Fungicide Resistance In *Botrytis* Species

Seona Casonato[1]
Burnley College, Uni of Melbourne, Burnley Gardens, RICHMOND VIC 3121

INTRODUCTION

Growers in the nursery industry are faced with numerous problems everyday. One of these is the control of disease which can cost growers hundreds of thousands of dollars in lost revenue. Fungal diseases are a major concern and one fungus that constantly threatens crops is *Botrytis cinerea*.

Botrytis cinerea can affect a range of plant parts, particularly the flowers, when there is high relative humidity and cool temperatures (Jarvis, 1992; Fletcher, 1984). Conducive conditions are worsened by poor ventilation, hence it is beneficial for heating and ventilation at sunset when the vapour pressure deficit is low (Jarvis, 1992). Generally infection occurs after the plants have been harvested and during storage and transport. The first symptoms, however, are usually found on the plant in the greenhouse (Dirske, 1982; Fletcher, 1984). Commencement of the infection usually occurs due to the conidia of the pathogen and it serves as the initial inoculum point for the infection for most outbreaks of *Botrytis*. Once conditions are conducive for infection, the symptoms of the disease appear within a few days (Agrios, 1988; Jarvis, 1992).

Fungicides are an important element in controlling infection from *Botrytis*, however it is important to note that they do not substitute for poor sanitation practices and environmental management. Control of *Botrytis* is generally achieved by growers implementing a spraying program in conjunction with appropriate hygiene measures. Over the past decade however, some of the fungicides have not been successful in controlling *Botrytis* due to resistance problems. It appears that systemic fungicides cause the majority of resistance problems as they generally have a single-site mode of action (O'Connor, 1990). When resistance to the fungicides occurs, the disease is extremely difficult to control and whole crops can succumb to the infection of *Botrytis*.

Fungicide resistance occurs due to fungi developing and multiplying in the presence of the fungicide that would normally kill them. The fungicide acts as a selection pressure and when exerted on the fungi, mutations occur within the genes and this causes the fungicide resistance (O'Connor, 1990; Dekker, 1987). If a grower can determine at an early stage that the *Botrytis* isolates on their property are becoming resistant or that they are resistant, procedures can be implemented to combat the problem.

A trial was established to determine if isolates of *B. cinerea* from a commercial floriculture crop are resistant to certain fungicides. The trial will also investigate what are the best chemicals to use in a spraying program on this property.

METHODS AND MATERIALS

Paper Disc Technique. A spore suspension of 1×10^6 cfu ml^{-1} was made with 7-day-old cultures. The spore suspension (0.1 ml) was evenly seeded on 12 ml of

[1]Australian Region Rod Tallis Youth Award Winner 1997

potato dextrose agar (PDA). A 13-mm diameter sterile disc of Whatman No. 4 filter paper was soaked in a made-up mixture of commercial fungicide of known concentration. Twenty-one different fungicides were used with two being experimental — Ex 500 SU and KBR 2738. Some of the fungicides are not specifically used to control *Botrytis*, however, the grower for whom these trials were undertaken wanted them tested. Three concentrations were used:

1) Recommended rate
2) 0.5× recommended rate
3) 0.1× recommended rate

The filter paper discs were air dried slightly and then placed in the centre of the seeded PDA plate.

There were three replicates for each treatment. The plates were incubated at 21±1C with 8 h of light per 24 h for a period of 72 h.

After the incubation period, fungal activity was assessed by measuring the diameter of the inhibition zones surrounding the paper discs. If there is no inhibition at the recommended rate, the *Botrytis* was classified as resistant to the particular fungicide. For other concentrations, if there was no inhibition zone, the dosage was determined to be too low if at the full rate there had been an inhibition zone. The inhibition zones were measured three times to give an average diameter of the inhibition zone. They were placed back into the incubator for a further 7 days when they would be assessed again at 10 days after the initial inoculation.

RESULTS AND DISCUSSION

Results indicated that the fungicides performed best against the *Botrytis cinerea* at full and 0.5 times the recommended rate. The 0.1× of the recommended rate performed poorly in the majority of the fungicides so were dropped from subsequent testing.

Results indicated that at the recommended rate, the following fungicides performed the best at 72 h:

Total inhibition	Alto® (a.i. Cyproconazole)
	Scarla® (a.i. Pyrimethanil)
	Plantvax® (a.i. Oxycarboxin)
66.1% inhibition	Octave® (a.i. Prochloraz)
53.6% inhibition	KBR 2738 (a.i. Fenhexamid)

After 10 days, Scarla® had the greatest inhibition followed by Alto®, Octave®, then Plantvax®.

As shown in Figure 1, there was a significant range in the efficacy of the chemicals tested. An interesting result is that Plantvax® had total inhibition. Plantvax® is a systemic fungicide that is used primarily for the treatment of rust, its ability to also inhibit the growth of *Botrytis* is therefore surprising.

Results from the laboratory assay appear to indicate there is a resistance to the benzimidazoles (e.g. Benlate) which is almost universal. Other fungicides tested showing good efficacy were Octave, Thiram (a.i. Thiram), and the test fungicide KRB 2738. The other test fungicide Ex 500 SU showed very poor efficacy. Resistance occurred in one of the dicarboximide fungicides (Sumisclex® a.i. Procymidone) and the other (Rovral® a.i. Iprodione) had very poor efficacy. After 10 days, the Rovral® had no inhibition zone. Fungicides belonging to the demethylation-inhibiting

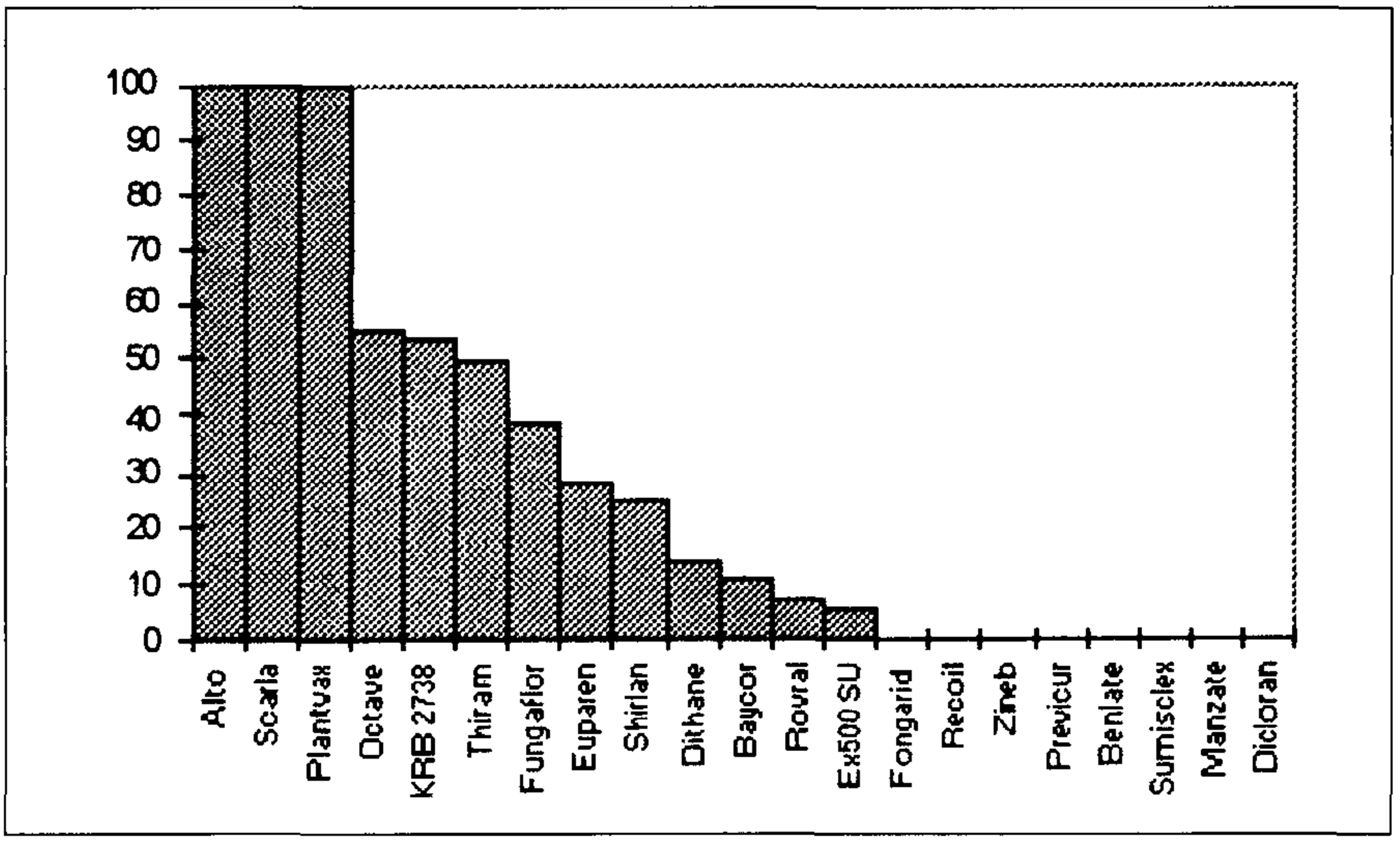

Figure 1: Inhibition zone percentage from 72-h plates at recommended rate.

fungicides (DMIs) appeared to be quite effective with such fungicide as Alto®, which is a triazole, performing well.

In the tests undertaken at 0.5 the recommended rate, Octave® with 48.1% inhibition on the plate and the experimental fungicide KBR 2738 with 43.8% inhibition were most effective and, even after 10 days, these were still the most effective plates.

These results indicate there are resistance problems with these *Botrytis* isolates. However, alternative fungicides may be used such as Alto® and Scarla®. Preliminary trials have indicated that there is the possibility that some fungicides may be effective at 0.5 rates as there appears to be good efficacy. It is important to note that these results must be carried out in the field as field results often vary significantly from laboratory tests. If an isolate is resistant to fungicides in the laboratory, it does not necessarily mean there will be resistance problems in the field.

Results show that benzimidazoles could be inappropriate to control *Botrytis* in the industry. Dicarboximides may be used with caution, however, other researchers have recommended that they only be used in peak periods of *Botrytis* infection. It appears that control of the fungus can be achieved by using chemicals from other fungicide groups, such as Alto® and Scarla® and also the test fungicide KBR 2738 which is soon to be released.

These results do highlight that effective control of *Botrytis* can not be achieved by fungicides alone and a fungicide spraying programme must be used in conjunction with environmental, sanitation, and cultural practices.

Trials are continuing and they will be carried out using the paper disc technique on *Botrytis* isolates that have had no fungicide applications. Another technique, the spore germination technique, will also be used. This measures the lengths of germ tubes of *Botrytis* on PDA plates amended with fungicides, and enables trials to be carried out within 24 h. This is highly beneficial to growers, who are then able to see outcomes of the trials within 48 h.

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