BOTRYTIS CINEREA BUNCH ROT OF TABLE GRAPES: COLONIZATION AND TIMING OF FUNGICIDE APPLICATION

Petrus Jurie de Kock

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Supervisor: Dr. G. Holz

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DECLARATION

I the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

SUMMARY

Colonization of table grape clusters by *Botrytis cinerea*, and the effects of various fungicide combinations on Botrytis postharvest bunch rot of table grapes were investigated in a series of trials over a 5-year period. Fungicide dip treatments *vs* fungicide sprays, as an alternative to cover inner parts of closed clusters with fungicide and to eradicate *B. cinerea* from infected floral parts, were also evaluated. The study demonstrates the ineffectiveness in the western Cape Province of early fungicide applications (bloom to marble size) to control Botrytis postharvest rot on table grapes. The disease was largely due to infection during storage by inoculum present in clusters at vérasion or at later stages. Therefore the most beneficial times for the application of fungicides were from bunch closure to ripening. Procymidone and prochloraz were more effectively applied as a dip treatment at vérasion than as a spray.

Although fungicides were applied at different times in various programmes, they were ineffective in inhibiting infection during storage. This was only achieved when grapes sprayed with fungicides were exposed to SO_2 during the storage period. Gamma radiation, combined with an SO_2 treatment, controlled postharvest Botrytis bunch rot of table grapes in cold storage more effectively than the standard practise of enclosing an SO_2 generator in boxes. Best control was obtained at a radiation dose of 2 kGy. Irradiation had no adverse effect on the quality of the grapes.

The advisability of a limited fungicide programme with only two dicarboximide treatments, namely at vérasion and before harvest, is discussed.

OPSOMMING

Die kolonisering van tafeldruiftrosse deur *Botrytis cinerea*, en die effek van verskillende fungisiedbespuitings op Botrytis naoesbederf van tafeldruiwe, is in 'n reeks van proewe oor 'n 5-jaar periode ondersoek. Fungisied doopbehandelings *vs* fungisiedbespuitings, as 'n alternatief om die binnedele van toe trosse met die chemikalie te bedek, en om *B. cinerea* vanaf geïnfekteerde blomdele uit te wis, is ook geëvalueer. Botrytis naoesbederf van tafeldruiwe kon nie in die westelike Kaapprovinsie met vroeë fungisiedbehandelings (blom tot albaster grootte) beheer word nie. Die siekte is die gevolg van infeksie gedurende opberging deur inokulum aanwesig op trosse tydens deurslaan of latere ontwikkelingstadia. Daarom was die mees gepaste tyd vir fungisiedtoediening vanaf korrelset tot met rypwording. Tydens deurslaan was procymidone en prochloraz meer effektief indien as 'n doopbehandeling toegedien.

Alhoewel fungisides op verskillende tye, en in verskillende programme toegedien is, kon dit nie infeksie gedurende opberging stopsit nie. Dit is wel verkry wanneer fungisiedbespuite druiwe aan SO_2 tydens opberging blootgestel is. Gammabestraling, in kombinasie met SO_2-beroking, beheer Botrytis naoesbederf van tafeldruiwe meer effektief as die standaard praktyk waar 'n SO_2-vel in die verpakking ingesluit word. Die beste beheer is verkry met bestraling teen 'n dosis van 2 kGy. Gammabestraling het nie die kwaliteit van die druiwe nadeling beïnvloed nie.

Die voordele van 'n beperkte fungisiedspuitprogram met slegs twee dikarboksimied-toedienings, naamlik met deurslaan en voor oes, word bespreek.

CONTENTS

1. COLONIZATION OF TABLE GRAPES BY *BOTRYTIS CINEREA* IN THE WESTERN CAPE PROVINCE

ABSTRACT

Colonization of table grape clusters by *Botrytis cinerea* was studied in the western Cape Province. Periods of greatest susceptibility were determined by estimating the following: natural occurrence of *B. cinerea* in clusters during different developmental stages; infection and Botrytis postharvest decay of grapes inoculated at defined phenological stages and covered with polyester bags during the growing season; Botrytis postharvest decay of clusters protected with the dicarboximide fungicide procymidone against natural infections at various stages of cluster development. No evidence was found for a relation between early infection and subsequent disease development or postharvest decay. Postharvest Botrytis decay was largely due to infection during storage by inoculum present in clusters at véraison or during later stages. The findings indicated that fungicide sprays should be applied during véraison and before harvest.

INTRODUCTION

Botrytis bunch rot, caused by *Botrytis cinerea* Pers., is the major decay of stored grapes in the western Cape Province of South Africa (Marais, 1985; Lourens, 1986). If not adequately controlled, Botrytis postharvest decay can cause substantial losses of table grapes during storage and export (Maude, 1980; Bulit & Dubos, 1988).

Strategies to reduce Botrytis bunch rot during storage and export are largely aimed at preventing berry infections during the growing season with fungicide sprays and to eradicate spores on grapes during storage with sulphur dioxide (SO_2) fumigation (Harvey, 1955a; Nelson, 1983; Pearson, Riegel & Massey, 1985; Eckert & Ogawa, 1988). The rationale for this is the association of the disease with early-season latent infections (McClellan & Hewitt, 1973; Nair, 1985; Nair & Parker, 1985) and infections of mature grapes favoured by late season rains or prolonged periods of high relative humidity (Harvey, 1955b; Jarvis, 1980). However, some aspects regarding the contribution of these two modes of infection to the development of Botrytis bunch rot of grapes are uncertain (Bulit & Dubos, 1988; English *et al.*, 1989).

Locally, little is known about the time of infection and its relation to postharvest Botrytis bunch rot of table grapes. The present study was therefore undertaken to identify the principal times of colonization of table grape clusters under local climatic conditions and hence the optimum times for protection of bunches against infection by *B. cinerea*.

MATERIALS AND METHODS

Inoculum

B. cinerea was isolated from infected grapes and maintained on potato-dextrose agar (PDA) at 20°C. Inoculum was prepared by transferring spores and mycelia from stock cultures to PDA and incubating these at 25°C under a diurnal regime (12 h white fluorescent light/12 h dark). Conidia were harvested by flooding 10 to 14-d-old cultures with sterile distilled water, dislodging conidia with a glass rod and filtering the suspension through two layers of cheesecloth. Spore counts were made with a haemacytometer and suspensions were diluted to approximately 1.2 spores x 10^6/ml. Clusters or berries were sprayed with inoculum until prior to runoff. Viability of inoculum at each inoculation period was verified by pipetting 1 ml-volumes onto water agar plates. Inoculum was spread evenly onto the surface with a sterile glass rod and the extent of spore germination determined after 24 h incubation at 20°C.

Vineyards

Commercial vineyards with a known history of Botrytis bunch rot in the Paarl and Hex River Valley areas were selected for the studies. Vines were trained to a slanting trellis and micro-irrigated. A standard program for the control of downy and powdery mildew, comprising of spray mixtures applied at a rate of 350 l/ha at 10 cm shoot length, 500 l/ha at flowering, 750 l/ha at pea size and 1000 l/ha from vérasion until harvest was followed by farmers in all vineyards. Sprays against downy mildew started at 10-15 cm shoot length and were applied with mistblowers biweekly until pea size. Fungicides used were mancozeb (Dithane M45 80% WP, FBC Holdings), folpet (Folpan 50% WP, Agrihold), fosetyl-Al/mancozeb (Mikal M 44/26% WP, Maybaker Agrikem) or mancozeb/oxadixyl (Recoil 56/8% WP, Bayer). Applications against powdery mildew started at 2-5 cm shoot length and were applied biweekly with mistblowers until 3 wk before harvest. Fungicides used were penconazole

(Topaz 10% EC, Ciba-Geigy), pyrifenox (Dorado 48% EC, Maybaker Agrikem) and triadimenol (Bayfidan 25% EC, Bayer).

Infection periods

Temperature and rainfall were recorded at weather stations at Bellevue Experimental Farm (Paarl) and De Doorns (Hex River Valley). Infection periods during each growing season were determined on the basis of the infection criteria of Sall, Teviotdale & Savage (1981). A rainy period was considered as conducive to the natural development of *B. cinerea* if more than 5 mm rain was recorded during 24 h (relative humidity ≥92%; average temperature 15-22°C), or if 1-5 mm rain fell on each of two consecutive days (relative humidity ≥92%; average temperature 15-22°C). In some vineyards temperature in and outside polyethylene bags, and the persistence of free surface water in grape clusters were monitored during the 24 h-incubation period with a CR 21 Micrologger (Campbell Scientific, Inc.: leaf wetness sensor model 731; temperature probe model 101). Conditions in the bags were considered favourable to infection when average temperatures were 15-22°C and free surface water was recorded for ≥15 h.

Colonization of grapes at phenological stages

Periods of greatest susceptibility were determined by estimating the following: natural occurrence of *B. cinerea* in clusters during different developmental stages; infection and Botrytis postharvest decay of grapes inoculated at defined phenological stages and covered with polyester bags during the growing season; Botrytis postharvest decay of clusters protected with the dicarboximide fungicide procymidone against natural infections at various stages of cluster development. The dicarboximides are known for their effective control of Botrytis bunch rot of wine grapes (Pearson & Riegel, 1983; Nair, Emmett & Parker, 1987)

Trial 1: During the 1984/85 season 480 clusters in each of four different vineyards (cv. Barlinka) were covered at the beginning of the blossoming period with polyester bags (Pallin, Formosa Harmony Intl. Inc.) that were sealed with wire ties to prevent natural infection by *B. cinerea*. The bags, which are made from non-woven pressed fibre, are partially water repellant, keep berries dry and dry very rapidly when partly wet. Preliminary studies showed that these bags do not interfere with fruit set or the development of established infections (P.J. de Kock, *unpublished*). The vineyards were regularly sprayed against downy (fosetyl-AL/mancozeb) and

powdery mildew (penconazole). Protected clusters were left unsprayed.

At defined phenological stages of cluster development Pallin bags were removed from all clusters. Half of the clusters were left uncovered for 10 d to enable natural infection by *B. cinerea* before the bags were replaced. The other clusters were immediately inoculated with *B. cinerea* and covered with polyethylene bags that were sealed with wire ties. These bags contained a little water to maintain high humidity during the infection period. Polyethylene bags were removed approximately 24 h after inoculation and the Pallin bags replaced.

Establishment of natural *B. cinerea* infections and susceptibility of clusters were monitored by random sampling of flowers or berries (50 per treatment) from each vineyard 10 d after replacement of Pallin bags. Pallin bags were removed and the clusters scrutinized for any signs of Botrytis infection. Flowers or berries were then cut at the rachis from the clusters, and the bags replaced. Flowers or berries were surface-disinfested for 1 min in 5% NaOCl and dried on filter paper in a laminar flow bench. Berries were halved with a sterile scalpel, the cut surface placed on PDA and incubated at 25°C in the dark. The percentage of flowers or berries with sporulating conidiophores of *B. cinerea* was recorded after 21 d.

The relation between time of infection and bunch rot was determined by monitoring pre- and postharvest decay of the different treatments. At harvest, bags were removed and bunches examined for symptoms of Botrytis bunch rot. Unblemished bunches were packed as for export with a SO_2 generator (0.3-0.55 g sodium metabisulfite affixed to a paper sheet [Laszlo *et al.*, 1981; Nelson, 1983]) inside a polyethylene bag in boxes (Patent no. RSA 75/6116). Grapes were stored at -0.5°C for 21 d followed by 7 d and assessed for Botrytis decay.

Trial 2: The relation between time of infection and bunch rot in vineyards of the cv. Barlinka was determined during the 1985/86 season as described for trial 1. Fungicides against downy and powdery mildew were applied as in the previous season. Natural infection was monitored by random sampling of 100 flowers (with calyptra dehisced) or berries at full bloom, pea size and véraison from the sprayed vines. Flowers or berries were cut, surface-disinfested and dried as described before. They were then placed on moist filter paper in 14 cm-diameter glass Petri dishes (5/dish), the dishes were covered to maintain about 95% relative humidity and incubated at 25°C in the dark. The percentage of flowers or berries with sporulating conidiophores of *B. cinerea* was recorded after 14 d.

Trial 3: During the 1985/86 season procymidone (Sumisclex 25% SC,

Agricura) or procymidone/sulphur (Sumisclex/sulphur 3/90% DP, Agricura) was used in various programs consisting of 3-9 applications, with each application being made at a defined phenological stage of cluster development between full bloom and ripening (Table 6). Vines (cultivar Barlinka) were sprayed with procymidone at 500 g a.i./ha in 1000 l of water/ha with an air-assisted handgun (pressure 2000 KPa) fitted with hollow cone nozzles. Procymidone/sulphur was applied at 525 g a.i./ha with a powder duster (Hatsuta multi-purpose power unit, Am-8 model "Blowmic"). Fungicide treatments were made to single row plots, each consisting of six mature vines. Each treatment was conducted as a complete randomized design with six replicates. In addition to the procymidone applications, vines were regularly sprayed against downy (fosetyl-AL/mancozeb) and powdery mildew (penconasole).

The effect of timing dicarboximide applications on infection of clusters was determined by monitoring pre- and postharvest bunch rot. At harvest, 16 bunches from the centre vines in each plot were collected and examined for bunch rot. The grapes were then divide in two equal groups. One group was packed with an SO_2 generator in polyethylene bags as described above and the other in polyethylene bags without an SO_2 generator. Grapes were stored as described before and assessed for postharvest decay.

Trial 4: The trial was conducted during 1987/88 in vineyards of table grape cultivars Waltham Cross and Barlinka. Mancozeb, folpet and mancozeb/oxadixyl were applied against downy mildew, and pyrifenox and penconazole against powdery mildew. Starting at pre-bloom, clusters (500 per cultivar) were additionally sprayed every 14 d with procymidone to protect them from natural infection against *B. cinerea*. The fungicide was applied with a knapsack sprayer (CP 3, Cooper, Pegler & Co.) fitted with hollow cone nozzles as described previously. At intervals of 18-21 d before a defined phenological stage of cluster develpoment, fungicide applications were interrupted. Half of the clusters were left unsprayed and unprotected to facilitate natural infection, whereas the other clusters were inoculated with *B. cinerea*. Inoculated clusters were covered with polyethylene bags as described above. Fungicide applications on all treatments were resumed 7-14 d after inoculation and applications were made until harvest.

At harvest, bunches were examined for Botrytis bunch rot. Unblemished bunches were packed, stored (without an SO_2 generator) and postharvest Botrytis decay determined as described above.

Trial 5: During the 1988/89 season bunches (cv. Barlinka) free of symptoms of Botrytis rot were selected at harvest from a vineyard which had not received sprays

against the disease. Regular fungicide sprays against downy and powdery mildew consisted of mancozeb/penconazole applications until pea size, followed with sulphur dusts until harvest. The bunches were divided in four equal groups. Two of the groups of bunches were surface disinfested by dipping in either 70% ethyl alcohol (10 sec) or NaOCl (1 g/l, 10 sec), then dried immediately under a flow of air. These bunches, as well as those of an untreated group, were packed in polyethylene bags in boxes without an SO_2 generator. Those of the last group were packed with an SO_2 generator. Grapes were stored at -0.5°C for 21 d followed by another 7 d at room temperature (approximately 25°C) and assessed for Botrytis decay.

Assessment of postharvest decay

Postharvest Botrytis decay was assessed according to the evaluation rating proposed by Unterstenhöfer (1963) for the infection of berries by *Plasmopara viticola* and the percentage decay of each replicate calculated with the formula of Kremer & Unterstenhöfer (1967). In some instances the percentage decay of each bunch was determined on a mass basis and the average decay per treatment calculated.

RESULTS

Inoculum

Spores used at each inoculation were highly viable and germinated freely on PDA. Germination varied between 80-98%, but was never less than 75%.

Colonization of grapes at phenological stages

Trial 1: Periods favourable to natural infection and chances for infection of clusters by *B. cinerea* in Pallin bags during the 1984/85 season are given in Table 1. Infection periods during each 10-d period when Pallin bags had been removed were recorded in the Paarl vineyards at pea size, marble size, vérasion and when ripe. In contrast, in the Hex River Valley infection periods occurred only during pea and marble size. Conditions in Pallin bags during the 24 h periods after inoculation were generally conducive to infection, except during full bloom in the Hex River Valley when an average temperature of 28.7°C was recorded.

TABLE 1. Timing of inoculation of table grape (cv. Barlinka) clusters with *Botrytis cinerea* during the 1984/85 season and periods favourable to infection

Location of vineyard	Timing of inoculation				
	Full bloom	Pea size	Marble size	Véraison	Ripe[a]
Paarl	09/11	06/12	03/01	31/01	28/02 (17/03)
Hex River Valley	13/11	10/12	08/01	04/02	11/04
	Chance for infection in polyethylene bags[b]				
Paarl	+	+	+	+	+ (+)
Hex River Valley	-	+	+	+	+
	Chance for natural infection of uncovered clusters[c]				
Paarl	-	+	+	+	- (+)
Hex River Valley	-	+	+	-	-

[a] Periods for vineyard A and B, respectively.

[b] Clusters were covered with polyester bags during the growing season. They were replaced with polyethylene bags during the 24 h incubation period.

[c] Clusters were left uncovered for 10 d to allow for natural infection by the pathogen.

The percentage of infected flowers or berries is given in Table 2. All clusters were free of Botrytis bunch rot at each sampling. The pathogen developed from material collected from vineyard A at each growth stage. In this vineyard significantly more naturally-infected berries were recorded at pea size than any other sampling period. In vineyard B no naturally-infected berries were collected during pea and marble size.

In the vineyards from the Hex River valley natural infections were not detected until véraison, and then only at very low frequencies.

Infected material was recovered during each sampling from inoculated clusters from vineyards in the Paarl area, but not from those sampled during full bloom and marble size (vineyard A) in the Hex River Valley. Highest incidences occurred on berries inoculated at pea size, except at vineyard B in the Paarl area where the highest percentage of infected berries was recovered from clusters inoculated at the ripe stage.

TABLE 2. Percentage table grape (cv. Barlinka) flowers or berries infected with *Botrytis cinerea* 10 d after inoculation, or after being subjected to natural infection

Timing of inoculation or natural infection	Flowers or berries infected (%)[a]							
	Paarl				Hex River Valley			
	Vineyard A		Vineyard B		Vineyard A		Vineyard B	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Full bloom	10	16	6	2	0	0	0	0
Pea size	33	35	0	25	0	31	0	25
Marble size	18	2	0	12	0	0	0	3
Véraison	16	13	12	10	0	12	4	10
Ripe	13	17	14	49	2	18	1	7
LSD[b]	14.93	20.08	11.78	21.38	2.02	4.29	1.75	7.74

[a] Clusters were covered with polyester bags during the growing season. Expt 1 = clusters left uncovered for 10 d at each phenological stage to allow for natural infection. Expt 2 = clusters sprayed with inoculum of *B. cinerea* and covered with polyethelene bags for 24 h. Each treatment consisted of 50 flowers or berries collected at random from clusters.

[b] According to Student analysis of variance LSD (P = 0.1).

All bunches were free from Botrytis bunch rot at harvest. However, no disease developed in any of the bunches during the normal storage period. The bunches were therefore kept at room temperature (approximately 25°C) for another week when Botrytis bunch rot was evident in some bunches.

Percentage Botrytis bunch rot of the different treatments is given in Table 3. Bunch rot developed in bunches from all treatments, and was generally more severe in inoculated than naturally infected bunches. Significantly more decay occurred on bunches from vineyard B from Paarl inoculated when ripe than inoculated during any other stage of cluster development. On the other hand, on bunches from vineyard A from the Hex River Valley, significantly more postharvest decay developed when they were inoculated during pea size than at full bloom, marble size or vérasion. Differences in decay of bunches, subjected to natural infection during different stages of cluster development, were not significant.

Lesions were scattered on the berry surface, but were not observed on peduncles. Lesions that could have originate from the stylar end were not seen.

TABLE 3. Percentage *Botrytis cinerea* postharvest decay of table grapes (cv. Barlinka) covered with polyester bags[a] during the 1984/85 growing season and inoculated or subjected to natural infection at defined phenological stages of cluster development.

Timing of inoculation or natural infection	Postharvest decay (%)[b]							
	Paarl				Hex River Valley			
	Vineyard A		Vineyard B		Vineyard A		Vineyard B	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Full bloom	38.3	34.6	19.9	55.0	0.8	20.7	4.6	22.5
Pea size	24.8	52.2	19.6	60.5	5.0	45.2	1.3	25.0
Marble size	16.7	39.5	18.5	45.8	5.0	6.4	3.3	24.0
Véraison	22.1	33.8	17.9	39.8	8.8	14.8	1.3	20.0
Ripe	25.8	37.3	31.7	84.4	6.3	24.7	5.0	6.5
LSD[c]	25.08	29.95	13.35	16.45	14.08	22.30	5.62	19.1

[a] See Table 1. Bunches were stored with an SO_2 generator for 43 d.

[b] Forty-eight bunches per treatment. Percentage decay calculated according to the formula of Kremer & Unterstenhöfer (1967).

[c] According to Student analysis of variance LSD (P = 0.1).

Trial 2: During the 1985/86 season three infection periods (data not shown) were recorded in the Paarl area. The first occurred at 5 November, 2 d prior to sampling at full bloom. The others were recorded during 29-31 December, 10 d before sampling at marble size, and during 13-14 January, approximately 2 wk before sampling at vérasion. In the Hex River Valley periods conducive to infection occurred approximately 1 wk before the full bloom-sampling and during 1-4 and 19 December, approximately 2 wk and 1 d prior to the pea size-sampling respectively. Dry weather prevailed during the rest of the season.

Percentage flowers or berries from both vineyards naturally infected by *B. cinerea* is given in Table 4. All clusters were free of any signs of Botrytis infection at each sampling period. However, the pathogen developed from apparently healthy and surface-disinfested flowers and berries obtained from both areas. In the Paarl vineyard highest incidence of natural infection was recorded during pea size. It was recorded only during flowering and marble size in the Hex River Valley, and then at very low incidences.

TABLE 4. Percentage table grape (cv. Barlinka) flowers or berries naturally infected by *Botrytis cinerea* at different stages of cluster development during the 1985/86 growing season

Growth stage	Sampling date	Flowers or berries infected (%)[a]	
		Vineyard A[b]	Vineyard B[c]
Full bloom	07/11	22	-
Full bloom	20/11	-	17
Pea size	11/12	27	-
Pea size	20/12	-	0
Marble size	10/01	13	-
Marble size	17/01	-	9
Véraison	05/02	13	-
Vérasion	26/02	-	0
LSD[d]		38.05	20.12

[a] One-hundred flowers or berries were collected at random at each phenological stage.

[b] Vineyard A = Paarl area.

[c] Vineyard B = Hex River Valley area.

[d] According to Student analysis of variance LSD (P = 0.1).

Except for inoculations made during véraison in the Hex River Valley, conditions in Pallin bags were generally conducive to infection by *B. cinerea* (data not shown). At harvest, all the bunches appeared free of infection loci. As during the 1984/85 trial, no bunch rot developed during the normal storage period. Boxes with grapes were therefore kept at room temperature (approximately 25°C) to enhance the development of *B. cinerea*.

Postharvest decay on bunches inoculated during the 1985/86 growing season is given in Table 5. Significantly more decay was recorded on bunches inoculated at véraison than during marble size (Paarl area) and those inoculated during véraison than at full bloom (Hex River Valley).

As observed during the 1984/85 season, lesions developed scattered on the berry surface. No Botrytis growth occurred on the peduncles or developed from the stylar end.

TABLE 5. Percentage *Botrytis cinerea* postharvest decay of table grapes (cv. Barlinka) covered[a] with polyester bags during the 1985/86 growing season and inoculated at defined phenological stages of cluster development

Timing of inoculation[d]	Postharvest decay (%)[b]	
	Paarl	Hex River Valley
Full bloom	17.9	63.7
Pea size	10.9	76.8
Marble size	5.7	69.2
Véraison	30.3	87.9
LSD[c]	19.58	19.51

[a] See Table 1. Bunches were stored with an SO_2 generator for 43 d.

[b] Forty-eight bunches per treatment. Percentage decay calculated according to the formula of Kremer & Unterstenhöfer (1967).

[c] According to Student analysis of variance LSD (P = 0.1)

Trial 3: Timing of procymidone application in three different vineyards during the 1985/86 season and periods conducive to the development of natural *B. cinerea* infection are given in Table 6. Infection periods occurred in all vineyards before the first application was made at full bloom. In the Paarl vineyards infection periods also occurred prior to application 4 (12 d after application 3 made at pea size), 2 wk before application 6 (1 wk after application 4) and 3 to 6 d before harvest (1 to 4 d after application 10). In the Hex River Valley vineyard an infection period was recorded on the day before application 2 (applied 3 wk after full bloom), 4 d before application 3 (applied at pea size) and 2 d before harvest (3 d after application 12).

The effect of time and frequency of procymidone application on the incidence of Botrytis postharvest bunch rot is given in Table 7. At harvest bunches from all treatments were free from any visible *B. cinerea* infection foci. Except for the Hex River Valley vineyard, Botrytis bunch rot developed during storage on bunches from all treatments that were packed without an SO_2 generator. On these grapes, procymidone treatments caused a drastic and significant reduction in the natural occurrence of the pathogen. Reduction in infection between the different procymidione programmes was not significant, except for vineyard B in the Paarl (programme H vs L and N).

TABLE 6. Timing of procymidone application in three vineyards during the 1985/86 season and periods favourable to natural infection with *Botrytis cinerea*

	Timing of fungicide application											
Vineyard[a]	Full bloom	3 wk after full bloom	Pea size	3 wk after pea size	6 wk after pea size	Véraison	1 wk after véraison	2 wk after véraison	3 wk after véraison	4 wk after véraison	5 wk after véraison	6 wk after véraison
A	07/11	26/11	17/12	07/01	-	28/01	06/02	10/02	18/02	25/02	-	-
B	12/11	26/11	18/12	07/01	-	28/01	06/02	10/02	18/02	25/02	05/03	11/03
C	14/11	04/12	23/12	15/01	04/02	18/02	24/02	05/03	11/03	18/03	25/03	01/04

	Infection periods (date)				
	November	December	January	February	March
A	5	29, 30	13, 14	19, 22	
B	5	29, 30	13, 14	19, 22	15
C	8, 9	1, 3, 4, 19			29

[a] Vineyards A and B = Paarl area; vineyard C = Hex River Valley area.

Natural occurrence of *B. cinerea* was drastically reduced by SO_2. With these grapes, nearly the same amount of decay occurred on bunches sprayed after full bloom than on those sprayed from vérasion onwards (vineyard A programme C vs K, L and O; Vineyard B programme B vs L and N; vineyard C programme A vs J, L and N).

Trial 4: During the 1987/88 season infection periods in the Waltham Cross and Barlinka vineyards were recorded only during pea size, whereas conditions in Pallin bags favoured infection during each inoculation period (data not shown). Percentage of postharvest decay after storage of each treatment is given in Table 8.

Notwithstanding the differential periods of inoculation and establishment of natural infection during cluster development, high incidences of decay were recorded from all treatments. Significantly more decay developed on Waltham Cross bunches inoculated at véraison than on those inoculated at other growth stages. On Barlinka on the other hand, postharvest rot on bunches inoculated during vérasion was significantly less than on those inoculated at any other stage of cluster development. Differences in decay between treatments subjected to natural infection were not significant.

TABLE 7. The effect of time and frequency of procymidone application in three different vineyards[a] on the incidence of postharvest *Botrytis cinerea* decay of table grapes (cv. Barlinka) during the 1985/86 growing season

Pro-gramme No	Timing of application[c]	Number of application	Postharvest decay (%)[b]					
			Vineyard A		Vineyard B		Vineyard C	
			$-SO_2$	$+SO_2$	$-SO_2$	$+SO_2$	$-SO_2$	$+SO_2$
1	A-F, H, J, L	9	-	-	-	-	0.00	0.42
2	A-D, F, H, J, L	8	-	-	16.08	0.73	-	-
3	A-D, F, H, J	7	5.77	1.25	-	-	-	-
4	B-F, H, J, L	8	-	-	-	-	2.32	0.00
5	B-D, F, H, J, L	7	-	-	25.90	0.00	-	-
6	B-D, F, H, J	6	7.73	3.27	-	-	-	-
7	D-F, H, J, L	6	-	-	-	-	1.67	0.00
8	D, F, H, J, L	5	-	-	34.22	0.00	-	-
9	D, F, H, J	4	4.58	1.25	-	-	-	-
10	F, H, J, L	4	-	-	24.07	4.32	4.58	0.00
11	F, H, J	3	15.02	1.15	-	-	-	-
12	F, H, J, L	4	-	-	14.03	0.00	3.67	0.37
13	F, H, J	3	18.15	1.27	-	-	-	-
14	F-L	7	-	-	13.87	0.00	0.00	0.00
15	F-J	5	8.08	2.08	-	-	-	-
16	Untreated	0	81.43	1.27	64.78	5.00	34.43	7.17
LSD[d]			15.32	4.31	18.55	5.53	12.81	6.48

[a] Vineyards A and B = Paarl area; vineyard C = Hex River Valley area.

[b] Forty-eight bunches per treatment. Bunches were stored either with, or without an SO_2 generator for 42 d. Percentage decay calculated according to the formula of Kremer & Unterstenhöfer (1967).

[c] A = full bloom; B = 3 wk after full bloom; C = pea size; D = 3 wk after pea size; E = 6 wk after pea size; F = véraison; G = 1 wk after véraison; H = 2 wk after véraison; I = 3 wk after véraison; J = 4 wk after véraison; K = 5 wk after véraison; L = 6 wk after véraison. For dates see Table 6.

[d] According to Student analysis of variance LSD (P = 0.1).

Trial 5: Periods favourable for the establishment of latent Botrytis infections, and the effect of surface disinfestation of clusters before storage on infection by *B. cinerea*, are given in Table 9. Successive infection periods favourable to the establishment of latent infections occurred during the critical stages of full bloom, pea size and vérasion. Dry weather prevailed before harvest. Surface disinfestation of clusters with ethanol, NaOCl and SO_2 caused a drastic and significant reduction in infection by *B. cinerea* during storage in spite of the high probability of latent infections. As in the previous experiments, lesions developed primarily on the berry surface, and rarely on peduncles. Lesions developing from the stylar end were not observed.

TABLE 8. Percentage *Botrytis cinerea* postharvest decay of table grapes regulary sprayed[a] with procymidone during the 1987/88 growing season and inoculated or subjected to natural infection at defined phenological stages

Timing of inoculation or natural infection	Postharvest decay (%)[b]			
	Waltham Cross		Barlinka	
	Expt 1	Expt 2	Expt 1	Expt 2
Full bloom	75.9	74.7	95.2	76.7
Pea size	81.3	79.2	95.9	74.7
Véraison	98.0	83.8	67.4	75.4
Ripe	87.5	75.3	100.0	68.3
LSD[c]	6.25	8.73	5.14	12.72

[a] Clusters were sprayed (Waltham Cross, 8 applications; Barlinka, 9 applications) every 7-14 d from prebloom until harvest. Expt 1 = applications were stopped 13-31 d before the defined phenological stage, clusters were inoculated and fungicide applications resumed after 7-14 d; expt 2 = clusters subjected to natural infection during periods left unsprayed.

[b] Forty-eight bunches per treatment. Bunches were stored without an SO_2 generator for 35 d. Percentage decay calculated according to the formula of Kremer & Unterstenhöfer (1967).

[c] According to Student analysis of variance LSD (P = 0.1).

TABLE 9. Effect of pre-storage surface disinfestation on *Botrytis cinerea* postharvest decay of table grapes (cv. Barlinka), harvested from a commercial vineyard[a] when ripe

Treatment	Postharvest decay (%)[b]
Ethyl alchohol[c]	7.3
Sodium hypochlorite[d]	4.5
SO_2[e]	5.2
Untreated	67.4
LSD[f]	6.45

[a] Infection periods in the vineyard were recorded on the following dates: 2 and 8 November; 10, 18, 19, 24 and 27 December; 31 January; 1, 12, 24 and 28 February; 1, 12 and 13 March.

[b] Forty-eight bunches per treatment. Bunches were stored without an SO_2 generator for 56 d. Percentage decay was assessed on a mass basis.

[c] Dipped in 70% ethyl alchohol for 10 sec and dried under a flow of air.

[d] Dipped in NaOCl (1 g/l) for 10 sec and dried under a flow of air.

[e] Sodium metabisulfite (0.3 g) affixed to a paper sheet.

[f] According to Student analysis of variance LSD (P = 0.1).

DISCUSSION

Evidence for the importance of early berry infections by *B. cinerea* and its relation to late season bunch rot is primarily circumstantial. On wine grapes in California (McClellan & Hewitt, 1973) and Australia (Nair, 1985; Nair & Parker, 1985), early rot or midseason bunch rot is ascribed to the ability of *B. cinerea* to infect immature grape berries via senescing flower parts, thus resulting in latent infections. At vérasion or later the fungus resumes growth and rots the grape. Savage & Sall (1982), however, were unable to detect the fungus in immature berries. Recently Pezet & Pont (1986) studied the effect of floral infections and the latency of *B. cinerea* on the cultivar Gamay. They demonstrated the presence of radioactively-marked mycelial filaments in pedicels and young berries of laboratory-inoculated clusters. McClellan & Hewitt (1973) found that inoculations with conidia increased later fruit infection only if made during bloom and that fungicide applications during bloom reduced infections that appeared months later. This finding was later substantiated in the Hunter Valley by Nair *et al.* (1987). Other researchers have questioned the need for fungicide application on wine grapes at bloom and have indicated good control of the disease with only two sprays beginning at vérasion (Lafon, Verdu & Bulit, Piglionica, Tarantini & Ferrara and Perez Marin, according to Pearson & Riegel, 1983; Pearson & Riegel, 1983).

In this study on table grapes, no evidence was found for a relation between early infections and subsequent disease development or postharvest decay. Postharvest Botrytis decay was largely due to infection during storage by inoculum present in clusters at vérasion or during later stages. This is clearly illustrated by the drastic reduction of infection on stored grapes by surface disinfestation, and by the experiments with Pallin bags.

During the 1984/85 season in the Hex River Valley, conditions were generally unfavourable for infection by *B. cinerea*. The pathogen could not be isolated from blossoms and from berries subjected to natural infection during the green berry stages. In spite of this, postharvest decay on bunches from these treatments did not differ significantly from those exposed to inoculum at more advanced stages of cluster development. Although the clusters were protected by Pallin bags, inoculum might have entered at the end of the season when bags had been damaged by wind. This might account for the development of postharvest decay on covered bunches that were free of *B. cinerea* when sampled during bloom.

The results of the procymidone timing trial also comfirmed the important role that late-arriving inoculum might play in the development of postharvest *B. cinerea*

bunch rot on table grape. Notwithstanding favourable infection periods that occurred during full bloom, pea size and vérasion, no significant difference in postharvest decay was found between programmes with applications during all the critical stages of cluster development and applications made from vérasion onwards. This does not necessarily imply that infections during bloom or during the green berry stages do not occur in the western Cape Province. Instead, infections occurring after vérasion mask those that occur earlier. Unripe grape berries are less susceptible to rot by *B. cinerea* than are ripe berries. The pathogen can, however, penetrate immature fruit at any growth stage (Nelson 1951; Kosuge & Hewitt, 1964; Bessis, 1972; Hill *et al.*, 1981). The fact that *B. cinerea* was consistently isolated from apparently healthy and surface-disinfested flowers and berries at all stages of cluster development, substantiate these findings and confirm the occurrence of latent infection. However, lesions that developed during storage occurred scattered over the the berry surface, and were rarely seen on peduncles. There was also no evidence of berry infections having arisen from latent infections of the stigma, as observed elsewhere (McClellan & Hewitt, 1973; Sparapano *et al.*, 1981; Nair, 1985; Nair & Parker, 1985).

On wine grapes, especially on cultivars with tight berry clusters, first symptoms of Botrytis bunch rot are generally evident by vérasion when sugar levels begin to increase (Sall *et al.*, 1981; Bulit & Dubos, 1988). Colonization of loose floral debris within clusters by the fungus has been observed and most probably serve as foci for infection at vérasion (Gessler & Jermini, 1985; Nair & Parker, 1985; Northover, 1987). The abscence of early bunch rot in table grape vineyards might be due to the grape bunches being looser, thereby allowing abscissed floral parts to drop from the bunch. As the berries are less compressed and the bunches better aereated, they may dry more rapid after a wet spell than in wine grape clusters. Spores of *B. cinerea* require prolonged periods of free moisture on surfaces of grape berries to germinate and to infect (Nelson, 1951). Also berry contact areas that are more susceptible to infection due to altered epicuticular wax (Marois *et al.*, 1986), would be less abundant on table grape berries.

Locally no control programme for Botrytis bunch rot of table grape based on the behaviour of the pathogen is followed and fungicides are applied on a routine basis of 5-11 applications during the growing season (P.J. de Kock, *unpublished*; Chambers, 1988) This study showed that properly timed fungicide sprays should provide a sound basis for control of Botrytis postharvest bunch rot. Therefore fungicide sprays at vérasion and before harvest, integrated with cultural practices (Gubler *et al.*, 1987; English *et al.*, 1989), would help to reduce inoculum, or its

effectivity on table grape at harvest.

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2. CONTROL OF *BOTRYTIS CINEREA* BUNCH ROT OF TABLE GRAPES: TIMING APPLICATION OF FUNGICIDES IN THE WESTERN CAPE PROVINCE

ABSTRACT

The effects of various fungicide combinations and the number of treatments on *Botrytis cinerea* postharvest bunch rot of table grapes were investigated in a series of trials over a 5-year period. Fungicide dip treatments *vs* fungicide sprays, as an alternative to cover inner parts of closed clusters with fungicide and to eradicate *B. cinerea* from infected floral parts, were also evaluated. This study demonstrates the ineffectiveness in the western Cape Province of early fungicide applications (bloom to marble size) to control Botrytis postharvest rot on table grapes. The disease was largely due to infection during storage by inoculum present in clusters at véraison or at later stages. Therefore the most beneficial times for the application of fungicides were from bunch closure to ripening. Procymidone and prochloraz were more effectively applied as a dip treatment at véraison than as a spray. The advisability of a limited fungicide programme with only two dicarboximide treatments, namely at véraison and before harvest, is discussed.

INTRODUCTION

Botrytis bunch rot, caused by *Botrytis cinerea*, is an annual threat to the quality of table grapes grown in the western Cape Province of South Africa (Marais, 1985; Lourens, 1986). It classically occurs during storage and has been attributed to infection of mature grapes following late-season rains (Harvey, 1955; Jarvis, 1980) and latent infection established earlier in the flowers (McClellan & Hewitt, 1973; Nair, 1985; Nair & Parker, 1985). In a recent study on colonization of table grape clusters under local climatic conditions (Part 1), no relation between infection during the early stages of cluster development and postharvest decay was found. Postharvest Botrytis bunch rot was largely due to infection during storage by inoculum present in clusters at véraison or at later stages.

Locally, no control programme for Botrytis bunch rot of table grape based on the behaviour of the pathogen is followed and fungicides are applied on a routine basis of 5-11 applications during the growing season (P.J de Kock, *unpublished*;

Chambers, 1988). Such attempts to maintain a protective coating of chemical on grapevines are not only uneconomical, but also bear little relation to the actual infection process. It has also been hypothesized that as the berries increase in size, penetration of fungicide into tightening clusters might become increasingly difficult (P.J de Kock, *unpublished*). Floral parts colonized by *B. cinerea* (Gessler & Jermini, 1985; Nair & Parker, 1985; Northover, 1987) could therefore remain unexposed and inner surfaces inadequately protected.

The objective of the present investigation was to identify the fungicide most effective against *B. cinerea*, to achieve maximum control of the disease with minimum use of fungicide, and to evaluate alternative means of fungicidal protection of bunches after closure.

MATERIALS AND METHODS

Inoculum

B. cinerea isolated from infected grapes was maintained on potato-dextrose agar (PDA) at 20°C. Inoculum was prepared, the viability thereof verified and clusters or berries inoculated as described previously (Part 1).

Vineyards

Commercial vineyards with a known history of Botrytis bunch rot in the Paarl and Hex River Valley areas were selected for the studies. Vines were trained to a slanting trellis and micro-irrigated. A standard program for the control of downy and powdery mildew, comprising of spray mixtures applied at a rate of 350 l/ha at 10 cm shoot length, 500 l/ha at flowering, 750 l/ha at pea size and 1000 l/ha from vérasion until harvest was followed by farmers in all vineyards. Sprays against downy mildew started at 10-15 cm shoot length and were applied every 14 d with mistblowers until pea size. Fungicides used were mancozeb (Dithane M45 80% WP, FBC Holdings), folpet (Folpan 50% WP, Agrihold), fosetyl-Al/mancozeb (Mikal M 44/26% WP, Maybaker) and mancozeb/oxadixyl (Recoil 56/8% WP, Bayer). Applications against powdery mildew started at 2-5 cm shoot lenght and were applied every 14 d mistblowers until 3 wk before harvest. Fungicides used were penconazole (Topaz 10% EC, Ciba-Geigy), pyrifenox (Dorado 48% EC, Maybaker) and triadimenol (Bayfidan 25% EC, Bayer).

Fungicides

The following fungicides were evaluated against *B. cinerea*: procymidone (Sumisclex 25% SC, Agricura), iprodione (Rovral 25% SC, Maybaker; Astryl 20% EC, Maybaker), vinclozolin (Ronilan 50% SC, Ronilan 50% EC, BASF), captab (Kaptan 50% WP, AECI), procymidone/sulphur (Sumisclex/sulphur 3/90% DP, Agricura) prochloraz (Sportak 45% EC, FBC Holdings), folpet (Folpan 50 50% WP, Makhteshim-Agan), benomyl (Benlate 50% WP, Agricura), chlorothalonil (Bravo 50% SC, Shell Chemical Division), thiram (Pomarsol 75% WP, Bayer), Mon 19001 (50% WP, Monsanto), chlozolinate (Serinal 40% SC, Shell Chemical Division) and iprodione/sulphur (Rovral/sulphur 3/90% DP, Maybaker). Fungicides formulated as emulsifiable or suspension concentrates were applied at 500 g a.i./ha in 1000 l of water/ha to run-off with an air-assisted handgun (pressure 2000 kPa) fitted with hollow cone nozzles. Dusting powders were applied at 525g a.i./ha with a powder duster (Hatsuta multi-purpose power unit, Am-8 model "Blowmic").

Experimental design and assessment of postharvest rot

Unless otherwise mentioned, fungicide treatments were made to single row plots, each consisting of six mature vines. Each treatment was conducted as a complete randomized design with six replicates. Postharvest Botrytis decay was assessed according to the evaluation rating proposed by Unterstenhöfer (1963) for the infection of berries by *Plasmopara viticola* and the percentage decay of each replicate was calculated with the formula of Kremer & Unterstenhöfer (1967). In some instances the percentage decay of each bunch was determined on a mass basis and the average decay per treatment calculated.

Evaluation of dicarboximides

In addition to the regular fungicide sprays against downy and powdery mildew (folpet and penconazole, respectively), procymidone, iprodione and vinclozolin were applied on a 6-schedule spray during 1984/85 on vines of the cultivar Barlinka. Applications were made at full bloom, 3 wk after full bloom, pea size, 3 wk after pea size, véraison and 1 wk before the first harvest.

At harvest eight bunches were collected from the centre vines in each plot. Bunches were examined for bunch rot and unblemished bunches packed as for export with an SO_2 generator (0.3-0.55 g sodium metabisulfite affixed to a paper

sheet [Laszlo *et al.*, 1981; Nelson, 1983]) inside a polyethylene bag in boxes (Patent no. RSA 75/6116). Grapes were stored at -0.5°C for 21 d followed by 7 d at 10°C and a further 7 d at room temperature (approximately 25°C). The percentage decay of each treatment was then determined.

Fungicide timing studies

To determine the critical phenological stage for protection against infection by *B. cinerea*, fungicides were used in several programmes. These comprised 1-9 applications, each being made at a defined stage of cluster development between bloom and ripening. The experiments were conducted during the 1984/85, 1986/87 and 1988/89 seasons. Dates and phenological stages at which fungicide applications were made are given in Table 2 and 3.

At harvest, 16 bunches were collected from the centre vines in each plot and examined for bunch rot. Unblemished bunches were packed, stored and the extent of decay determined as described above.

Fungicide dip treatments

Fungicide dip treatments *vs* fungicide sprays as an alternative to cover inner parts of closed clusters with fungicide and to eradicate *B. cinerea* from infected floral parts were evaluated in a series of experiments.

Trial 1: The effect of different fungicides on eradicating *B. cinerea* from infected necrotic flowers in grape clusters was determined during the 1986/87 season. The medium tight-cluster wine grape cultivar Riesling was selected to determine whether dead floral parts infected with *B. cinerea* contribute to postharvest bunch rot. Clusters were inoculated at full bloom to ensure a high proportion of infected flowers. At set periods after inoculation the clusters were dipped for 5 sec in a fungicide suspension (1000 mg a.i./l) of either procymidone, iprodione, prochloraz or captab. The periods were: 24 h after inoculation; 48 h after inoculation; 24 h after inoculation followed by a dip treatment every 7 d until 10 d before harvest; 48 h after inoculation followed by a dip treatment every 7 d until 10 d before harvest. Control treatments received no dip treatments. Fungicide sprays against downy mildew (folpet and fosetyl-AL/mancozeb) and powdery mildew (triadimenol) were applied as described before.

Inoculated clusters were inspected at 7 d intervals for dead floral parts. At each

inspection 40 necrotic flowers were collected at random and incubated on water agar (WA) in Petri dishes at 25°C in the dark. The percentage of flowers with sporulating conidiophores of *B. cinerea* was recorded after 14 d incubation. Follow-up fungicide treatments were applied after sampling of flowers.

At harvest 16 bunches were collected from the centre vines in each plot and examined for bunch rot. Unblemished bunches were packed in polyethylene bags in standard export boxes and stored for 33 d at -0.5°C, followed by 11 d at 10°C. SO_2 generators were enclosed to minimize the effect of late-arriving inoculum. Percentage Botrytis decay of each bunch was determined after storage on a mass basis and the average percentage decay for each treatment was calculated.

Trial 2: The effect of fungicide spray and dip treatments on inoculum administered to clusters at different stages of bunch closure was evaluated during the 1987/88 season. Clusters of the table grape cultivar Barlinka were inoculated at either full bloom, pea size or véraison. To ensure proper flower infection and formation of sufficient necrotic flowers, clusters inoculated at full bloom were first treated with fungicide at early pea size. Clusters inoculated at pea size or at véraison were treated 2 d after inoculation. Fungicides evaluated were procymidone, iprodione, prochloraz and folpet. They were applied either as a spray or a dip as described previously. Follow-up fungicide treatments were applied at véraison and 1 wk before harvest.

At the following periods 20 necrotic flowers were collected from each cluster: from clusters inoculated at full bloom 27 d after inoculation and 18 d after the first fungicide treatment; from clusters inoculated at pea size 1 d after inoculation and 18 d after the first fungicide application. Flowers were incubated on WA in Petri dishes as described above and the percentage flowers with sporulating conidiophores of *B. cinerea* recorded after 14 d.

At harvest 16 bunches were collected from the centre vines in each plot and examined for bunch rot. Unblemished bunches were packed in polyethylene bags in boxes without an SO_2 generator, stored for 33 d at -0.5°C followed by 11 d at 10°C. The percentage Botrytis rot of each bunch was determined on a mass basis and the average percentage decay for each treatment calculated.

Trial 3: The ability of fungicide spray and dip treatments to protect inner surfaces of bunches after closure against infection by *B. cinerea* was evaluated on bunches (cultivar Barlinka) treated at vérasion and inoculated thereafter. Vines were sprayed weekly during the 1987/1988 season with procymidone (during bloom)

and vinclozolin (during pea size) to protect clusters against natural infection. The spray programme commenced at pre-bloom and was stopped 3 wks before véraison. At véraison, procymidone, iprodione, benomyl or prochloraz were applied to bunches either by spraying or as a dip as described before. No fungicide was administered to bunches of the control treatment. All the bunches were inoculated with *B. cinerea* at intervals of 1, 7, 14 and 21 d after fungicide application.

Thirty-two bunches from the vines in each plot were collected 28 d after inoculation, packed in polyethylene bags in boxes stored for 34 d at -0.5°C, followed by 7 d at 10°C. To evaluate the fungicidal effect of the chemicals on late arriving inoculum, SO_2 generators were not enclosed in the boxes. The percentage Botrytis postharvest decay of each bunch was determined on a mass basis and the average percentage decay for each treatment calculated.

Trial 4: The effect of fungicide dip treatments on inoculum present on ripe bunches was evaluated during storage. Sound unblemished bunches of the table grape cultivar Barlinka, free from Botrytis bunch rot and obtained from a packhouse in Paarl during the 1987/1988 season, were inoculated by spraying with a *B. cinerea* spore suspension, dried, and packed in polyethylene bags in boxes. SO_2 generators were not enclosed in the polyethylene bags. Boxes with grapes were kept at room temperature. At 1, 12, 18, 24, 36 and 72 h after inoculation bunches were unpacked, dipped for 5 sec in either procymidone, iprodione, benomyl or prochloraz (1000 mg a.i./l), dried, and repacked. Control bunches were dipped in sterile distilled water. The grapes were then stored for 5 d at 25°C followed by 42 d at -0.5°C. The percentage Botrytis postharvest decay of each bunch was determined on a mass basis and the average percentage decay for each treatment calculated.

Infection periods

Temperature and rainfall for the 1984-88 growing seasons were recorded at weather stations at Bellevue Experimental Farm (Paarl), the Oenological and Viticultural Research Institute (Stellenbosch) and De Doorns (Hex River Valley). Infection periods during each growing season were determined on the basis of the infection criteria of Sall, Teviotdale & Savage (1981). A rainy period was considered as conducive to the natural development of *B. cinerea* if more than 5 mm rain was recorded during 24 h (relative humidity ≥92%; average temperature 15-22°C), or if 1-5 mm rain fell on each of two consecutive days (relative humidity ≥92%; average temperature 15-22°C). In some vineyards temperature in and outside polyethylene bags, and the persistence of free surface water in grape clusters were monitored

during the 24-h incubation period with a CR 21 Micrologger (Campbell Scientific, Inc.: leaf wetness sensor model 731; temperature probe model 101). Conditions in the bags were considered favourable to infection when average temperatures were 15-22°C and free surface water was recorded for ≥15 h.

RESULTS

Effectiveness of dicarboximides

Although infection periods (data not shown) were recorded before both harvests, Botrytis bunch rot was not seen on the bunches at harvest. Percentage postharvest decay of the different treatments is given in Table 1. All fungicides gave good control of postharvest decay, but only procymidone reduced the percentage decay significantly.

TABLE 1. Control of postharvest *Botrytis cinerea* decay of table grapes (cv. Barlinka) with dicarboximide fungicides[a] applied on a 6-schedule spray programme during the 1984/85 growing season in the Hex River Valley

	Post harvest decay (%)[b]	
Fungicide	**First harvest**	**Second harvest**
Procymidone	3.23	2.00
Iprodione	7.53	22.40
Vinclozolin SC	6.67	8.33
Vinclozolin EC	6.67	8.47
Untreated	38.12	45.10
LSD[c]	31.16	39.02

[a] Applied at full bloom (21/11), 3 wk after full bloom (11/12), pea size (01/01), 3 wk after pea size (22/01), véraison (12/02) and 1 wk before harvest (05/03). First harvest 12/03; second harvest 28/03.

[b] Forty-eight bunches. Bunches were stored with an SO_2 generator for 35 d. Percentage decay calculated according to the formula of Kremer & Unterstenhöfer (1967).

[c] According to Student analysis of variance LSD (P = 0.1).

Fungicide timing studies

In the 1984/85 growing season, infection periods occurred during late full bloom, early pea size, late pea size, vérasion and 1 wk before harvest (data not shown). Notwithstanding the more or less evenly distributed occurrence of these periods, all treatments gave good control of Botrytis postharvest rot (Table 2). The extent of rot on nonsprayed vines and those treated with either a six-spray schedule (five sprays between bloom and véraison; treatment No 1, 2), or a two-spray schedule applied after véraison (treatment No 5) differed significantly. However, differences in Botrytis postharvest decay between the differently scheduled procymidone applications were not significant. Applications made during the early stages of cluster development (bloom-véraison) were not essential to control postharvest bunch rot.

In the 1986/87 season infection periods occurred at the end of flowering and marble size (data not shown). However, no significant difference in the amount of decay was found between the unsprayed treatment and the differently-scheduled procymidone applications when bunches were stored with a SO_2 generator (Table 3). When stored without the SO_2 generators percentage decay of treatments No 1 and 4 differed significantly from the unsprayed treatment. Bunch rot control on vines that received four evenly-distributed applications (treatment No. 1) was also significantly better than on those sprayed only at full bloom. A spray programme with four procymidone applications (full bloom, pea size, véraison, 1 wk before harvest) reduced infection to the same extent as a programme with only two late season sprays (véraison, 1 wk before harvest). Finally, least control was recorded when sprays were applied pre-véraison.

The 1988/89 season generally favoured the natural development of *B. cinerea* and consecutive infection periods occurred during full bloom, pea size and vérasion (Table 3). On Barlinka, the various fungicide programmes, except No. 11, which was applied only pre-vérasion, reduced infection during storage significantly when compared with the untreated control. The exception was Waltham Cross, where only programme No. 5 had significantly less decay than the untreated control. Although different fungicides were applied in various programmes, they were ineffective in inhibiting infection during storage. This was achieved only when stored grapes were exposed to SO_2.

TABLE 2. Effect of time and frequency of procymidone application on the incidence of postharvest *Botrytis cinerea* decay of table grapes (cv. Barlinka) during two growing seasons

Treatment No.	Timing of application: Full bloom	3 wk after full bloom	Pea size	3 wk after pea size	Véraison	Wk after véraison: 1	2	3	Number of applications	Postharvest decay (%)[a]: -SO_2	+SO_2
1984/85 growing season[b]											
1	+	+	+	+	+	-	-	+	6	-	0.83
2	+	+	+	+	+	-	-	+	6	-	2.40
3	+	+	+	+	+	-	-	+	6	-	4.03
4	+	+	+	+	+	-	-	+	6	-	2.87
5	-	-	-	-	+	+	+	+	4	-	1.28
6	-	-	-	-	-	+	-	+	2	-	0.42
7	-	-	-	-	+	-	-	+	2	-	5.88
8	-	-	-	-	-	-	-	-	0	-	38.12
LSD[d]										-	22.17
1986/87 growing season[c]											
1	+	-	+	-	+	-	+	-	4	16.85	0.00
2	+	-	-	-	-	-	-	-	1	51.73	1.67
3	-	-	+	-	-	-	-	-	1	40.42	1.73
4	-	-	-	-	+	-	+	-	2	23.75	0.83
5	-	-	-	-	-	-	-	-	0	67.43	10.00
LSD[d]										30.16	19.06

[a] Forty-eight bunches per treatment. Bunches were stored with, and without an SO_2 generatorfor 35 d. Percentage decay calculated according to the formula of Kremer & Unterstenhöfer (1967).

[b] Trial conducted in a vineyard in the Hex River Valley. Fungicide applied on 21/11 (full bloom), 11/12 (3 wk after full bloom), 01/01 (pea size), 22/01 (3 wk after pea size), 12/02 (véraison), 19/02 (1 wk after véraison), 26/02 (2 wk after véraison), 05/03 (3 wk after véraison), 12/03 (harvest).

[c] Trial conducted in a vineyard in the Paarl area. Fungicide applied on: 25/11 (full bloom), 18/12 (pea size), 09/02 (véraison), 25/02 (2 wk after véraison), 04/03 (harvest).

TABLE 3. The effect of time and frequency of different fungicide applications during the 1988/89 season[a] on the incidence of postharvest *Botrytis cinerea* decay of table grapes

Programme No	Fungicide application[b]					Postharvest decay(%)[c]			
						Waltham Cross		Barlinka	
	Full bloom	Pea size	3 wk after pea size	Véraison	1 wk before harvest	$+SO_2$	$-SO_2$	$+SO_2$	$-SO_2$
1	Ct	Ct	Ct	I	I	0.05	32.50	0.25	25.83
2	F	F	F	I	I	0.06	36.00	0.50	48.00
3	B	B	B	I	I	0.00	32.33	0.37	22.67
4	T	T	T	I	I	0.00	16.17	0.16	36.17
5	I	F	F	I	I	0.00	32.83	0.12	44.17
6	P	P	P	P	P	0.00	41.50	0.13	25.00
7	M	M	M	M	M	0.05	39.83	0.37	61.33
8	Cz	Cz	Cz	Cz	Cz	0.06	38.83	0.23	21.50
9	-	-	-	I	I	0.00	32.17	0.34	45.33
10	I	I	I	-	-	0.08	56.67	1.41	66.50
11	I	I	I	I	I	0.00	43.17	0.66	33.50
12	I	I	I	I/S	I/S	0.00	26.17	0.38	12.67
13	-	-	-	-	-	0.06	45.17	1.49	81.83
LSD[c]						NS	20.02	NS	18.04

[a] Infection periods occurred in the Waltham Cross vineyard during 2 and 8 November; 10, 18, 19 and 24 December; 31 January; 1 February. They were recorded in the Barlinka vineyard during 2 and 8 November; 10, 18, 19 and 24 December; 31 January; 1, 12, 24 and 28 February; 1 March.

[b] Fungicide application on Waltham Cross: 16/11 (full bloom), 08/12 (pea size), 05/01 (3 wk after pea size) 31/01 (véraison) and 14/02 (1 wk before harvest). Fungicide application on Barlinka: 17/11 (full bloom), 09/12 (pea size), 06/01 (3 wk after pea size) 09/02 (véraison) and 28/02 (1 wk before harvest). Fungicides were: Ct = chlorothalonil; I = iprodione; F = folpet; B = benomyl; T = thiram; P = procymidone; M = Mon 19001; Cz = chlozolinate; I/S = iprodione/sulphur.

[c] According to Student analysis of variance LSD (P = 0.1).

Fungicide dip treatments

Trial 1: The percentage dead flowers supporting conidiophore formation of *B. cinerea* after each sampling period is given in Table 4. As indicated by the high incidences of infected flowers sampled from untreated clusters, conditions in the polyethylene bags were generally favourable for infection. Incidences of infected flowers obtained at each sampling from untreated clusters from which all floral debris were regularly removed were nearly constant at each sampling.

TABLE 4. Percentage dead flowers, obtained at intervals after inoculation[a] and fungicide application from clusters of the medium-tight cluster wine grape cultivar Riesling, with sporulating conidiophores of *Botytis cinerea* after 14 d incubation on water agar

	Flowers infected (%)[b]			
	Interval after inoculation (days)			
Treatment[c]	**8**	**15**	**22**	**34**
Prochloraz				
A	60	23	20	25
B	50	18	22	5
C	60	75	66	65
D	83	28	18	4
Iprodione				
A	7	22	32	0
B	3	3	0	8
C	0	8	24	40
D	7	5	0	0
Procymidone				
A	30	13	22	8
B	20	20	18	20
C	7	56	62	60
D	13	18	14	12
Captab				
A	37	15	32	4
B	17	2	0	0
C	27	3	12	10
D	33	13	10	0
Untreated				
E	53	58	48	55
F	73	74	52	60

[a] Inoculated at full bloom during 1986/87 in a vineyard at the Viticultural and Oenological Research Institute, Stellenbosch. Dead flowers sampled until pea size (34 d after inoculation).

[b] Forty dead flowers were collected at random at each interval from clusters of each treatment.

[c] Treatments: A = fungicide dip 1 d after inoculation; B = fungicide dip 2 d after inoculation; C = fungicide dip 1, 8, 15 and 22 d after inoculation; D = fungicide dip 2, 8, 15, and 22 d after inoculation; E = all dead flowers and floral debris removed at each interval; F = clusters inoculated but untreated with fungicide.

Iprodione and captab caused a drastic, although not consistent, reduction in the percentage dead flowers that supported sporulating conidiophores of *B. cinerea*. No or only a few infected flowers were collected at each sampling when these fungicides were applied on a 7-d schedule until pea size. Due to a drastic reduction in aborted flowers of all treatments by day 34, sampling was discontinued after pea size. Virtually no dead flowers could be found in bunches of the different treatments at marble size.

Except for two days of rainy weather on 21 and 22 January (2 d after fungicides were applied), no further infection periods were recorded. Bunches of the different treatments were free from grey mould at harvest, and no Botrytis postharvest decay was detected in bunches of any of the treatments after 44 d storage.

Trial 2: Cool wet weather conducive to the development of *B. cinerea* prevailed for the 5 d-period before and during the day of the first sampling of flowers. On the afternoon after the first fungicide treatment, 19.2 mm of rain fell whereas more infection periods occurred respectively 7 and 2 d before the next sampling. Therefore very high incidences (65-97%) of dead flowers that supported conidiophore formation of *B. cinerea* were recorded at each occasion. None of the fungicides caused a significant reduction in the percentage infected flowers (data not shown).

Treated bunches were thoroughly scrutinized for dead floral parts at vérasion. However, none was found, nor was Botrytis bunch rot detected at that stage.

After the wet spell during early pea size, no further infection periods were recorded. When packed, bunches from all the treatments were free from visible signs of *B. cinerea* infection.

Postharvest decay of bunches treated with fungicides at different stages of bunch closure is given in Table 5. The dicarboximides significantly reduced postharvest decay on bunches inoculated at full bloom and treated from pea size onwards. A similar trend was found on bunches inoculated at véraison and treated thereafter. When clusters were inoculated at pea size and treated afterwards, only procymidone, applied as a spray, had a significant effect. The fungicide gave best control during late season (inoculation at véraison) when applied as a dip treatment.

Prochloraz dip and folpet spray treatments gave a consistent reduction of postharvest decay irrespective of application frequency or the developmental stage at which clusters were inoculated. Prochloraz was ineffective when applied as a spray.

Trial 3: The ability of fungicide spray and dip treatments to protect inner surfaces of bunches after closure against infection by *B. cinerea* is given in Table 6. Procymidone gave a consistent and better control than the other fungicides, whereas a procymidone dip treatment protected bunches for a longer period against infection by *B. cinerea* than a spray. Iprodione, applied either as a spray or a dip, was not as effective as procymidone, and gave no control on bunches inoculated 21 d after the fungicide had been applied. Benomyl and prochloraz were ineffective in controlling bunch rot.

TABLE 5. Control of *Botrytis cinerea* postharvest decay of table grapes (cv. Barlinka) by different methods of fungicide application at defined stages of cluster development during the 1987/88 growing season[a]

	Postharvest decay (%)[b]					
	Full bloom[c]		Pea size[d]		Véraison[e]	
Fungicide	Spray	Dip	Spray	Dip	Spray	Dip
Procymidone	46.5	49.4	57.8	73.8	45.9	27.8
Iprodione	48.9	57.3	77.2	79.9	54.0	49.0
Prochloraz	78.3	45.3	86.9	45.0	73.3	41.4
Folpet	44.4	47.2	49.1	57.1	40.9	52.9
Benomyl	-	63.3	-	91.8	-	71.9
No treatment	81.4		94.1		97.6	
LSD[f]	22.04		18.18		24.02	

[a] Trial conducted in a vineyard in the Paarl area.

[b] Forty-eight bunches per treatment. Bunches were stored without an SO_2 generator for 35 d. Percentage decay was determined on a mass basis.

[c] Inoculated at full bloom; fungicides applied at pea size, véraison and 1 wk before harvest.

[d] Inoculated at pea size; fungicides applied at pea size, véraison and 1 wk before harvest.

[e] Inoculated at véraison; fungicides applied at véraison and 1 wk before harvest.

[f] According to Student analysis of variance LSD (P = 0.1).

TABLE 6. Protection of table grapes[a] (cv. Barlinka) against infection with *Botrytis cinerea* by fungicides applied at véraison by either a spray or a dip treatment

	Postharvest decay (%)[b]							
	Interval inoculated after fungicide treatment (days)							
	1		7		14		21	
Fungicide	Spray	Dip	Spray	Dip	Spray	Dip	Spray	Dip
Procymidone	31.8	42.8	38.8	47.7	56.7	44.4	78.9	66.9
Iprodione	39.0	66.2	53.7	54.4	69.8	51.0	90.6	95.2
Benomyl	74.5	86.7	78.1	73.9	82.6	92.6	74.2	85.9
Prochloraz	82.8	94.4	84.4	89.5	89.9	92.6	84.4	84.8
Untreated	62.0		88.7		96.5		95.2	
LSD[c]	16.11		33.92		24.87		25.27	

[a] Trial conducted during the 1987/88 growing season in a vineyard in the Paarl area.

[b] Twenty-four bunches per treatment. Bunches were harvested when ripe (28 d after véraison) and stored without an SO_2 generator for 35 d. Percentage decay was determined on a mass basis.

[c] According to Student analysis of variance LSD (P = 0.1).

TABLE 7. Postharvest *Botrytis cinerea* decay of table grapes (cv. Barlinka) harvested when ripe and inoculated[a] at set intervals after fungicide treatment

	Postharvest decay (%)[b]					
	Interval of fungicide application after inoculation (h)					
Fungicide	**1**	**12**	**18**	**24**	**36**	**72**
Procymidone	55.7	67.1	76.5	64.1	76.8	79.1
Iprodione	66.1	66.1	73.6	67.7	77.8	74.3
Benomyl	66.2	82.8	68.8	72.3	78.4	84.9
Prochloraz	20.7	40.6	46.2	50.2	54.0	64.7
Untreated	65.8	81.9	72.5	77.0	83.0	83.2
LSD[c]	15.78	18.59	14.28	16.31	11.10	15.20

[a] Bunches inoculated and treated after harvest.

[b] Eighteen bunches per treatment. Bunches were stored without an SO_2 generator for 47 d. Percentage decay was determined on a mass basis. Means transformed to arcsine.

[c] According to Student analysis of variance LSD (P = 0.1).

Trial 4: Postinfection activity of the different fungicides against *B. cinerea* is given in Table 7. Only prochloraz had a curative effect and reduced decay significantly when applied up to 72 h after inoculation.

DISCUSSION

This study demonstrates the ineffectiveness in the western Cape Province of early fungicide applications (bloom-marble size) to control *B. cinerea* postharvest rot on table grapes. Fungicide timing studies on vines combined with SO_2 fumigation during storage showed that postharvest decay did not relate to infections during flowering and green berry stages. There was also no evidence that berry infections arose from latent infections of the stigma, as observed on wine grapes in other countries (McClellan & Hewitt, 1973; Nair & Parker, 1985). A build-up of spores occurred on clusters during the period vèraison until harvest and fungicide applications restricted to this periode reduced the inoculum significantly. These findings confirmed those of a previous study (Part 1) which showed that postharvest bunch rot of table grapes is largely due to inoculum present at vérasion or during later stages. Other researchers have reported good control of Botrytis bunch rot of wine grapes at harvest using only two sprays beginning at vérasion (Lafon, Verdu &

Bulit, Piglionica, Tarantini & Ferrara and Perez Marin, according to Pearson & Riegel, 1983; Pearson & Riegel, 1983).

Although fungicides were applied at different times in various programs, they were ineffective in inhibiting infection during storage. This was only achieved when grapes sprayed with fungicides were exposed to SO_2 during the storage period. Sulphur dioxide does not kill the fungus inside the berry, but only eradicate spores on the surface (Harvey, 1955; Peiser & Yang, 1985; Marios *et al.*, 1986). On unsprayed bunches it drastically reduced the amount of Botrytis rot observed after storage. The killing effect was more pronounced when bunches were from vines sprayed with fungicides. Thus, apart from controlling preharvest bunch rot, late season fungicide applications enhance the effectivity of SO_2. This synergistic effect might be due to the lower spore levels against which SO_2 must operate.

In clusters of both the medium tight-clustered wine grape cultiver Riesling and table grape cultivar Barlinka, dead flowers infected with *B. cinerea* were found until marble size. At vérasion most of the dead flowers had abscissed. Clusters of table grape cultivars, which are not as tight as wine grapes, allow aborted flowers to drop from the bunch. Successive dip treatments with iprodione and captab eradicated the fungus successfully from dead flowers of Riesling. In spite of this, no indication was found that infected flowers contribute to the development of postharvest bunch rot of table grape.

Except for procymidone and prochloraz, no real advantage was found when bunches were dipped in fungicides to ensure better coverage. The eradicative effect of prochloraz dip treatments could be well used during late season when early winter rains precludes spraying.

In different parts of the world, dicarboximide-resistant *B. cinerea* developed in vineyards that had been treated for several years with these fungicides (Leroux & Clerjeau, 1985; Löcher, Lorenz & Beetz, 1987; Northover, 1988). Dicarboximides are currently applied at extensive rates in table grape vineyards in the western Cape Province (P.J. de Kock, *unpublished*; Chambers, 1988). Therefore, implementation of resistance management strategies for dicarboximide fungicides in grapes seems appropriate. This investigation shows that satisfactory control of bunch rot can be achieved with two dicarboximide treatments, i.e. at vérasion and before harvest. The downy mildew fungicides mancozeb, captan and folpet, which are normally applied during bloom and the green berry stages, proved to be as effective against bunch rot as the dicarboximides. The inclusion of these fungicides in the limited fungicide programme as outlined here will give additional activity against the pathogen during

the early season and might help to avoid the rapid build-up of strains of *B. cinerea* resistant to the dicarboximides.

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3. THE USE OF GAMMA RADIATION FOR CONTROL OF POSTHARVEST *BOTRYTIS CINEREA* BUNCH ROT OF TABLE GRAPES IN COLD STORAGE

ABSTRACT

Gamma radiation, combined with an SO_2 treatment, controlled postharvest Botrytis bunch rot of table grapes in cold storage more effectively than the standard practise of enclosing an SO_2 generator in boxes. Best control was obtained at a radiation dose of 2 kGy. Irradiation had no adverse effect on the quality of the grapes.

INTRODUCTION

Botrytis cinerea, the main decay pathogen of table grapes in storage (Hewitt, 1974; Nelson, 1985), is responsible for annual losses of more than R5 million (Marais, 1985; Lourens, 1986) in South Africa. The decay is largely due to spores present in bunches at harvest, or to the formation of late season latent infections (Parts 1 and 2). Fungicide application on grape vines at defined stages of cluster development is advocated for the control of *B. cinerea* (Eckert & Ogawa, 1988), but cannot prevent postharvest rot (Parts 1 and 2). Although postharvest fumigation with sulphur dioxide (SO_2) eradicates spores and prevents contact spread (nesting) effectively (Gentry & Nelson, 1968; Nelson & Ahmedullah, 1972; Nelson, 1983; Peiser & Yang, 1985; Kokkalos, 1986), the fungus still causes spoilage (Parts 1 and 2; Marios *et al.*, 1986; Eckert & Ogawa, 1988). Alternative control methods should be found to minimize losses due to *Botrytis* decay during storage and export.

The ability of gamma radiation to penetrate fruits and inactivate pathogens in established lesions deep in the host tissues (Beraha *et al.*, 1957; Eckert & Ogawa, 1988), offers a potential for therapeutic treatment of established infections. A study was therefore made to determine the feasibility of employing gamma radiation for the control of postharvest Botrytis bunch rot table grapes in cold storage.

MATERIALS AND METHODS

Table grapes

Experiments were conducted during the 1986/87-1988/89 seasons on table grapes of different cultivars, harvested as for export from commercial vineyards and packed in packhouses in the Paarl and Hex River Valley. The grapes, which were free from visible symptoms of Botrytis bunch rot when packed, were kept in polyethylene bags in boxes (Patent no. RSA 75/6116) used for export. SO_2 generators (0.3-0.55 g sodium metabisulfite affixed to a paper sheet [Lazlo *et al.*, 1981; Nelson, 1983]) were enclosed in polyethylene bags when appropriate.

Trial 1

Grapes of the cultivars Waltham Cross, Dan ben Hannah, Bien Donné, Alphonse Lavalleé and Barlinka were packed with a quarter of a standard SO_2 generator enclosed to suppress the development of Botrytis bunch rot during storage and transit. They were kept in cold storage for 2-13 d (depending on cultivar) before being sent by road (cold transit for 2 d) to Iso-Ster, Johannesburg, for gamma radiation. Boxes of each cultivar were divided in three equal groups and irradiated at doses of 1.5, 2 or 3 kGy. Grapes were kept after radiation for 4 wk at -0.5°C, followed for another week at 10°C and assessed for Botrytis bunch rot.

Trial 2

Boxes with grapes of the cultivars Waltham Cross and Barlinka were divided in three equal groups: with SO_2 generators enclosed; without SO_2 generators; without SO_2 generators, but grapes inoculated after packaging by spraying with a spore suspension of *B. cinerea* as described previously (Part 1). Boxes were kept in cold storage for varying periods, depending on the cultivar, before being sent by road (cold transit) for gamma radiation to Hepro, Cape Town or Iso-Ster, Johannesburg. Each treatment was then divided in three separate groups and the boxes irradiated at 1.5, 2 or 3 kGy. Grapes were kept after radiation for 4 wk at -0.5°C, followed for another week at 10°C before being assessed for Botrytis decay.

Trial 3

Bunches of Waltham Cross and Barlinka were packed in boxes without SO_2 generators, or with either 1/8, 1/4, 1/2, 3/4 or a complete SO_2 generator enclosed. They were kept in cold storage for 24 h and sent by road (cold transit) to Hepro, Cape Town, for gamma radiation. All treatments, exept the control, were irradiated at 2 kGy. Grapes were kept after radiation for 4 wk at -0.5°C, followed for another 2 wk at 10°C before being assessed for Botrytis decay.

Disease assessment

Postharvest Botrytis decay was assessed according to the evaluation rating proposed by Unterstenhöfer (1963) for the infection of berries by *Plasmopara viticola* and the percentage decay of each replicate was calculated with the formula of Kremer & Unterstenhöfer (1967). In some instances the percentage decay of each bunch was determined on a mass basis and the average decay per treatment calculated.

RESULTS

Trial 1

The percentage postharvest decay observed in the four experiments is given in Table 1. Gamma radiation caused no consistent reduction in decay of the different cultivars. Significant control of decay at all the dosages was obtained only with Barlinka.

No meaningfull differences in quality were observed between irradiated and non-irradiated grapes (data not shown). Like decay, gamma radiation had a differential effect on some of the cultivars. In the first experiment, radiation at 2 and 3 kGy caused a change in colour of the berries of grapes from Bien Donné. The yellow-green berries turned brown with dark longitudinal stripes. In the second experiment, no change in berry colour was observed on this cultivar. Radiation at 3 kGy resulted in a high percentage of soft berries of Waltham Cross, but only in the first experiment.

Trial 2

The percentage postharvest Botrytis decay on inoculated and uninoculated grapes is given in Table 2. Gamma radiation caused a significant reduction in the effectivity of inoculum on inoculated Barlinka bunches, but decay was still unacceptibly high. On uninoculated Barlinka grapes packed with or without SO_2 generators, irradiation significantly reduced Botrytis bunch rot. Best control was achieved when Barlinka grapes were packed with SO_2 generators and irradiated at 2 kGy.

No meaningfull differences in quality were observed between irradiated and unirradiated grapes (data not shown). Some berries developed cracks and were covered with bacteria and yeasts, whereas berries of Waltham Cross irradiated at Hepro, Cape Town, turned caramel-brown.

TABLE 1. Percentage *Botrytis cinerea* postharvest decay of table grapes[a], harvested during the 1986 season as for export from commercial vineyards and gamma irradiated by Iso-Ster, Johannesburg before cold storage

	Postharvest decay (%)[b]								
	Expt 1[c]			Expt 2[d]		Expt 3[e]		Expt 4[f]	
Radiation dose	WX	DbH	BD	DbH	BD	AL	WX	WX	Bar
1.5 kGy	9.60	73.32	41.64	36.82	25.88	70.44	15.28	14.02	4.88
2 kGy	11.86	64.80	28.78	24.36	17.38	38.92	10.86	10.00	2.88
3 kGy	12.98	64.64	40.06	22.84	11.74	35.98	7.28	3.67	3.07
Control	18.42	67.66	48.44	30.98	26.28	71.88	6.14	23.05	21.67
LSD[g]	NS	NS	NS	NS	14.49	25.44	NS	13.40	12.07

[a] Table grape cultivars: WX = Waltham Cross; DbH = Dan ben Hannah; BD = Bien Donné; AL = Alphonse Lavalleé; Bar = Barlinka.

[b] Forty bunches per treatment. Bunches were stored with a quarter of an SO_2 generator for 35 d. Percentage decay was calculated according to the formula of Kremer & Unterstenhöfer (1967).

[c] Expt 1 = grapes harvested in Paarl area on the 16 January, and irradiated on 30 January.

[d] Expt 2 = grapes harvested in the Hex River Valley area on 21 January, and irradiated on 14 February.

[e] Expt 3 = grapes harvested in the Hex River Valley area on 19 February, and irradiated on 6 March.

[f] Expt 4 = grapes harvested in the Hex River Valley area on 8 April, and irradiated on 15 April.

[g] According to Student analysis of variance LSD (P = 0.1).

TABLE 2. Percentage *Botrytis cinerea* postharvest decay of table grapes harvested during the 1987 growing season as for export from commercial vineyards and gamma irradiated by different institutions[a] before cold storage

	Postharvest decay (%)[b]			
	Waltham Cross[c]		Barlinka[d]	
Treatment and radiation dose	Hepro	Iso-Ster	Hepro	Iso-Ster
Inoculated[e], stored without SO_2				
1.5 kGy	63.40	100.00	57.26	97.72
2 kGy	63.08	99.52	44.13	72.48
3 kGy	54.53	100.00	53.25	98.85
Control	70.89	99.58	90.85	100.00
LSD[f]	18.60	1.17	10.79	4.87
Uninoculated, stored without SO_2				
1.5 kGy	32.00	37.58	45.69	53.80
2 kGy	13.53	29.73	32.81	47.12
3 kGy	33.76	27.08	43.12	50.95
Control	22.73	50.73	78.40	72.48
LSD[f]	14.54	21.31	13.14	10.93
Uninoculated, stored with SO_2				
1.5 kGy	0.17	10.37	7.38	37.42
2 kGy	0.19	7.62	5.13	39.00
3 kGy	0.25	5.53	8.54	37.78
Control	0.86	14.55	18.97	62.17
LSD[f]	1.04	10.76	6.14	19.58

[a] Hepro, Cape Town; Iso-Ster, Johannesburg.

[b] Forty-eight bunches per treatment. Bunches were stored for 42 d. Percentage postharvest decay in grapes treated by Hepro was determined on a mass basis. Percentage postharvest of grapes treated by Iso-Ster was calculated with the formula of Kremer & Unterstenhöfer (1967).

[c] Grapes irradiated by Hepro were harvested in the Paarl area on 18 February and irradiated on 19 February. Grapes irradiated by Iso-Ster were harvested in the Hex River Valley area on 17 March and irradiated on 24 March.

[d] Grapes irradiated by Hepro were harvested in the Paarl area on 19 March and irradiated on 20 March. Grapes irradiated by Iso-Ster were harvested in the Paarl area on 19 March and irradiated on 25 March.

[e] Inoculated after packaging by spraying with a spore suspension of the pathogen.

[f] According to Student analysis of variance LSD ($P = 0.1$).

Trial 3

The percentage postharvest Botrytis decay on irradiated grapes exposed to different SO_2 treatments is given in Table 3. On Waltham Cross, best control was achieved when irradiated grapes were stored with either a 3/4 or a complete SO_2 generator enclosed. However, reduction in decay was not significant when compared with the non-radiated control. On Barlinka no extra benefit was achieved by irradiation.

Irradiation had no adverse effect on the quality of the grapes. Some berries developed cracks and were covered with bacteria and yeasts, whereas berries of Waltham Cross turned caramel-brown.

TABLE 3. Percentage *Botrytis cinerea* postharvest decay of table grapes[a] irradiated at 2 kGy with gamma radiation before storage, and fumigated with SO_2 during cold storage

Radiation treatment and SO_2 generator[c]	Postharvest decay (%)[b]	
	Waltham Cross[d]	Barlinka[d]
Irradiated		
One eight	35.25	7.73
One quarter	21.86	3.62
Half	6.81	3.42
Three quarter	2.97	4.43
Full	5.24	3.94
None	60.32	30.11
Non-irradiated		
Full	13.23	4.76
LSD[f]	10.38	7.85

[a] Table grapes harvested during the 1989 season as for export from commercial vineyards.

[b] Forty-eight bunches per treatment. Bunches were stored for 42 d. Percentage postharvest decay was determined on a mass basis.

[c] Portion of a standard SO_2 generator (0.3 g sodium metabisulfite affixed to a paper sheet) enclosed in polyethylene bags.

[d] Harvested in the Paarl area on 27 February and irradiated by Hepro on 2 March.

[e] Harvested in the Paarl area on 21 March and irradiated by Hepro on 22 March.

[f] According to Student analysis of variance LSD (P = 0.1).

DISCUSSION

Gamma radiation combined with an SO_2 treatment, controlled postharvest Botrytis decay of table grapes in cold storage more effectively than the standard practise (Combrink *et al.*, 1978; Laszlo *et al.*, 1981) of enclosing an SO_2 generator in boxes. Best control was obtained at a radiation dose of 2 kGy. Shirzad & Langerak (1982) observed the same effect with a similar combination, but they stored grapes at a constant temperature of 10°C during the experiment. My findings also confirmed those of Bramlage & Couey (1975) (according to Thomas, 1986) who showed that gamma radiation alone was less effective in controling decay than the standard SO_2 sheet.

Browning of Waltham Cross berries at a dosage of 3 kGy was similar to that observed by Matthee & Marais (1963) at higher dosages. Although better control was achieved at 3 kGy in some of the experiments, irradiation at that dosage would be unpractical. Bacterial and yeast growth on the surface of berries radiated at 3 kGy indicate that some micro-organismes survive the high radiation dosage (Nelson, Maxie & Eukel, 1959).

The temperature at which table grapes were irradiated, seems to play an important role in the quality and colour of the berries. Although a longer period elapsed between packing and radiation of the table grapes sent to Johannesburg, no colouring of the cultivar Waltham Cross was observed. The change in colour might also be due to gibberellic acid which is applied during pea-size to ensure a uniform berry-size (Combrink *et al.*, 1974). No loss of other quality aspects such as texture was observed. The cultivar Waltham Cross is known to have loose berries, but berry fall-off was not promoted by radiation in this investigation, as recorded elsewhere (Kim *et al.*, 1969, according to Thomas, 1986).

The application of radiation to packed table grapes offers a possibility to control postharvest *B. cinerea* decay. Unfortunately government regulations at present prohibit the export of irradiated food from South Africa.

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