Fungicide Efficacy Against *Botrytis cinerea* at Different Positions on Grape Shoots

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Fungicide efficacy at different positions on grape shoots was determined on vinelets inoculated with *Botrytis cinerea*. To ensure proper fungicide coverage, vinelets (table grape cultivar Dauphine, wine grape cultivar Merlot noir), prepared from cuttings, were sprayed in a spray chamber at the recommended dosages with iprodione, pyrimethanil, cyprodinil/fludioxonil and fenhexamid. Vinelets for the control treatment were left unsprayed. After 24 h the vinelets were dusted with dry, airborne conidia in a settling tower to reduce the sporadic occurrence of the pathogen on shoots and incubated for 24 h at high relative humidity (±93%) to promote infection. Fungicide efficacy was determined by observing intact vinelets for symptom expression at nodes, internodes, leaf blades, petioles and inflorescences, and by determining surface colonisation (shoots left unsterile) and penetration (surface-disinfested shoots) by isolation studies. Symptoms of *B. cinerea* decay did not develop on sprayed and unsprayed vinelets that were kept in dry chambers during the 2-week observation period. The pathogen, however, developed from the isolated parts. On the unsprayed vinelets for both cultivars *B. cinerea* incidences recorded on two media and the two sterility regimes were significantly higher on leaf blades than on the petioles, shoot pieces and inflorescences. The isolation studies showed that the different fungicides were highly and nearly equally efficient in reducing superficial *B. cinerea* and latent infection at the various positions. In the case of leaf blades *B. cinerea* incidence was significantly reduced by each fungicide on both cultivars. This was not the case for the other parts, which yielded *B. cinerea* at low incidences under the two sterility regimes. The study showed that the fungicides used in this study, if applied properly to shoots at the pre-bloom stage, should effectively reduce the amount of *B. cinerea* in leaves and completely prevent the infection of nodes, internodes and inflorescences.

Botrytis cinerea Pers.: Fr., a pathogen of grapevine (*Vitis vinifera* L.), can infect most of the plant's organs. However, studies with *B. cinerea* on various aspects such as timing of fungicide application, biological control, host resistance and disease prediction models usually consist of investigations on bunches. The rationale for this is that the most prominent phase of the disease is found on berries (Harvey, 1955; McClellan & Hewitt, 1973; Nair, 1985). Incidence of disease severity is usually estimated by using rating scales on mature berries (Kremer & Unterstenhüfer, 1967; Pearson & Riegel, 1983; De Kock & Holz, 1991, 1994). These studies have resulted in the recommendation of four window periods for the control of *B. cinerea* in bunches (Pearson & Riegel, 1983; Nair, *et al.*, 1987; Northover, 1987; De Kock & Holz, 1991,1994; LeRoux, 1995).

Estimations of the amount of *B. cinerea* occurring at different positions on leaves and bunches in vineyards in the Western Cape province (Gütschow, 2001; Gütschow & Holz, 2002) showed that levels are higher during early season than generally assumed. Leaves and bunches were asymptomatic at pea size and bunch closure, but they carried high to very high amounts of *B. cinerea* at the various positions. Amounts of the pathogen were lower at véraison and harvest. Exceptions were leaf blades, which consistently carried high amounts, and the berry cheek, which constantly carried low amounts of the pathogen. These findings suggest that control of *B. cinerea* infection by cultural, chemical and biological means can only be achieved by reducing inoculum at the correct infection court and appropriate developmental stage. It is therefore important to consider the efficacy of fungicides on grapevine in terms of control at different morphological parts and infection courts. Little information is available on fungicide efficacy against dry, airborne *B. cinerea* conidia on different parts of grapevine. The aim of this study was to determine infection and fungicide efficacy at specific positions on shoots of grape vinelets inoculated with dry, airborne conidia of *B. cinerea*. It has been postulated that, following air and water dispersal, infection by solitary conidia should play a prominent role in the epidemiology of *B. cinerea* on grapevine (Coertze & Holz, 1999; Holz *et al.*, 2000; Coertze, *et al.*, 2001).

MATERIALS AND METHODS

Vinelets and fungicide sprays

Cuttings, obtained during July to August from two vineyards (table grape cultivar Dauphine; wine grape cultivar Merlot noir), were kept overnight in captab (5000 µg a.i./mL) (500 WP, Zeneca Agrochemicals SA) and stored at 4°C in moist perlite in plastic bags. Before an experiment, cuttings were removed from the bags and placed in warm water (50°C) for 30 minutes to eradicate harmful organisms and to stimulate even budbreak (Goussard & Orffer, 1979). Each cutting was cut into one-bud cuttings approximately 6 cm in length, which were inserted in holes in foamalite strips. The foamalite strips with cuttings were placed in stainless

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steel trays (53 x 28 x 2 cm) filled with tap water and kept at 25°C in a growth room at high relative humidity (85% RH) to initiate budbreak. Approximately 2 weeks after budding had commenced, when five to six leaves unfolded and the inflorescences were clearly visible (Eichhorn-Lorenz, Stage 12), the trays with vinelets were divided into two groups. Vinelets of one group were left unsprayed. The other trays with vinelets were placed in a spray chamber and sprayed at recommended dosages (Nel *et al.*, 1999) with iprodione (Rovral Flo 255 SC, Aventis), fenhexamid (Teldor 500 SC, Bayer), cyprodinil/fludioxonil (Switch 62.5 WG, Syngenta) or pyrimethanil (Scala 40 SC, Aventis). Application was conducted through a window in the spray chamber that consisted of a steel framework (800 x 1410 x 660 mm [height x length x width]) covered with plastic. The fungicides were applied to prerunoff with a gravity-feed mist-spray gun (ITW DEVILBISS Spray Equipment Products) used at 2 bar. To ensure maximum coverage, the spray mist was allowed 1 min to settle on the vinelets, after which the trays were removed from the chamber and air-dried. After each spray, the chamber was well ventilated and cleaned before the next application. Following fungicide treatment, the vinelets were kept for 24 h at 22°C before inoculation.

Inoculation

A virulent isolate of *B. cinerea* (Coertze & Holz, 1999), obtained from a naturally infected grape berry, was maintained on potato-dextrose agar (PDA) at 5°C. For the preparation of inoculum, the isolate was first grown on canned apricot halves. Conidiophores from the colonised fruit were transferred to PDA in Petri dishes and incubated at 22°C under a diurnal regime (12 h near ultraviolet light; 12 h dark light). Dry conidia were harvested with a suction-type collector from 14-day-old cultures and stored at 5°C until use. Storage time did not affect germination (Spotts & Holz, 1996); the dry conidia could therefore be used in all experiments. For inoculation of the sprayed and unsprayed (control treatment) vinelets, 3 mg dry conidia were dispersed by air pressure into the top of an inoculation tower (Plexiglass, 3 x 1 x 1 m [height x depth x width]) according to the method of Salinas *et al.* (1989) and allowed to settle onto the vinelets that were positioned in the foamalite strips in two trays. Petri dishes with water agar (WA; 12 g Biolab agar, 1000 mL H_2O) and PDA were placed on the floor of the settling tower at each inoculation and percentage germination was determined after 6 h incubation at 22°C (100 conidia per Petri dish, three replicates). Germination varied between 92 and 99%. Following inoculation, the trays were placed in 12 ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). Each chamber was considered as a replicate and contained three trays with 20 vinelets per tray. The chambers were kept for 24 h at 22°C with a 12-h photoperiod. These conditions provided circumstances commonly encountered in nature by the pathogen on grapevine surfaces, namely dry conidia on dry surfaces under high relative humidity. Studies (Gütschow, 2001) with dry conidia of *B. cinerea* on grape vinelets under similar conditions showed that germination, surface colonisation and penetration reached a maximum during this period.

Assessment of *B. cinerea*

Following incubation, the sprayed and unsprayed vinelets were divided into three groups. Vinelets of the one group were surface sterilised in 70% ethanol for 5 sec, those of the other two groups were left unsterile. Vinelets of one unsterile group (one vinelet per replicate, per treatment) were placed in dry chambers and kept for 14 days at 22°C with a 12 h photoperiod. The vinelets were monitored for symptom expression and the development of *B. cinerea* at the internodes, nodes, leaf blades, petioles and inflorescences. Vinelets of the other unsterile group, and the sterile group, were used for isolation. From each of these vinelets four shoot segments consisting of a node and internode (approximately 20 mm each) and 10 each of leaf blades, petioles and inflorescenses were removed. Five each of the leaf blades, petioles, inflorescences and two each of the shoots segments were placed on Kerssies's *B. cinerea* selective medium (Kerssies, 1990) in Petri dishes. The remainder of the material was placed on water agar (WA) medium supplemented with paraquat [0.1 mL Paraquat/l WA] (WPK Paraquat, 200 g/L [bipyridyl], WPK Agricultural). The plates were incubated at 22°C under diurnal light and the sections were monitored daily for symptom expression and the development of *B. cinerea*. The presence of *B. cinerea* was recorded on the unwounded area of the different tissues only. During incubation the different treatments provided conditions that facilitated the development of conidia occurring on the surface of the shoots, or by mycelia in the tissue. Previous studies (Coertze & Holz, 1999; Coertze *et al.*, 2001; Gütschow, 2001; Volkmann, 2001) with grape bunch tissue on these media showed that no superficial mycelial growth developed on the sections during the first 3 to 5 days of incubation. Hyphal growth usually occurred from cells underlying the cuticle into the medium after 5 days, which indicated direct penetration by superficial conidia and the development of mycelia in the host tissue. On sections from unsterile shoots, disease expression was therefore the result of infection by surface inoculum and the development of mycelia in host tissue. *B. cinerea* incidences on segments from a specific position on the shoot therefore gave an indication of infection at that position as influenced by the amount of superficial conidia and mycelia. Surface sterilisation completely eliminated *B. cinerea* from the shoot surface (Sarig *et al.*, 1996; Coertze & Holz, 1999; Coertze *et al.*, 2001) and prevented the development of surface inoculum. Development of the pathogen from surface-sterilised shoot parts therefore gave an indication of infection at a specific position as influenced by mycelia in host tissue. Paraquat terminates host resistance in the cells of the cuticular membrane without damaging host tissue and thus facilitates the development of latent mycelia (Baur *et al.*, 1969; Cerkauskas & Sinclair, 1980; Pscheidt & Pearson, 1989; Grindrat & Pezet, 1994). The number of segments yielding sporulating *B. cinerea* colonies were recorded and the incidences calculated after 9 days. The incidences were used to quantify the amount of *B. cinerea* occurring superficially or in the tissue at the various positions on the shoots.

Statistical analysis

The experimental design was a split plot repeated in four blocks. The main plot treatments were cultivar, medium and fungicide treatment. The sub-plot treatment was surface-sterilised and not sterilised. Statistical computations were performed using SAS (SAS Institute Inc, Cary, NC). The data of the experiment, which was repeated, was subjected to analyses of normality of residuals ($P > 0.05$ = normality) using the Shapiro and Wilk test for nor-

mality (Shapiro & Wilk, 1965). The data were examined further by using the analysis of variance (ANOVA) and the treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor & Cochran, 1980). Levene's test for homogeneity of variance was performed to test the seasonal variability in observations of comparable magnitude.

RESULTS

Disease expression on intact shoots

No symptom of *B. cinerea* decay developed at any of the positions on shoots of the sprayed and unsprayed vinelets that were kept in dry chambers during the 2-week observation period.

Occurrence of *B. cinerea* on shoots used for isolation

The development of *B. cinerea* from the different positions followed a similar, constant pattern in both cultivars. Lesions were observed after 3 days on leaf blades and mostly developed from the area alongside the veins and from the leaf basis. On the petioles, shoot segments and inflorescences lesions were usually noted after 5 days. On shoot segments lesions developed first and more often from the nodes than the internodes. Levene's test for homogeneity of variance ($P>0.05$) indicated that the seasonal variability in data from each of the two complete experiments were of comparable magnitude and hence a combined analysis was validated. In the comparison of data no significant differences were found in *B. cinerea* incidence between cultivars, and in incidences between tissues incubated on the two media (Table 1). However, the ANOVA of data showed that the interaction of cultivar, fungicide treatment, medium, sterility regime and position had a highly significant effect ($P < 0.01$) on *B. cinerea* incidence. On the unsprayed vinelets for both cultivars, incidences recorded on the two media and the two sterility regimes were significantly higher on leaf blades than on the petioles, shoot segments and inflorescences (Table 2). *B. cinerea* incidences at the latter positions fluctuated on the two media and sterility regimes, but were consistently at a low level on the shoots. In both cultivars surface sterilisation of unsprayed vinelets reduced *B. cinerea* incidence at the different positions. This treatment reduced *B. cinerea* incidences on shoot segments to 6.3% on the paraquat medium and to 0% on the Kerssies medium. On leaf blades, which carried *B. cinerea* at high levels in the unsterile regime, incidences on both media were still high (≥60%) after surface sterilisation. The isolation and incubation studies showed that the different fungicides were highly and nearly equally efficient in reducing the amount of *B. cinerea*. In the case of leaf blades on unsprayed vinelets, which carried the pathogen at high levels under the two sterility regimes, incidences were significantly reduced by each fungicide on both cultivars. This was not the case on petioles, shoot segments and inflorescences, which yielded *B. cinerea* at low incidences under the two sterility regimes. Furthermore, on vinelets sprayed with fungicides, *B. cinerea* sporadically developed on one of the media from leaf blades and petioles, but seldom from shoot segments and infloresences.

TABLE 1

Analysis of variance of data for the effect of cultivar, fungicide treatment, medium, sterility regime and position on the percentage parts isolated from shoots on vinelets that developed *Botrytis cinerea*.

Source of variation	Df	MS	SL
Replicate	7	597.991	0.0034
Cultivar (C)	1	227.813	0.2695
Fungicide Treatment (FT)	4	45,194.648	0.0001
C x FT	4	79.961	0.7856
Medium (Med)	1	80.000	0.5122
C x Med	1	61.250	0.5663
FT x Med	4	1,932.930	0.0001
C x FT x Med	4	196.992	0.3773
Error(C x FT x Med)	133	185.256	
Sterility Regime (SR)	1	4,500.000	0.0001
C x SR	1	180.000	0.3661
FT x SR	4	1,912.695	0.0001
C x FT x SR	4	33.320	0.9618
Med x SR	1	0.313	0.9699
C x Med x SR	1	525.313	0.1237
FT x Med x SR	4	34.883	0.9585
C x FT x Med x SR	4	22.383	0.9816
Error(C x FT x Med x SR)	140	218.973	
Position (P)	3	17,989.479	0.0001
SR x P	3	265.833	0.0731
C x P	3	51.146	0.7188
C x SR x P	3	187.500	0.1781
FT x P	12	11,865.065	0.0001
FT x SR x P	12	51.445	0.9424
C x FT x P	12	103.294	0.5416
C x FT x SR x P	12	82.487	0.7302
Med x P	3	257.500	0.0805
Med x SR x P	3	63.646	0.6432
C x Med x P	3	248.750	0.0891
C x Med x SR x P	3	93.646	0.4828
FT x Med x P	12	341.680	0.0004
FT x Med x SR x P	12	58.633	0.9069
C x FT x Med x P	12	205.326	0.0443
C x FT x Med x SR x P	12	290.716	0.0026
Error(C x FT x Med x SR x P)	840	114.159	

DISCUSSION

Laboratory studies with asymptomatic mature leaves obtained from vineyards (Gütschow, 2001; Gütschow & Holz, 2002) showed that they generally carry high amounts of *B. cinerea*. Young leaves on shoots of grape vinelets, and older leaves from vineyard shoots also remained asymptomatic after inoculation with *B. cinerea* (Gütschow, 2001). In this study fungicides were applied in a spray chamber to shoots on vinelets and inoculation was conducted with dry, airborne conidia in a spore settling tower. These systems ensured proper fungicide coverage (G. Holz, unpublished data) and reduced sporadic occurrence of *B. cinerea* (Coertze & Holz, 1999; Coertze *et al.*, 2001), thereby allowing uniform evaluation of fungicide efficacy at the different positions on shoots. The isolation studies confirmed that solitary conidia readily penetrated leaf tissue and that latent infection was established at very high levels in leaf blades. Latent infection levels were low in petioles, nodes, internodes and inflorescenses. The fungicides, which belonged to different chemical classes, all effectively reduced the amount of *B. cinerea* in leaves and completely prevented infection of nodes, internodes and inflorescences.

The finding that leaf blades carry high levels of latent *B. cinerea* mycelia suggest that leaf infection is an important pri-

TABLE 2

Means[v] of the effect of the interaction of cultivar x fungicide treatment x medium x sterility regime x position on the percentage parts isolated from shoots on vinelets, sprayed with fungicides and inoculated with airborne *Botrytis cinerea* conidia, that yielded the pathogen on two different media[w].

	Dauphine								Merlot noir							
	Shoot Segment		Leaf Blade		Petiole		Inflorescence		Shoot Segment		Leaf Blade		Petiole		Inflorescence	
Fungicide Treatment	K	P	K	P	K	P	K	P	K	P	K	P	K	P	K	P
Control																
NS[x]	12.5 i-l	18.7 g-j	77.5 bc	95.0 a	30.0 f	47.5 e	27.5 fg	10.0 j-m	6.3 klm	12.5 i-l	67.5 cd	100.0 a	30.0 f	25.0 fgh	12.5 i-l	42.5 e
S[y]	0.0 m	6.3 klm	60.0 d	80.0 b	10.0 j-m	20.0 f-j	12.5 i-l	7.5 klm	0.0 m	6.3 klm	65.0 d	80.0 b	7.5 klm	22.5 f-i	10.0 j-m	12.5 i-l
Iprodione																
NS	0.0 m	0.0 m	0.0 m	0.0 m	12.5 i-l	0.0 m	0.0 m	0.0 m	6.3 klm	0.0 m	5.0 klm	0.0 m	5.0 klm	2.5 l-m	0.0 m	0.0 m
S	0.0 m	0.0 m	0.0 m	2.5 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	20.0 f-j	0.0 m	5.0 klm	0.0 m
Pyrimethanil																
NS	0.0 m	0.0 m	5.0 klm	7.5 klm	5.0 klm	2.5 lm	0.0 m	0.0 m	0.0 m	0.0 m	5.0 klm	10.0 j-m	0.0 m	5.0 klm	2.5 lm	5.0 klm
S	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	5.0 klm	0.0 m	2.5 lm	2.5 lm	0.0 m
Cypro/fludio[z]																
NS	0.0 m	0.0 m	7.5 klm	0.0 m	10.0 j-m	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	10.0 j-m	5.0 klm	10.0 j-m	5.0 klm	0.0 m	0.0 m
S	0.0 m	0.0 m	2.5 lm	0.0 m	2.5 lm	0.0 m	0.0 m	0.0 m	0.0 m	0.0 m	5.0 klm	2.5 lm	12.5 i-l	0.0 m	0.0 m	0.0 m
Fenhexamid																
NS	0.0 m	0.0 m	15.0 h-k	0.0 m	5.0 klm	2.5 lm	0.0 m	0.0 m	0.0 m	0.0 m	7.5 klm	7.5 klm	7.5 klm	0.0 m	0.0 m	0.0 m
S	0.0 m	0.0 m	5.0 klm	2.5lm	0.0 m	0.0 m	0.0 m	0.0 m	6.3 m	0.0 m	7.5 klm	2.5 lm	0.0 m	0.0 m	0.0 m	0.0 m

[v]Values in each column followed by the same letter are not significantly different according to the Student's t-test at P = 0.05. [w]K = Kerssies medium, P = paraquat medium.
[x]NS = not sterile. [y]S = surface sterilised. [z]Cypro/fludio = Cyprodinil/fludioxonil.

mary infection event and plays an important role in the epidemiology of the disease on grapevine. The role of latent *B. cinerea* mycelia on leaves in the epidemiology of grey mould on perennial strawberries is well established (Braun & Sutton, 1987; Sutton, 1998). Young leaves are highly susceptible to infection. However, these infections become latent and the fungus colonises the tissues and sporulates as the leaves senesce and die (Braun & Sutton, 1987, 1988). Conidia formed on dying and necrotic leaves are the principal source of inoculum for *B. cinerea* fruit rot epidemics. In a comparison of sanitation and fungicides for management of *B. cinerea* fruit rot of strawberries, Merteley *et al.* (2000) showed that leaf sanitation (removal of senescent and necrotic leaves) reduced fruit rot incidence compared to untreated controls. In grapevine sclerotia are a source of conidia that result in primary infection of young tissue (Nair & Nadtotchei, 1987). Thomas (1983) showed that the bulk of sclerotia recovered from vineyard soils in the Western Cape province developed on vine leaves and shredded prunings. Sclerotia were formed on infected leaves that form a mat on the soil in autumn, or are covered under soil during winter (Thomas *et al.,* 1981). The optimum temperature for sclerotial germination followed by infection was between 20°C and 25°C (Nair & Nadtotchei, 1987). Primary leaf infection that become latent is therefore likely prior to bloom when daily air temperature during spring reaches 20°C. Although these infections mostly remain asymptomatic (Gütschow, 2001), spots may develop on young leaves and the leaf infection sites can produce conidia abundantly during wet periods (Thomas, 1983; Nair & Hill, 1992), thereby contributing to the inoculum load that causes primary infection of nodes, internodes and inflorescences during the prebloom stage. These conidia can also contribute to the high inoculum load, which was shown with spore traps to occur early season in Western Cape vineyards (G. Holz, unpublished data), and to the high occurrence of the pathogen at various positions in bunches from bloom to bunch closure (Holz *et al.*, 1997, 1998; Gütschow & Holz, 2002). Most producers appear to be adhering to the recommendations (Pearson & Riegel, 1983; Nair *et al.*, 1987; Northover, 1987; De Kock & Holz, 1991,1994; LeRoux, 1995) that recommend four applications per season, namely at the end of flowering, at bunch closure, at véraison and three weeks before harvest. The findings that young leaves are highly susceptible to infection, that inflorescences are infected by *B. cinerea* shortly after budburst, that the amount of natural latent *B. cinerea* mycelia in leaves (Gütschow 2001) and inoculum levels in bunches (Gütschow 2001; Gütschow & Holz, 2002) are at their highest shortly after bloom and lowest prior to harvest, suggest that the timing of fungicide application should be reconsidered. Thus, to effectively reduce *B. cinerea* in grapevine, three preventative applications are recommended to reduce primary infection events: (a) between budding and pre-bloom to counteract primary leaf infection; (b) during bloom to pea-size stage to reduce the amount of the pathogen in clusters and to prevent colonisation of floral debris; and (c) at bunch closure to reduce the amount of *B. cinerea* at various positions of the inner bunch, especially for cultivars with tight bunches. The fungicides used in this study, if applied properly to shoots at pre-bloom, should effectively reduce the amount of *B. cinerea* in leaves and prevent infection of nodes, internodes and inflorescences.

It has been hypothesised that the development of bunch rot is most pronounced in cultivars that develop dense canopies and compact fruit clusters (Savage & Sall, 1983). This has resulted in the recommendation of various cultural practices that influence canopy management in order to reduce disease incidence and severity (Gubler *et al.*, 1987). The removal of basal leaves alters the microclimate within the grapevine canopy (English *et al.*, 1989) and reduces the development of Botrytis bunch rot (Gubler *et al.*, 1987). Debris provides an initial nutrient source for *B. cinerea* and increases available inoculum for subsequent infections (Savage & Sall, 1984). This study showed that grapevine leaves carry high levels of latent *B. cinerea* inoculum and that they could serve as a reservoir for inoculum and subsequent infections. Leaves removed from vines during pre-bloom and during the early stages of bunch development should therefore be removed from the vineyard to reduce conidial and mycelial inoculum for infection of susceptible bunch parts.

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