02% detergent and 0.1% glycerol. The detergent Mannoxol OT (British Drug House, Poole, England), known to be a very effective formulating agent, had to be discarded at this stage because it almost completely prevented germination of rust uredospores on treated leaves, thus preventing the study of the specific effect of TPP. On the other hand, Tween 20 at 0.02% had little influence on infection of broadbean by *U. fabae* or of bean by *U. phaseoli*.

In all further experiments, TPP was mixed routinely with 0.01% Tween 20 and 0.1% glycerol and sprayed on the leaves 2 days before inoculation unless otherwise stated.

Cycloheximide (Sigma Chemical Co., St. Louis, MO 63178) and α-amino-oxyacetate (AOA) (Janssen Chimica, Beerse, Belgium) were tested for their possible reversal effect on the TPP-induced inhibition of rust infection; they were dissolved in water and sprayed on the leaves 1 day before inoculation.

The various phases of the infection process on inoculated leaves were observed by fluorescence microscopy after staining with Calcofluor (3,7). Rates of spore germination and appressorium formation were recorded at 1-day intervals after inoculation and calculated as percentages of 150 spores observed randomly on the surface of TPP-treated or untreated leaves.

Further fungal development in leaves was observed using the staining technique with aniline blue described by Niks (5). Fifty infection sites were observed daily after inoculation in each of four independent experiments. The number of rust pustules was evaluated electronically by using a Quantiment 720 System 23 (Cambridge Instruments Co., Dortmund, Germany).

RESULTS

When bean leaves were sprayed with TPP (125 μg/ml) 2 days before inoculation, subsequent formation of pustules by *U. phaseoli* was prevented by 95–97% (Fig. 1). Rates of spore germination (70–90% of total number of observed spores) and appressorium formation (55–65% of the number of germinated spores) were similar on TPP-treated bean leaves and on the untreated

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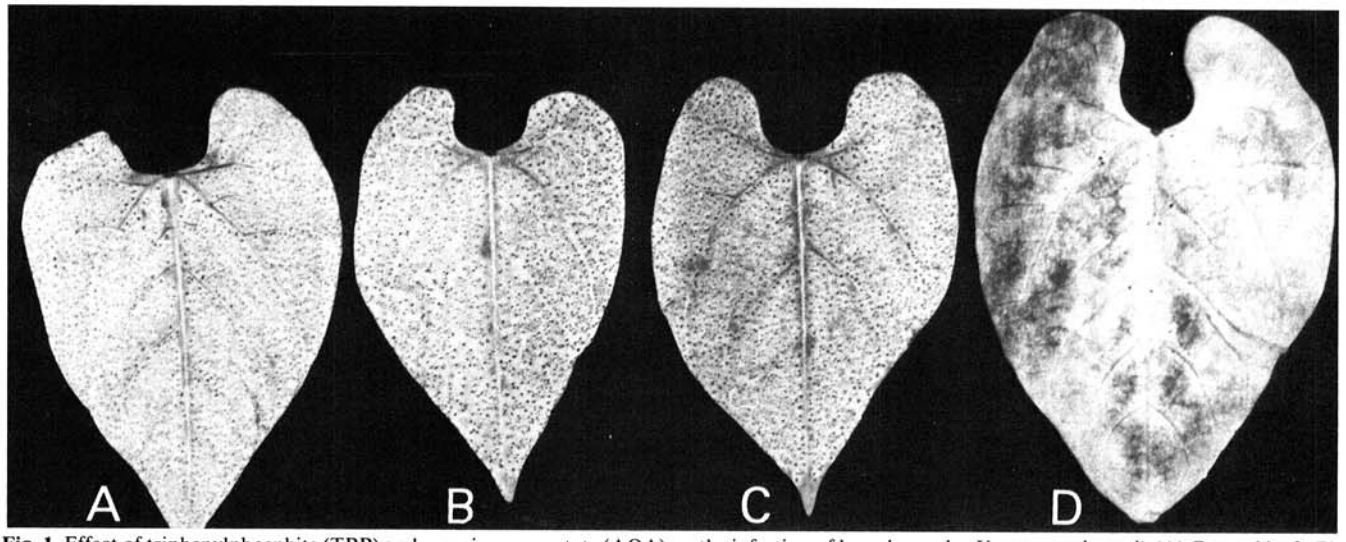

Fig. 1. Effect of triphenylphosphite (TPP) and α-aminooxyacetate (AOA) on the infection of bean leaves by *Uromyces phaseoli*. **(A)** Control leaf, **(B)** leaf treated with AOA (12.5 μg/ml) 1 day before inoculation, **(C)** leaf treated successively with TPP (125 μg/ml) 2 days before inoculation and with AOA (12.5 μg/ml) 1 day before inoculation, and **(D)** leaf treated with TPP (125 μg/ml) 2 days before inoculation. Observations were made 6 days after inoculation.

controls. Furthermore, no differences were observed in the length of *U. phaseoli* germ tubes, their growth on the leaf surface, or subsequent penetration of the leaves.

To obtain a comparable high level of inhibition of *U. fabae*, the concentration of TPP applied to broadbean leaves had to reach 500 μg/ml; under such conditions, germination of uredospores and formation of appressoria were partially impeded.

Therefore, further work concentrated on bean rust, to investigate the stage where internal growth of *U. phaseoli* was arrested. Formation of substomatal vesicles, development of infection hyphae, and subsequent development of haustorial mother cells proceeded normally within 1–2 days in TPP-treated bean leaves. However, no haustoria were observed among about 800 aborted infections examined 2–4 days after inoculation of treated leaves, whereas many haustoria formed within 2 days in untreated control leaves.

Numerous secondary hyphae were observed at the infection foci of control leaves 3–4 days after inoculation, whereas such hyphae were seen only in association with a few residual actively growing foci in TPP-treated leaves.

The events responsible for stopping the infection process were thus expressed at the level of mesophyll cells with which a contact was established by the fungus; this suggested the specific triggering of a resistance mechanism in TPP-treated plants. To test this hypothesis, we studied the effect of several compounds known to suppress resistance mechanisms in plants, thus inducing susceptibility to fungal or viral pathogens in resistant varieties.

When bean leaves previously sprayed with TPP (125 μg/ml) were treated with AOA (12.5 to 50 μg/ml), pustule formation was largely restored; at this concentration, AOA had no visible effect on the infection of control leaves (Fig. 1).

The effect of specific inhibitors of protein synthesis was also tested. When used at a concentration of 2.5 μg/ml, cycloheximide largely restored the sensitivity of TPP-treated bean leaves to infection by *U. phaseoli* but showed no direct effect on the infection of control leaves. Under similar experimental conditions, chloramphenicol at 25 μg/ml or formycin B at 20 μg/ml did not change TPP-induced resistance.

Total phenolic compounds, evaluated by the method of Rathmell and Bendall (6), and total lignins, estimated by the technique of Hammerschmidt and Kuć (2), were not substantially different in TPP-treated leaves compared with control leaves.

DISCUSSION

TPP-induced inhibition of rust infection in bean did not reflect a direct effect of this chemical on the fungus itself. Inhibition resulted from induction of a resistance mechanism triggered in TPP-treated leaves that prevented formation of haustoria from haustorial mother cells. The suppression of the inhibitory effect of TPP by AOA may be in the involvement of the phenylpropanoid pathway in this process (4). Restoration of sensitivity by treatment with cycloheximide is an indication that de novo synthesis might be necessary to express the potential resistance capacity of TPP-treated cells. This latter effect has also been shown for the expression of genetic resistance of oat to crown rust (9).

In some respects, the mode of action of TPP on bean infection by *U. phaseoli* resembles that of another phosphorous-containing compound, aluminium phosethyl (TEPA) (G. Bompeix, *personal communication*). TEPA is mainly active in preventing infection of plants by Phycomycetes (1).

At low concentration, both TPP and TEPA show a specific mode of action by inducing an indirect mechanism that blocks fungal development at the host-parasite interface.

Differences, however, may be noticed between the mode of action of the two compounds. Upon inoculation of TEPA-treated tomato leaves, limited "blocking" necrotic reactions developed (1), whereas no necroses were associated with the inhibition of *U. phaseoli* in TPP-treated bean leaves.

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