

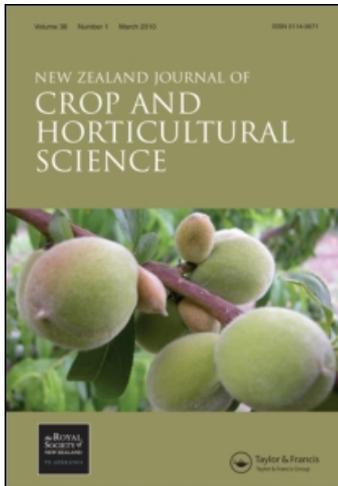
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Sensitivity of Australian *Sclerotinia sclerotiorum* isolates from bean fields to boscalid

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White mould caused by *Sclerotinia sclerotiorum* is one of the most damaging diseases of bean worldwide. High incidence of white mould can lead to complete crop loss through rejection by processors. Since 2004, in Australia, white mould is managed by the prophylactic application of the fungicide, boscalid during flowering. The sensitivity of *S. sclerotiorum* isolates ($n = 150$) was tested using a mycelial growth assay. The effective concentration of boscalid required to reduce mycelial growth by 50% (EC_{50}) was calculated using probit analysis. The frequency distribution of EC_{50} values was unimodal and similar to that of 11 non-exposed isolates collected from other crops or obtained from culture collections. No evidence of resistance or reduced sensitivity was found within the tested *S. sclerotiorum* population from Australian bean fields. This study provides valuable baseline data for monitoring changes in sensitivity to this fungicide.

Keywords: carboximide fungicides; fungicide resistance; white mould

Introduction

White mould, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most destructive diseases of bean worldwide (Boland & Hall 1987). The disease is most often found on the pods causing water-soaked areas which progress into soft, necrotic lesions, followed by the production of white cottony mycelia and eventually sclerotia (Abawi & Grogan 1979; Abawi et al. 1975). Losses from the disease can also be incurred from stem rot which causes weakening and lodging of plants (Boland & Hall 1987).

Control of white mould in Australian bean fields is problematic and often incomplete. This may be due to the long survival periods of sclerotia in soil, a wide host range encompassing many of the crops encountered in the typical

vegetable cropping rotation (e.g. pyrethrum, alkaloid poppy, potato, carrot) and weeds, large numbers of wind-borne ascospores, and the absence of appreciable host resistance within commercial bean cultivars (Abawi & Grogan 1979; Boland & Hall 1987; McDonald & Boland 2004). Management of white mould within Australian bean fields therefore is highly reliant upon the use of fungicides at early flowering stage to prevent infection of the senescing petals, followed by up to two subsequent applications at 7- to 10-day intervals.

Boscalid (Filan[®]; Nufarm Australia) was first registered for use on Australian bean fields in December 2004, following the withdrawal of procymidone (Sumislex; Sumitomo Chemical Australia) due to safety concerns.

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Current resistance management guidelines suggest no more than three applications of boscalid (500 g/ha) within a season (Australian Pesticides and Veterinary Medicines Authority, PER10276). However, currently boscalid is the only fungicide registered for white mould control within Australian bean fields, making fungicide resistance development likely, and important for the future management of white mould. In addition to being integral to disease management programmes of bean production, boscalid is also used in the management of other fungal diseases on crops commonly found in the production rotation, such as pyrethrum (Pethybridge et al. 2008). Hence, *S. sclerotiorum* populations are exposed potentially to numerous applications of boscalid and are highly likely to develop resistance or reduced sensitivity (Kuhn 1984).

Boscalid is a member of the carboximide group of fungicides that act on the mitochondrial respiration pathway at succinate-ubiquinone reductase (complex II) (Kuhn 1984; Avenot et al. 2008). Resistance to boscalid within plant-pathogenic fungi was first reported within *Alternaria alternata* isolates from Californian pistachio orchards (Avenot & Michailides 2007). Subsequently, boscalid resistance has also been reported in *Botrytis cinerea* from apple (Kim & Xiao 2010), *Podosphaera xanthii* from cucumber (Miyamoto et al. 2010), *Corynespora cassiicola* from cucumber (Miyamoto et al. 2009) and many others.

The objective of this study was to characterize the baseline sensitivity to boscalid of *S. sclerotiorum* populations from Australian bean fields and to quantify any evidence of resistance or reduced sensitivity in comparison with non-exposed populations. This information will be critically important for assessment of temporal changes in sensitivity to boscalid within *S. sclerotiorum* populations.

Method

One hundred and fifty isolates of *S. sclerotiorum* were obtained from diseased pods from 55 bean

fields across northern Tasmania during 2008 and 2009. These isolates were likely to have been exposed to boscalid for three cropping seasons, since the introduction of this fungicide into bean production in December 2004. Of these isolates, 102 were collected from 23 bean fields in 2008 and 48 from 32 fields in 2009. For comparison, 11 *S. sclerotiorum* isolates were also included in the study which had not been exposed to boscalid. These isolates were sourced from pyrethrum fields within the same production district as the bean fields prior to the introduction of boscalid, and culture collections, originally isolated from lettuce, sunflower and carrot. To obtain the isolates from bean fields, diseased pods with sclerotia were selected. Single sclerotia were surface sterilized with 1% sodium hypochlorite (20% commercial bleach) for 2 min, rinsed three times with sterile distilled water, and placed on water agar. The identity of each isolate was confirmed by morphological examination following hyphal tip culturing (Willets & Wong 1980).

Sensitivity of each isolate to boscalid was assessed using a mycelial growth assay at concentrations of 0, 0.1, 0.05, 0.5, 5 and 50 µg boscalid/ml in potato dextrose agar (PDA). Stock solutions of boscalid were made by dissolving technical formulations of boscalid (BASF Corporation, Research Triangle Park, NC, US) in 100% ethanol and sterile distilled water. The concentration of ethanol within the amended media did not exceed 1 ml/l. An appropriate aliquot of stock solution was then mixed with 400 ml PDA which had been autoclaved and cooled to 60 °C, before being poured into 9 cm diameter polystyrene Petri dishes and allowed to dry overnight.

Three replicated plates of each boscalid concentration were inoculated with a 4 mm agar plug of each isolate removed from the edge of a 7-day-old colony grown on PDA at 20 °C in the dark. Mycelial growth was measured after 48 h of incubation in darkness at 20 °C along a premarked diameter.

The sensitivity of isolates to boscalid was assessed using probit analysis. Using this

analysis, the effective concentration of active ingredient required to cause 50% inhibition (EC_{50}) of mycelial growth was calculated. Inhibition was calculated as $1 - (\text{mean growth rate on unamended media})$. Probit analysis was conducted using a generalized form of the macro described by Hsiang et al. (1997) for the Statistical Analysis System (Version 9.1).

Results

The distribution of sensitivity to boscalid was unimodal, with EC_{50} values for all isolates $\leq 0.6 \mu\text{g/ml}$ (Fig. 1). Three isolates collected in 2008 had slightly higher EC_{50} values ($0.4\text{--}0.6 \mu\text{g/ml}$), but this is likely to reflect natural variability within the *S. sclerotiorum* population, rather than reduced sensitivity. Moreover, there was no evidence of reduced sensitivity in isolates collected in 2009 in comparison with those collected in 2008. Among the 102

S. sclerotiorum isolates collected in 2008, 75 (73.5%) had EC_{50} values lower than $0.2 \mu\text{g/ml}$. For isolates collected in 2009, 46 (96%) of the 48 isolates tested had EC_{50} values below $0.2 \mu\text{g/ml}$. In contrast, the EC_{50} values of *S. sclerotiorum* isolates belonging to the non-exposed populations ranged from $0.017\text{--}0.159 \mu\text{g/ml}$.

Discussion

To our knowledge, this is the first report of the distribution of sensitivities to boscalid within the *S. sclerotiorum* populations from bean fields in Australia. These results indicated that the isolates tested were highly sensitive to boscalid based on the unimodal frequency distribution of EC_{50} values, the similarity between the EC_{50} values of *S. sclerotiorum* isolates collected from bean and those collected from non-exposed populations, and the similarity to baseline sensitivity for boscalid in other

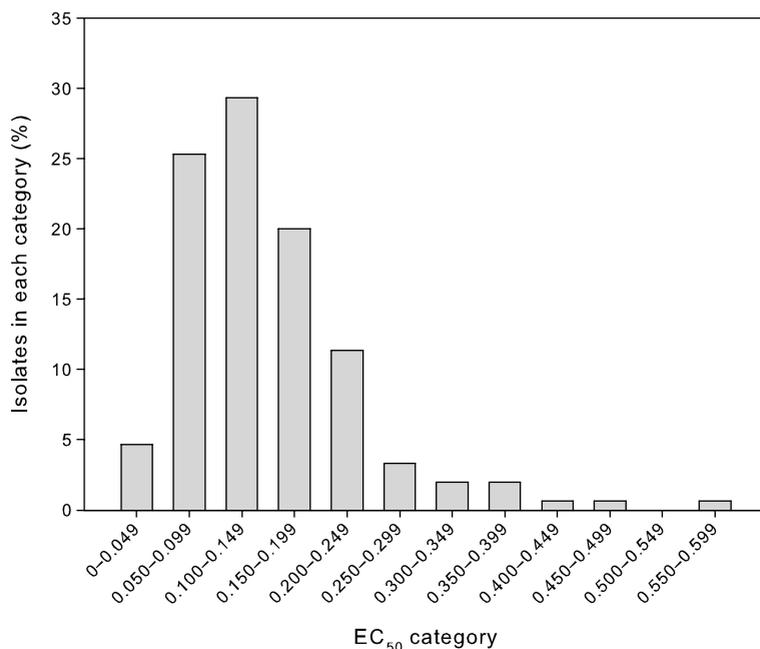


Figure 1 Frequency distribution of effective concentrations of boscalid to inhibit 50% of mycelial growth (EC_{50}) for *Sclerotinia sclerotiorum* isolates collected from bean fields in 2008 ($n=102$) and 2009 ($n=48$). EC_{50} values for the 11 isolates previously not exposed to boscalid ranged from $0.017\text{--}0.159 \mu\text{g/ml}$, with a mean of $0.095 \mu\text{g/ml}$.

plant pathogenic fungi. Liu et al. (2009) reported the baseline sensitivity of 161 isolates of *S. sclerotiorum* from rapeseed to boscalid ranging between 0.002–0.391 µg/ml with a mean of 0.042 µg/ml. In contrast, much higher EC₅₀ values have been recorded for fungi in which resistance to boscalid has been characterized. For example, 38 of 46 *Alternaria alternata* isolates from pistachio orchards in Californian were highly insensitive to boscalid, with EC₅₀ values of at least 500 µg/ml. In these orchards, control failures had not been observed, however boscalid had been applied two or three times per season for up to 4 years (Avenot et al. 2008).

Our results indicate that poor control or putative control failures in Australian bean fields were unlikely to be due to reduced sensitivity to boscalid. Additional factors such as extremely high levels of within-field inoculum, poorly timed fungicide applications and/or fungicide application efficiency due to sub-optimal environmental conditions or application technique are likely to contribute to incomplete disease control. This information provides important baseline sensitivity data which will enable temporal assessment of boscalid sensitivity within the *S. sclerotiorum* population to minimize losses caused by white mould.

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