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COMMUNICATION

A BENZOTHIADIAZOLE DERIVATE PROMOTES EXPERIMENTAL CONTROL OF BACTERIAL SPECK (*Pseudomonas syringae* pv. *tomato*) BY ACTIVATING CONSTITUTIVE HOST DEFENSES[1]

Reginaldo da Silva Romeiro[2]
Dora Marchiori Silva Neves[2]
Rodrigo Serpa Vieira Leite[2]

RESUMO

UM DERIVADO BENZOTIAZÓLICO PROPORCIONA CONTROLE EXPERIMENTAL DA MANCHA-BACTERIANA-PEQUENA (*Pseudomonas syringae* pv. *tomato*) ATIVANDO DEFESAS CONSTITUTIVAS DO HOSPEDEIRO

Plantas de tomateiro (TopSeed), com trinta dias de idade, produzida: em casa de vegetação, foram atomizadas com uma solução aquosa de BTH, um derivado tiabendazólico, a 250µg/ml, uma semana antes de serem artificialmente inoculadas com dois patógenos desafiadores: *Alternaria solani* (inóculo equivalente a 10^3 conídios/ml) e *Pseudomonas syringae* pv. *tomato* (inóculo equivalente a OD_{540} = 0,1). Sete dias após a inoculação, o número de lesões por folíolo foi estimado em ambos os casos e comparado com o de plantas controle, não expostas ao ativador químico. Obteve-se uma boa proteção quando o patógeno desafiador era *Pseudomonas syringae* pv. *tomato*, mas o composto foi ineficaz contra *A. solani*.

Palavras-chaves: *Lycopersion esculentum*, BTH, doenças do tomateiro.

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[2] Deptº de Fitopatologia da UFV. 36571-000 Viçosa, MG. E-mail: rromeiro@mail.ufv.br, dmsneves@mail.ufv.br, rsvleite@alunos.ufv.br

ABSTRACT

Thirty day old tomato plants (TopSeed), greenhouse grown, were sprayed with a water solution of BTH, a benzothiadiazole derivate, at 250µg/ml, one week before being artificially inoculated with two challenging pathogens: *Alternaria solani* (inoculum equivalent to 10^3 coniadia/ml) and *Pseudomonas syringae* pv. *tomato* (inoculum equivalent to $OD_{540} = 0.1$). In both cases, a number of lesions per leaflet was counted seven days later and compared with the number in the control plants, which had not been exposed to the activator. A good protection was achieved when the challenger pathogen was *Pseudomonas syringae* pv. *tomato*, but the compound was ineffective against *Alternaria solani*.

Kew words: *Lycopersicon esculentum*, BTH, tomato diseases.

The intensive and continuous use of pesticides in large amounts is need for growing tomatoes under Brazilian tropical conditions (*12, 19*) and long-term consequences of this practice may be serious damages to human health and to the environment.

Recently, a new class of fungicides has been developed as a result of the so-called speculative chemistry which was carried out in an attempt to make novel cyano imines (*2*). The so-called fourth-generation fungicides, most having a benzothiadiazole nature, do not have a direct effect on plant pathogens (*17*) but act by activating constitutive plant defenses. For instance, Bartholomew et al. (*2*) mention that 4-chloro-N-[cyano(ethoxy)methyl]benzamide is highly active for the control of Oomycete fungi. Lawton et al. (*10*) report benzothiadiazole (BTH) as a novel chemical activator of disease resistance in tobacco, wheat and other important agricultural plants, and that it acts by activating systemic acquired resistance (SAR) in *Arabidopsis thaliana*, turning the plant broadly resistant to infection by turnip crinkle virus, *Pseudomonas syringae* pv. *tomato* and *Peronospora parasitica*. Gorlach et al. (*7*) concluded that BTH protect wheat systemically against powdery mildew (*Erysiphe graminis* f.sp. *tritici*) infection by affecting multiple steps in the life cycle of the pathogen.

So far, the control of bacterial plant diseases has been accomplished mostly by using exclusion procedures because no efficient chemical products have been developed for this specific purpose to date. A new chemical that acts in an indirect way and might also control bacterial diseases is something new and its efficiency must be tested under Brazilian conditions. After learning about BTH and its potentiality, it is necessary to test it by using several pathosystems under greenhouse and field conditions.

Material and methods. All plant pathogenic microorganisms used in this study were obtained from the culture collection of the Department

of Plant Pathology of the Universidade Federal de Viçosa, Brazil. Bacteria were routinely grown on medium 523 of Kado and Heskett (*8*) and preserved at –80°C after emulsification in 15% (v/v) glycerol (*6*), while fungi were grown on PDA (*20*) and preserved in sterile soil, according to procedure recommended by Smith and Onions (*16*).

For "in vitro" assay, a procedure similar to the one used by Neves et al. (*13*) was chosen. Semisolid culture medium (1.0% w/v) was melted and allowed to equilibrate in a waterbath at 50°C for 30 minutes. One hundred µl from a turbid propagule suspension of the pathogens (approximately 10^9 c.f.u/m for *P. syringae* pv. *tomato* and 10^3 conidia/ml for *A. solani*) were added to each tube and, after homogenization, the mixture was poured in plates to form a 1mm deep layer. After solidification of the gel, disks of filter paper (0.5mm in diameter) were set at 5 equidistant places, and 100µl of a water suspension of BTH [(Benzol(1,2,3-)-thiadiazole-7-carbothioic acid S-methyl ester)] were added to each disk. Plates were incubated for 48 hours at 28°C.

For "in vivo" assay, thirty-day old tomato plants (Top Seed, Lot No. 4681), greenhouse grown, were sprayed with a water solution of BTH (250µg/ml), while control plants were sprayed with water only. One week later, the plants were moved to a moist chamber for 24 hours; a suspension of propagules ($OD_{540} = 0.1$ for the bacterial challenger and 10^3 conidia/ml for the fungal challenger) was sprayed onto the phylloplane of the test plants. Ten replicates per treatment and 3 plants per replicate were used. Seven days after inoculation, the number of lesions/leaflet/plant/replicate was recorded and averages estimated.

Results and discussion. Plants exposed to the plant activator (BTH) did not show necrotic symptoms of phytotoxicity before inoculation. Nevertheless, as soon as the challenger pathogens got in contact with the phylloplane, phytotoxicity showed up even before disease symptoms became evident.

The chemical activator showed no "in vitro" direct inhibitory effect against *P. syringae* pv. *tomato* and *A. solani* in the "in vitro" assay.

Figure 1 shows that in the greenhouse test, in which tomato plants were inoculated with both pathogens one week after being exposed to BTH, the benzothiadiazole derivate provided a good protection against the bacterial challenger (F test significant, $p < 0.01$) but not against the fungal one (F test non-significant, $p > 0.01$).

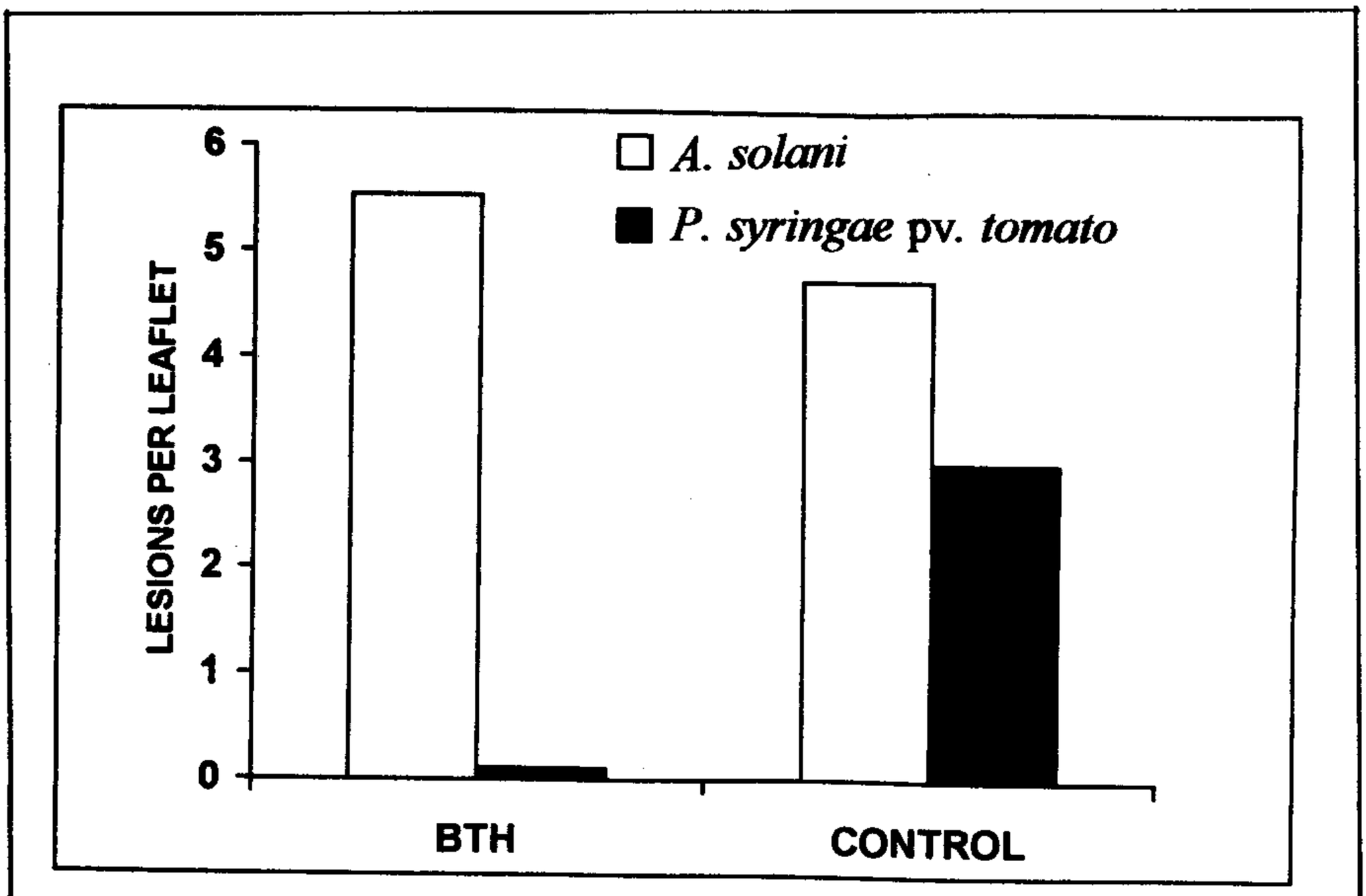

FIGURE 1 – Experimental control of bacterial speck and late blight of tomato by using BTH ([Benzol(1,2,3-)-thiadiazole-7-carbothioic acid S-methyl ester)] at 250 μg/ml, under greenhouse conditions.

No inhibition haloes were observed in bioassay plates, and we can assume the compound has no activity against both pathogens, at least within the limits and accuracy of the bioassay and at the tested concentration (250μg/ml). It was no surprise to find that BTH had no direct activity against the two challenging pathogens. Sticher et al. (*17*) as well as Kunz et al. (*9*) comment that chemical activators of plant defenses differ from the abiotic ones (SAR-inducing microrganisms, for instance), because they usually have no direct activity against pathogens themselves, and their efficiency is due to their ability to activate naturally-existing plant defenses.

Exposure of tomato plants to BTH did not bring about any visible phytotoxicity symptoms until after they were inoculated with the challenger pathogens. However, few days after inoculation, phytotoxic manifestations became evident, but they looked mild and consisted of large dark, dry, amorphous areas totally different from the typical symptoms of bacterial speck (*15*) and of black spot (*19*). In spite of the fact that symptoms of phytotoxicity were conspicuous, they did not show up in all plants nor in all leaves. Occurence of certain levels of toxicity was expected for chemical activators (*17*).

As shown in Figure 1, BTH was very efficient in controlling tomato speck but did not work properly for the fungal pathogen. This may not come as a surprise, considering the complexity of the SAR (Systemic Acquired Resistance) as a biological phenomenon and the yet embrionary state of art of the SAR-inducing mechanisms.

SAR is assumed to be a type of induced disease resistance response in plants, characterized by broad spectrum disease control and an associated coordinate expression of a set of SAR genes (*5, 7, 10, 18, 21*). SAR may be induced by either microrganisms and microbial extracts or by specific chemical activators. When SAR-inducing agents have a biotic nature, resistance has a tendency to be broad non-specific (*4, 11, 22*). On the other hand, chemically induced SAR seems to be more specific and narrower (*17*). In this experiment (Figure 1), BTH was very efficient in controlling tomato speck and did not work against the fungal pathogen. That is no surprise, considering the SAR complexity and a very limited understanding of the SAR-inducing mechanisms.

In plant pathology, the use of chemical compounds for the control of fungal diseases of plants is a well-developed area (*1*), which is not true for bacterial diseases. Nowadays the control of plant bacteriosis is mostly anchored in exclusion and, eventually, in eradication. An efficient technology for the chemical control of plant bacterial diseases is still not available and antibiotics are recommended only under restrict situations (*3, 14, 15*). Therefore, the efficiency of BHT opens a new perspective for the chemical control of bacterial disease of plants.

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