**Title** Postharvest harpin or *Bacillus thuringiensis* treatments suppress citrus black spot in 'Valencia'

oranges

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Citation Crop Protection, Volume 29, Issue 7, July 2010, Pages 766-772

**Keywords** Plant–pathogen interaction; Induced resistance; Biological control; Plant defense mechanisms

## **Abstract**

Citrus black spot (CBS) caused by Guignardia citricarpa represents an important threat to citriculture in Brazil. Limited information is available regarding potential biological control agents and new alternative compounds that may provide protection of orange fruits against the disease. In this study, the effects of commercial products based on Bacillus thuringiensis var. kurstaki (Bt) bacterium, Bt pure isolates and Harpin protein (Messenger®) on the postharvest control of CBS, were evaluated in 'Valencia' sweet orange fruits harvested for three consecutive years in a citrus grove. The fruits were sprayed with the following products: DiPel® WP (Bt, subspecies, kurstaki strain HD-1, 16,000 International Units mg<sup>-1</sup>, 32 g active ingredient kg<sup>-1</sup>) (1, 20 and 50 mg ml<sup>-1</sup>), Dimy Pel<sup>®</sup> WP (Bt, subspecies, kurstaki, strain HD-1, 17,600 IU mg<sup>-1</sup>, 26 g active ingredient 1<sup>-1</sup>) (2, 20 and 50 mg ml<sup>-1</sup>), Messenger (3% harpin protein) (1 and 2 mg ml<sup>-1</sup>) and fungicide Tecto Flowable SC (thiabendazole, 485 g 1<sup>-1</sup>) (0.8 g active ingredient 1<sup>-1</sup>), besides the Bt isolates, Bt- HD-567, Bt-DiPel and Bt-Dimy  $(9 \times 10^8 \text{ CFU ml}^{-1})$ . Ten days after treatment, the number of newly developed CBS lesions and pycnidia produced were evaluated using fifty fruits per treatment. The Dimy Pel® and Messenger® reduced the number of new developed CBS lesions on fruits in up to 67% and 62%, respectively. All applied treatments drastically decreased the number of pycnidia produced in the CBS lesions on orange fruits with 85% to 96% reductions compared to the untreated control. Volatile compounds produced by the isolates Bt- HD-567, Bt-Dimy and Bt- DiPel, reduced the number of lesions on treated fruits by 70%, 65% and 71% compared to the control, respectively. In addition, the survival of Bt isolates on orange fruit surfaces were evaluated by recovering and quantifying the number of CFU every seven days for up to 28 days. The declines in survival rates on orange fruit surfaces were drastic for the three strains of Bt in the first week. The CFU numbers of all applied isolates declined by 4 to 5 orders of magnitude after storage at room temperature for 28 days. In vitro assays revealed that the Bt isolates significantly reduced the mycelial growth of the pathogen, ranging from 32% to 51%, compared to the control, whereas no inhibitory effect was observed in the presence of Messenger<sup>®</sup>.