Available online at www.sciencedirect.com
SCIENCE DIRECT®
Crop Protection 24 (2005) 625–631
Crop Protection
www.elsevier.com/locate/cropro

Fungicides for control of flower and berry infections of *Botrytis cinerea* in boysenberry

M. Walter[a,*], P. Harris-Virgin[b], C. Morgan[b], J. Stanley[b], K.S.H. Boyd-Wilson[a], G.I. Langford[a], M.S. Moore[c]

[a]*HortResearch, Canterbury Agricultural and Science Centre, P.O. Box 51, Lincoln, New Zealand*
[b]*HortResearch, Nelson Research Centre, P.O. Box 220, Motueka, New Zealand*
[c]*Bayer New Zealand Ltd, P.O. Box 11-332, Christchurch, New Zealand*

Received 18 May 2004; accepted 29 November 2004

Abstract

During 1998–2002 in New Zealand, fungicides were evaluated on boysenberry (*Rubus* spp. hybrid) for control of *Botrytis cinerea* in the laboratory (detached flowering lateral assay), and in the field (two small scale field evaluations and one large scale grower trial). Fungicides used were thiram (standard grower control), pyrimethanil, cyprodinil + fludioxonil, fenhexamid and three undisclosed plant extracts. Laboratory studies showed that fungicides, except for two plant extracts, reduced flower infections. Disease incidence was also affected by the timing of fungicide applications in response to artificial flower inoculations with *B. cinerea* conidia. In the field, all fungicides reduced *B. cinerea* berry infections, however some products (pyrimethanil, cyprodinil + fludioxonil, plant extract) affected plant growth and/or berry quality and their use was discontinued. Further grower trials of fenhexamid in comparison to standard fungicide applications (grower control) and an unsprayed control on four commercial properties showed that fenhexamid achieved similar reductions in disease levels as the grower control. The fenhexamid residue decay curve followed a first order kinetics with less than 0.05 mg/kg fenhexamid detected in fruit at harvest. The research contributed to the registration of fenhexamid for use in New Zealand boysenberry.

Keywords: Fenhexamid; Thiram; Boysenberry; *Botrytis cinerea*; *Rubus*

1. Introduction

Stylar *Botrytis cinerea* (Perk.: Fries) flower and drupelet infections account for over 90% of all boysenberry (*Rubus* spp. hybrid) berry infections (Walter et al., 1999). Epidemiological studies have shown that *B. cinerea* inoculum peaks over flowering and again at harvest (Walter et al., 1997). Current control strategies aim to reduce sporulation of *B. cinerea* on litter on the ground (Walter et al., 2004a), which is an important inoculum source (Walter et al., 1997), and to prevent flower infection by fungicide applications. In New Zealand, a wide range of fungicides is registered for *B. cinerea* control in boysenberry. However, resistance development and/or residues in fruit affect market-access for export boysenberry and therefore restrict the use of most fungicides in New Zealand boysenberry production. Depending on the export market, currently four different disease management spray programmes exist, based predominantly on copper, thiram, tolylfluanid and/or captan applications. The aim of the research was to evaluate potential fungicides and some newer fungicides, not yet registered in New Zealand for use in berry fruit, for their effect on *B. cinerea* control in boysenberry.

*Corresponding author. Tel.: +64 03 325 6600; fax: +64 03 325 6063.
E-mail address: mwalter@hortresearch.co.nz (M. Walter).

0261-2194/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2004.11.005

2. Materials and methods

2.1. Inoculum

Cultures of *B. cinerea* isolates BC125, BC126 and BC128, originally isolated from boysenberry fruit, were maintained and grown on oatmeal agar as described by Walter et al. (1999). Three-week old plates were flooded with sterile distilled water plus 0.01% Tween 80 (tween water). The resultant suspension was filtered through a double layer of lens tissue (Whatman 105) to remove mycelial fragments. Suspensions were centrifuged at 3000 rpm for two minutes, the supernatant decanted and the spore pellet resuspended in tween water to remove nutrients from the agar. This procedure was repeated twice. Conidial suspensions were then counted with the aid of a haemocytometer and adjusted to the required conidial concentration. A mix of equal parts of the 3 isolates was applied at the rate of 10^4 and 10^5 conidia/ml for artificial inoculation in the laboratory and field experiments, respectively, as described below.

2.2. Detached flowering lateral assay

Approximately 80 boysenberry floricane laterals (cv. 'Riwaka late') were picked (with flowering buds still closed) on 31st October 1996 from a grower's property in the Motueka area. In the laboratory, any open flowers were removed and laterals were cut to 400–500 mm lengths and put in groups of three (referred to as a plot) into 250 ml Earlenmeyer flasks containing 200 ml sterile distilled water in a growth room (20 °C) at 95–100% relative humidity with a 12 h photoperiod (Phillip's TLD 58W/33 0001 white). After 3 days, approximately 90% of flowers were open and all closed buds were removed. Each plot contained between 7 and 12 open flowers.

Fungicide treatments (Table 1) were applied prior to (24 and 1 h) and following (24 h) the *B. cinerea* inoculations using sterile airbrushes (Model 250-2, Badger Air Brush Co.). Both, fungicides and the *B. cinerea* conidial suspension, were applied to just before run-off and allowed to dry prior to re-incubation at high-humidity in the growth room. Each treatment was applied to five replicate plots. The total of 135 plots, consisting of 5 replicates $\times 9$ treatments $\times 3$ fungicide timings, was completely randomised during incubation (plots not touching). After 5, 7 and 10 days, flower parts (stamens and styles) were assessed visually for incidence of *B. cinerea* sporulation.

2.3. First field evaluation (Field 1)

Based on the laboratory results, fungicide treatments (Table 1) were further evaluated the following season (1997/98) in the boysenberry research block (cv. 'Riwaka late') in Motueka (Nelson region). Treatments were replicated six times within two rows, as random blocks. Each block consisted of three treated plants separated by 2 non-treated buffer plants. From the treated plants, only the centre plant was used for assessments.

On 9th November 1997, when 50% of flowers had petals open, 40% of flowers had developed to small green drupelet stage, and 10% of buds were still closed, 20–30 open flowers per plant were tagged. Then on the following day the fungicides and water were applied to plants, followed on the third day by an application of a conidial suspension (10^5 conidia/ml) of *B. cinerea*. The

Table 1
Fungicide treatments used in the detached flowering lateral assay, field evaluations and grower trial

Exp.[a]	Fungicide/Product	Supplier	Product rate (per litre water)
L, F1, F2, G	Nil	—	—
L, F1, F2, G	Water	—	—
L	Product A[b]	Yates NZ Ltd	5 ml
L, F1	Product B[b]	Yates NZ Ltd	20 ml
L, F1, F2	Product C[b]	Nufarm Ltd	10 ml
G	Captan (Captan Flo)[c]	Nufarm Ltd	2 ml
G	Tolylfluanid (Euparen® Multi)[d]	Bayer NZ Ltd	1.5 ml
L, F1	Pyrimethanil (Scala®)[e]	BASF NZ Ltd	2 ml
L, F1	Cyprodinil + fludioxonil (Switch®)[f]	Novartis Crop Protection	1 g
L, F1, F2, G	Fenhexamid (Teldor®)[g]	Bayer NZ Ltd	1.5 ml
L, F1, F2, G	Thiram (Thiram DF)[h]	Nufarm Ltd	3 g

[a]Experiment: L = Detached flowering lateral assay; F1 = Field 1; F2 = Field 2; G = Grower trial.
[b]Undisclosed water soluble/emulsifiable plant extracts.
[c]Captan Flo contained 480 g/litre captan in the form of a suspension concentrate.
[d]Euparen® Multi contained 500 g/kg tolyfluanid in the form of a water dispersible granule.
[e]Scala® contained 400 g/litre pyrimethanil in the form of a suspension concentrate.
[f]Switch® contained 375 g/kg cyprodinil and 250 g/kg fludioxionil in the form of a water dispersible granule.
[g]Teldor® contained 480 g/litre fenhexamid in the form of a suspension concentrate.
[h]Thiram DF contained 800 g/kg thiram in the form of water dispersible granule.