
Efficacy of post-harvest fungicide sprays and fan-drying for the control of gray mould (*Botrytis cinerea*) in roses (*Rosacea hybridus* var. *annabella*)

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Botrytis cinerea is a fungal disease of many cut flowers, affecting their quality and hence, market value. The visual symptoms of the disease usually appear during the post-harvest period. The objectives of this study were to determine if the post-harvest fungicidal sprays; post-harvest Fan-drying, use of post-harvest fungicidal sprays in combination with fan drying, were effective in the control of *Botrytis cinerea* in cut roses. Post-harvest spray combinations of three specific botryticides, followed by fan drying were investigated. Fan drying significantly reduced the incidence of post-harvest *Botrytis* infection but this was not consistent over all three-fungicide treatments. With the exception of one fungicide treatment, there was a clear interaction between fungicide sprays and fan drying. A combination of specific botryticide sprays and fan drying appeared to be a good option for reducing post-harvest *Botrytis* infections. The treatments had no adverse effects on the quality or vase life of the rose variety used.

Key words: *Botrytis cinerea*, Fungicidal, Post-Harvest, Botryticide, Interaction, Fan-Drying.

Introduction

Gray mould is an important post-harvest, water-dependent and cool weather fungal pathogen of many greenhouse crops. The disease results in considerable losses in cut flowers with symptoms often developing during storage and transport. In roses, symptoms include browning and rotting of the petals, and this affects quality and results in economic loss to growers.

Roses account for approximately 70% of Zimbabwe's cut flower exports. Currently there are about 350 hectares under production. The cost of setting up greenhouses equipment is about 34 000 USD per hectare. This makes it

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difficult for the growers to break even. Thus, there is a need to develop effective gray mould control measures in order to reduce losses.

Economically, *Botrytis* is one of the most important post-harvest diseases of roses. The spores can adhere to flower buds and remain dormant for at least 3 weeks. Alternatively, if cut flowers are wet for several hours, the spores produce germ tubes and appresoria or even effect initial penetration of flower buds. In the above state, they can become temporarily non-aggressive (quiescent infections) until favorable conditions for development prevail (Bissett, 2002b). Tight packing of wet flowering stems and little air movement results in high humidity. Under these conditions, temperature fluctuations during transport can cause condensation. Such conditions in the boxes are conducive to *Botrytis* development since the physiological or biochemical changes taking place in the cut flower stems probably enhance *Botrytis* development (Hausbeck and Moorman, 1996).

The *Botrytis* fungus is genetically very variable and resistance to fungicides can develop rapidly. *Botrytis* has developed resistance to nearly all the chemical classes of fungicides in one part of the world or another. As a result, it is the classical “high resistance risk” fungus. This ability to develop resistant strains severely limits chemical control options, as there are relatively few fungicide classes active against the pathogen (Hausbeck and Moorman, 1996). For instance, the Benzimidazole class of systemic fungicides includes carbendazini (Bavistin), benomyl (Benlate) and thiophanate-methyl (tops-in-M).

Within two or three years of their introduction, strains of *Botrytis* highly resistant to all benzimidazoles were detected (Hausbeck and Moorman, 1996), Leroux, 1995. Resistance to benomyl and cross-resistance to other benzimidazole fungicides in *Botrytis* populations is now common. In Zimbabwe resistance to thiophanate-methyl has been detected in a *Botrytis* population at Southern Roses (Bissett, 2002a) and resistance to the benzimidazoles is likely to be widespread in *Botrytis* populations throughout Zimbabwe. *Botrytis* resistance to benzimidazole fungicides appears to be persistent, as it has been reported that a *Botrytis* population in a greenhouse where benzimidazole use ceased in the 1970’s still exhibited resistance 12 years later. Thus, once resistance to benzimidazoles has been detected there is no point in continued use of this class of fungicides (Hausbeck and Moorman, 1996). *Botrytis* resistance was detected within a few years after the introduction of the Dicarboximide class of fungicides (Hausbeck and Moorman, 1996; Leroux, 1995). *Botrytis* resistance to Dicarboximide fungicides (another class of fungicides e.g. Rovral) is not persistent and the frequency of resistant strains declines in the absence of a selection pressure. Dicarboximide resistance, however, reappears rapidly once the fungicides are reintroduced. There is also

evidence that incipient iprodione resistance may be present in *Botrytis* populations at Southern Roses (Bissett, 2002b). Pyrimethanil (Scala) is now a new translaminar botryticide, which possesses protective and some curative activity. Nevertheless, it is strictly recommended for preventive use in order to achieve good efficacy and to minimize the risk of resistance development in *Botrytis* populations (Rossenlenbioich and Stuebler, 2000). This restriction as well as the use in combination with other fungicides is necessary, as *Botrytis* strains resistant to anilinopyrimidines have already been reported in French and Swiss vineyards (Leroux, 1995). Given the problems of resistance associated with *Botrytis*, the newest option for *Botrytis* control is fenhexamid (Teldor).

Fenhexamid, which is not yet registered in Zimbabwe, has contact activity and belongs to the new chemical class of hydroxanilides, which has a new mode of action (Rossenlenbioich and Stuebler, 2000). Although monitoring for *Botrytis* resistance in strawberry fields and vineyards for a number of years has failed to find evidence of resistance to fenhexamid, the product is considered to have a high resistance risk and only three sprays are recommended per season. Fenhexamid can serve as an essential tool in anti-resistance management strategies (Rossenlenbioich and Stuebler, 2000).

Bissett (Bissett, 2002a) has shown that post-harvest *Botrytis* infection can be reduced considerably by drying the flowers after hydration is complete, but before grading. This can be achieved by placing the buckets containing harvested stems in front of fans for 30 minutes in the grading halls. No work has been carried out to determine the effect of using both fan drying and post-harvest sprays of specific botryticides on the incidence of post-harvest *Botrytis* infection.

Materials and methods

Pre-harvest: One bay (3 beds of 2 rows x 39m) of roses (cultivar Annabelle) was left unsprayed with fungicides active against *Botrytis cinerea* for approximately 6 weeks prior to the commencement of the trial. This length of time was required as weather conditions were not conducive to *Botrytis cinerea* development. This bay was not harvested for two days prior to the commencement of the experiment to ensure that there were sufficient stems at about the correct cut-stage. A total of 350 individual rose stems were cut and placed in buckets containing 10cm depth of standard post-harvest solution (0,5ml 3,5% sodium hypochlorite + 1ml teepol + 0,5g aluminium sulphate per litre of dam water). Each bucket contained 35 stems.

A 5 x 2 factorial arrangement of spray treatments and an unsprayed control treatment was made using buckets of 35 rose stems. The treatments were either fanned or not (five treatments x two fanning regimes) before being

bunched and boxed. There were three replications arranged in a randomized complete block design both in the box for simulated overseas transport and in the vase life room (Table 1 and Figure 1). The plot size was a bunch of 10 flowering stems of 35 cm length.

Table 1. Treatments combinations used in the experiment

No.	Treatment	Fan	Product per 100 litres water
1	No spray	no	----
2	No spray	yes	----
3	*V90	no	18mls
4	V90	yes	18mls
5	Rovral 250SC + V90	no	200mls + 18mls
6	Rovral 250SC + V90	yes	200mls + 18mls
7	Scala 400SC + V90	no	120mls + 18mls
8	Scala 400SC + V90	yes	120mls + 18mls
9	Teldor 500SC + V90	no	75mls + 18mls
10	Teldor 500SC + V90	yes	75mls + 18mls

*Volcano 90 (V90) - a non-ionic wetting agent, SC – Soluble Concentrate

Immediately after harvest, sprays were applied, until runoff, to the rose buds and uppermost leaves of the 35 stems in each bucket using a Factor15 knapsack sprayer fitted with a Lurmack 30 HC6 hollow cone nozzle. The knapsack was thoroughly cleaned between each treatment mix and application. After application, buckets of stems were placed in a holding room (15°C) and allowed to hydrate for 1hour 15 minutes.



Fig. 1. Layout of experiment

Key to experimental layout:

ROW	EXPERIMENTAL RANDOMIZATION	UNITS
TOP ROW: BLOCK 1	*T ⁷ _{VF} ; T ¹⁰ _{TNF} ; T ¹ _F ; T ³ _{RNF} ; T ⁴ _{SF} ; T ² _{VNF} ; T ⁸ _{RF} ; T ⁵ _{TF} ; T ⁶ _{NF} ; T ⁹ _{SNF}	
MIDDLE ROW: BLOCK 2	T ⁹ _{SF} ; T ¹ _{NF} ; T ⁵ _{TF} ; T ⁴ _{SNF} ; T ⁶ _F ; T ³ _{RF} ; T ¹⁰ _{TNF} ; T ⁸ _{RNF} ; T ² _{VF} ; T ⁷ _{NF}	
BOTTOM ROW: BLOCK 3	T ¹ _{NF} ; T ³ _{RNF} ; T ¹⁰ _{TF} ; T ² _{VNF} ; T ⁸ _{RF} ; T ⁴ _{SF} ; T ⁶ _F ; T ⁷ _{VF} ; T ⁵ _{TNF} ; T ⁹ _{SNF}	

*T¹⁻¹⁰: Treatment number; V: V90; F/NF: Fanned/Not Fanned; R: Rovral; S: Scala; T: Teldor

Bunches from treatment numbers 2, 4, 6, 8 and 10 were boxed separately from treatment numbers 1, 3, 5, 7 and 9. In each box bunches were arranged by blocks. Block 1 bunches were arranged at the bottom of boxes. Block 2 bunches were placed on top of block 1 bunches and block 3 bunches at the top on top of block 2 bunches. Thus, each of the two boxes contained 15 bunches. After the boxes had been sealed, they were placed on the pre-cooler in the coldroom (2°C) for 1 hour to ensure the rapid cooling to 2°C. The boxes were left in the cold room overnight, then the following morning the boxes were removed from the 2°C cold room at 0800 h and placed in the 15°C cold room at the Flora Marketing trial site until 1700 h. This 9 h period at 15°C was to stimulate the increase in temperature, which can be expected on the 9 h flight to overseas markets. At 1700 h, the boxes were once again placed in a 2°C cold room where they remained for two and a half days before being removed and the flowering stems placed in vases. This 2.5-day period at 2°C was to simulate the possible storage period at overseas destinations before the flowers are removed from boxes and sold.

After being held at 2°C for 2.5 days the boxes were transported to the Flora Marketing vase life room in Belgravia where the bunches of stems were placed into vases containing borehole water which had been sterilized with 0.5mls (3.5%) sodium hypochlorite per litre the day before. Two centimeters were cut off the bottom of each stem before the flowers were placed in the vase. The vases were arranged in blocks to remove any variation, which may have arisen due to temperature differences within the vase life room. Vases in block 1 were arranged on the top shelf, those in block 2 on the middle shelf and those in block 3 on the bottom shelf. Vase position within each block was determined randomly (Ryner, 1969). The vase life room was maintained at a temperature of 21 - 22°C and a relative humidity range of 49 – 64%. Light conditions were 12 hours light and 12 hours dark.

After 7 days in the vase life room the flowers in each vase were assessed for infection by Botrytis. The symptoms of infection were browning of the

petals (Figure 2). Note was also made of the condition of flowers to determine if any treatments had an adverse effect on the ability of flowers to open normally or caused other forms of phytotoxicity.

The numbers of flowers infected with *Botrytis* in each vase was converted to a percentage. Since the data were not normal, they were transformed (angular transformation) for analysis (Ryner, 1969). Analysis of variance (ANOVA) was done using GENSTAT Version 11. The Least Significant Differences (LSD) test at $P < 0.05$ was done.

Results

There were significant differences ($P < 0.05$) between the fungicide treatments (Table 2). Rovral and Teldor in combination with fan drying reduced *Botrytis* infection by about 9.6% compared to no fan drying and no signs of phytotoxicity or wilting were observed in any of the treatments. Rovral had the least *Botrytis* counts (9.2), followed by Teldor (13.0), though these two were not significantly different from each other.

Table 2. Mean percent *Botrytis* infected flowers assessed after 7days in the vase

Treatment	Mean <i>Botrytis</i> counts
Rovral	9.2 ^{a#}
Teldor	13.0 ^{ab}
V90	14.4 ^b
Scala	22.6 ^c
Nil (Control)	31.3 ^d
LSD: ($P < 0.05$) = 15.01	
SE = 5.05	

Within a column any means followed by the same letter are not significantly different at $P < 0.05$



Fig. 2. Gray mould infected stem (left) and healthy stem (right)

With the Scala treatment, no interaction between fan drying and fungicide treatment was apparent and high mean *Botrytis* counts were obtained (22.6). On the other hand, the V90 treatment (wetting agent) had significantly lower *Botrytis* infection compared to the Scala treatment.

Discussion

The Scala treatment had been regularly used over the last 3 years and the results obtained above could be linked to resistance development. The reduction of infection by fanning is supportive of O'Neill, Shtienberg and Elad (1997) and Hausbeck and Moorman, (1996) who indicate that dormant spores on the flower petal surfaces only become active in the presence of a free film of moisture. Hence, removal of that free moisture from the flower surfaces by fan drying reduced the incidence of *Botrytis* disease. Though the fungicide x fanning treatment interactions showed no significance, practically however, the drying, which is achieved by fanning the cut roses, has a great effect on reducing *Botrytis* infection as this removes any excess moisture on plant surfaces. The observation that the V90 treatment had significantly lower *Botrytis* infections compared to the Scala treatment might be a result of the wetting agent (V90) reducing the surface moisture of the cut flowers and hence leading to drier conditions. It is also possible that the wetting agent washed off some of the spores from the surfaces of the flowers, during its application, thus reducing the level of *Botrytis* infection.

Conclusion

Rovral was most effective in achieving post-harvest control of *Botrytis cinerea* in cut rose flower. Post-harvest fan drying is a useful method to reduce the incidence of *Botrytis* infections. A combination of a botryticide spray and fan drying is more effective than fan drying alone in preventing post-harvest *Botrytis* infections.

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