THE ECOLOGY OF *BOTRYTIS CINEREA* ON GRAPE IN THE WESTERN CAPE PROVINCE

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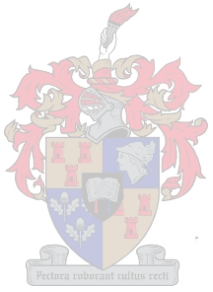

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DECLARATION

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

Botrytis cinerea Pers.: Fr., a pathogen of grapevine (*Vitis vinifera* L.), moves mainly through conidia by air currents in vineyards which are deposited intermittently on the surfaces of leaves, inflorescences and bunches. Little is known about the relationship between the inoculum dosage in air and incidence of Botrytis bunch rot, and how the relationship is influenced by environmental and host factors. To better understand this relationship, information is needed on the period over which conidia have accumulated, the time they are able to survive and remain infectious, time of symptom expression in relation to conidium arrival at the infection court and host surface wetness. The aims of this study were (i) to estimate the amount of viable *B. cinerea* occurring in air in vineyards, and at different positions on leaves, inflorescences and bunches of grape at different phenological stages, (ii) to determine the relationships between the number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines and the incidence of *B. cinerea* recorded from the different tissues, and (iii) to compare the efficacy of fenhexamid on leaves and inflorescences carrying natural *B. cinerea* inoculum with those inoculated with dry, airborne conidia.

Different techniques were used to detect viable *Botrytis cinerea* in air currents and on plant material obtained from table (cultivars Dauphine and Waltham Cross in Paarl- and Worcester-district) and wine grape (cultivars Chardonnay, Sauvignon Blanc and Merlot in Stellenbosch- and Malmesbury district) vineyards in the Western Cape province during 2001-02 and 2002-03. For four consecutive days during prebloom, bloom, pea-size, bunch closure, veraison and harvest, sets of Petri dishes with freshly prepared Kerssies' *B. cinerea* selective medium (spore traps) were left overnight in the bunch zone of vines. Plant material was collected from the vines on the fourth day. Leaves, infloresence and bunches were treated with paraquat to terminate host resistance and to promote the development of the pathogen on the tissues. The *B. cinerea* inoculum dosage in air, and the incidence at which the pathogen was detected at various positions on leaves and in bunches normally differed between vineyards. However, the various tests revealed that the pathogen generally occurred in a consistent pattern in air in the bunch zone of vines, on leaves and in bunches from all vineyards. The inoculum dosage in air in the bunch zone of the vine was generally highest during prebloom or during bloom, it decreased at pea size and mostly remained at a very low level at the later growth stages. The estimations of viable *B. cinerea* residing naturally on

leaves and in bunches, showed that their amounts depicted levels occurring in air in the bunch zone of the vine. Necrotic leaves occurring early season in vineyards were identified as an important source of secondary inoculum for dispersal to the developing bunches. Latent infections at the various positions in bunches were few at véraison and harvest. However, due to the necrotrophic ability of the pathogen, extensive berry rot (due to berry-to-berry contact) and thus severe bunch rot developed from a single berry that become symptomatic at the base of the pedicel/berry attachment zone. The *B. cinerea* occupation pattern explains why Botrytis bunch rot develops mostly from the inner bunch and why disease management strategies should concentrate on the bloom to pre-bunch closure stage and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season. Thus, to effectively reduce *B. cinerea* in grapevine, preventative applications are recommended to reduce two primary infection events: (a) between budding and pre-bloom to counteract primary leaf infection; (b) during late bloom or early pea size stage, to reduce the amount of the pathogen on leaves and infloresences and to prevent colonisation of floral debris. A third spray can be applied at bunch closure to reduce the amount of *B. cinerea* at various positions of the inner bunch, especially for cultivars with tight bunches.

The efficacy of fenhexamid on leaves and inflorescences carrying natural *B. cinerea* inoculum was compared with those inoculated with dry, airborne conidia. Shoots were obtained during late bloom from a vineyard (wine grape cultivar Merlot) in the Stellenbosch region. The shoots were divided into two main groups. One group of shoots was left uninoculated, the other shoots were inoculated by dusting with dry *B. cinerea* conidia in a settling tower. Before inoculation, equal numbers of shoots in each main group was sprayed with fenhexamid, or left unsprayed. Following inoculation and incubation, shoots of each treatment were divided in two equal groups. The one lot of shoots were rinsed in water. The other lot of shoots were immersed in paraquat solution to terminate host resistance and to promote the development of the pathogen from the tissues. For both uninoculated and inoculated shoots, irrespective of fungicide treatment, leaves remained asymptomatic at both the blade and petiole position for the water rinse treatment. No symptom of *B. cinerea* decay developed at any of the positions on leaves from shoots sprayed with fenhexamid. Spraying of shoots with fenhexamid completely suppressed *B. cinerea* infection and symptom expression on both uninoculated and inoculated inflorescens. For inoculated shoots, *B. cinerea* developed from approximately 50% of the laterals in the water rinse treatment. However, inflorescences rinsed in water remained asymptomatic.

The laboratory studies showed that fungicides, if applied properly to shoots and bunches under controlled conditions, effectively reduced the amount of *B. cinerea* at the various positions on leaves and inflorescence, and prevented infection and symptom expression at bloom. However, these goals are not achieved in vineyards where the fungicides are applied by conventional spraying methods. Therefore, more work is needed to evaluate fungicide application techniques by conventional spraying methods for proper fungicide coverage, and the reduction of *B. cinerea* in bunches.

OPSOMMING

Botrytis cinerea Pers.: Fr., 'n patogeen van druiwe (*Vitis vinifera* L.), beweeg hoofsaaklik deur middel van konidia in lugstrome deur die wingerd, en word dan afwisselend op die oppervlakte van die blare, bloeiwyses en trosse gedeponeer. Daar is nog min bekend oor die verhouding tussen die hoeveelheid inokulum in die lug en die voorkoms van *Botrytis* op die trosse, en hoe die verhouding deur omgewings- en gasheerfaktore beïnvloed word. Ten einde hierdie interaksie beter te verstaan, word inligting benodig oor die tydperk waarin die konidia akkumuleer, die tyd wat hulle oorleef en virulent bly, en die tyd van simptoom-uitdrukking in verhouding tot die verspreiding van die konidia by die infeksie-setel en benatbaarheid van die gasheer-oppervlakte. Die doel van hierdie studie was (i) om die hoeveelheid lewensvatbare *B. cinerea* wat in die lug voorkom, asook by verskeie posisies op blare, bloeiwyses en trosse by verskillende fenologiese stadiums te kwantifiseer, (ii) om die verhouding tussen die aantal aangetekende *B. cinerea* kolonies op spoorvangers wat in die trossone van die wingerd geplaas is, en die voorkoms van *B. cinerea*, aangeteken van verskeie weefsels, te bepaal, en (iii) om die effektiwiteit van fenhexamid op blare en bloeiwyses wat natuurlike *B. cinerea* inokulum dra, te vergelyk met dié wat met droë, luggedraagde konidia geïnokuleer is.

Verskillende tegnieke is gebruik om lewensvatbare *Botrytis cinerea* in lugstrome en op plantmateriaal van tafeldruiwe (kultivars Dauphine en Waltham Cross in Paarl- en Worcester-distrik) en wyndruiwe (kultivar Chardonnay, Sauvignon Blanc en Merlot in Stellenbosch- en Malmesbury distrik) in wingerde van die Wes-Kaap provinsie gedurende 2001-02 en 2002-03 te kwantifiseer. Petri bakkies met vars voorbereide Kerssies medium, selektief vir *B. cinerea* (spoorvangers), is vir vier agtereenvolgende dae gedurende vóórblom, blom, ertjiekorrel, trostoemaak, kleurbreek en oes, oornag in die trossone van wingerdstokke in betrokke wingerde, gelaat. Plantmateriaal is op die vierde dag versamel. Blare, bloeiwyses en trosse is met paraquat behandel ten einde die gasheerweerstand af te breek en ontwikkeling van die patogeen op die weefsel te bevorder. *B. cinerea* inokulum in die lug, en die frekwensie waarby die patogeen op verskeie posisies op blare en in die trosse voorgekom het, het normaalweg tussen wingerde verskil. Die verskeie toetse het getoon dat die patogeen normaalweg in 'n vaste patroon in die lug en die trossones van wingerde, asook op blare en in trosse van alle wingerde voorkom. Die inokulumkonsentrasie in die lug in die trossones van wingerdstokke was normaalweg die hoogste gedurende vóórblom of gedurende

blom. Die inokulumdruk het by ertjiekorrel verminder en meestal by 'n 'n baie lae vlak tydens die latere groeistadia gebly.

Die bepaling van lewensvatbare *B. cinerea* wat natuurlik op blare en in trosse gedeponeer is, het getoon dat hul hoeveelhede ooreenstem met vlakke wat in die lug in die trossone van die wingerd voorkom. Nekrotiese blare vroeg in die seisoen is 'n belangrike bron van sekondêre inokulum en speel dus 'n belangrike rol by die verspreiding van *Botrytis* tussen die ontwikkelende trosse. Latente infeksies by die verskeie posisies in trosse was laag by kleurbreek en oes. Weens die saprofitiese vermoëns van die patogeen, kan uitgebreide korrelvrot (a.g.v. korrel-tot-korrel kontak) en dus ernstige trosvrot, ontwikkel. 'n Enkele korrel kan by die basis van die pedisel/korrel vashegtingsone simptomaties raak, en vandaar na aangrensende korrels versprei. Die *B. cinerea* kolonisasiepatroon verduidelik waarom *Botrytis* trosvrot meestal vanaf die binneste tros ontwikkel en waarom siektebeheerstrategieë op die vóórblom- tot blomstadium gekonsentreer moet word, en op die inhibering van *B. cinerea* ontwikkeling in die binneste tros gedurende die vroeë stadia van die seisoen. Dus, om *B. cinerea* effektief tydens die twee primêre infeksie stadiums in wingerde te verminder, kan voorkomende toedienings aanbeveel word: (a) tussen knopvorming en vóórblom om primêre blaarinfeksie te verhoed; (b) gedurende láátblom en vroeë ertjiekorrel om die hoeveelheid inokulum op die blare en bloeiwyses te verminder, en die kolonisasie van blomdebris te voorkom. 'n Derde toediening kan tydens trostoemaak aangewend word om *B. cinerea* by verskeie posisies in die binneste tros te verminder, veral by kultivars met digte trosse.

Die effektiwitiet van fenhexamid op blare en bloeiwyses waarop natuurlike *B. cinerea* inokulum voorkom is vergelyk met dié wat met droë, luggedraagde konidia geïnokuleer is. Lote is vanaf 'n wingerd (wyndruif kultivar Merlot) in die Stellenbosch distrik tydens láátblom verkry en in twee hoofgroepe verdeel. Die een groep lote is geïnokuleer deur droë *B. cinerea* konidia in 'n afsettingstoring te strooi, terwyl die ander groep nie geïnokuleer is nie. Vóór inokulasie, is die helfte van die lote in elke groep met fenhexamid behandel, terwyl die ander helfte onbehandeld gelaat is. Ná inokulasie en inkubasie, is lote van elke behandeling verder in twee eweredige groepe verdeel. Die een groep lote is in water gespoel, terwyl die ander groep lote in 'n paraquatoplossing gedompel is om die gasheerweerstand te verwyder, en die ontwikkeling van die patogeen vanuit die weefsels te bevorder. Vir die waterspoelbehandeling van beide ongeïnokuleerde en geïnokuleerde lote, ongeag van die fungisiedbehandeling, het die blare asimptomaties by beide die bladoppervlakte en

blaarsteelposisie gebly. Geen simptome van *B. cinerea* verrotting het by enige van die blaarposisies van die lote, met fenhexamid gespuit, ontwikkel nie. Die spuit van die lote met fenhexamid het die *B. cinerea* infeksie en die simptoomontwikkeling op beide die ongeïnokuleerde en geïnokuleerde bloeiwyses heeltemal onderdruk. By die geïnokuleerde lote, het *B. cinerea* vanaf ongeveer 50% van die laterale in die waterspoelbehandeling ontwikkel, alhoewel, bloeiwyses wat in water afgespoel is, heeltemal asimptomaties gebly het.

Laboratoriumstudies het getoon dat fungisiedes, indien korrek toegedien op lote en trosse onder gekontroleerde toestande, tot effektiewe vermindering van *B. cinerea* getalle by die verskillende posisies op blare en bloeiwyses lei, en infeksie en simptoomuitdrukking tydens blom voorkom. Weens die feit dat die doelwitte nie behaal kan word in wingerde waar die fungisiede deur konvensionele spuitmetodes toegedien is nie, moet meer studies gedoen word om fungisied toedieningstegnieke, by konvensionele spuitmetodes, vir deeglike fungisiedbedekking en die vermindering van *B. cinerea* in trosse, te evalueer.

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CONTENTS

1. THE ECOLOGY OF *BOTRYTIS CINEREA* ON PLANT SURFACES, WITH EMPHASIS TO GRAPEVINE

INTRODUCTION

Botrytis cinerea belongs to the class Deuteromycotina (Fungi Imperfecti), order Moniliales and family Moniliaceae. It is the anamorph of *Botryotinia fuckeliana* (de Bary) Whetzel, which belongs to the Ascomycotina, order Helotiales and family Sclerotiniaceae. In 1729 Micheli first proposed the genus *Botrytis*, derived from the Greek 'botrus' meaning bunch of grapes. Later, in 1801, Persoon validated the genus and assigned five species to it under the binomial system of Linneaus, including one of Micheli's species, *B. cinerea*, named by von Haller (1771) (Jarvis, 1977). A large number of taxa were then added to the genus, many of which were made in error. Hennebert, in 1972, redefined the genus by reducing the 380 taxa to only 22 species, including *B. cinerea* (Jarvis, 1977).

B. cinerea has a very wide host range, unlike other species in the genus, such as *B. allii* Munn, that only infects *Allium* species. At least 235 host plant species have been reported for *B. cinerea* worldwide (MacFarlane, 1968). The host range of *B. cinerea* includes many economically important plant species. For example, *B. cinerea* is a serious threat to ornamentals such as roses and gerberas, vegetable crops such as onion, tomato and cucumber, as well as to fruits such as grapes, strawberries and kiwifruit (Elad, 1988b; Elmer *et al.*, 1995; Kerssies, 1993; Kerssies *et al.*, 1995; Stall, 1991; Sutton, 1990). The pathogen can cause devastating crop losses from seedling diseases to rots on near mature fruits just before harvest, such as on strawberries and grapes. In addition, seemingly unblemished produce can develop post-harvest rots in storage and during transport, for example in kiwifruit (Elmer *et al.*, 1995).

For *B. cinerea*, the host range is extremely wide and the potential for an alternative host plant to be an inoculum source is greater. However, it is unlikely that the same alternative host plant species, or numbers of species, will be available in all crops, even in the same geographical region. Thus, despite the broad host range of *B. cinerea*, the most consistently available inoculum source within a crop also comes, as with the more specialized forms, from the crop itself. The fungus exists in the different habitats as mycelia, micro- and macro-conidia, chlamydospores, sclerotia, apothecia and ascospores.

SURVIVAL

The disease cycle of *B cinerea* and the growth habit and phenologies of its host plants are often inextricably linked. Dormant fungal structures play a central role in each of these disease cycles. Each part of the fungus thallus can serve as a survival structure.

Sclerotia

B. cinerea over-winter and over-summer in the form of hard, black structures called sclerotia, either on the surface or within colonized plant tissue (Flaherty *et al.*, 1992; Tronsmo & Raa, 1977). Sclerotia are resistant to extremes in temperature (Coley-Smith, 1980; Nair & Martin, 1987). The ultra structural features of sclerotia suggest that they are well adapted for relatively long periods of survival (Coley-Smith, 1980; Nair & Martin, 1987). Nair and Martin (1987) reported that the two features likely to facilitate survival were the deposition of melanin on the surface and the presence of inner cells that are equipped with electron-dense storage bodies. Sclerotia can survive in soil (Agrios, 1997; López-Herrera *et al.*, 1994; Nair & Nadtotchei, 1987) and may be spread through tillage operations (Ellerbrock & Lorbeer, 1977). Sclerotia will produce large numbers of conidia during wet periods (Hewitt, 1974; Jarvis, 1980) that result in primary infection of young tissue (Nair & Nadtotchei, 1987). Thomas *et al.* (1981) showed that the bulk of sclerotia recovered from vineyard soils in the Western Cape province developed on vine leaves and shredded prunings. Honda and Mizumura (1991) found that sclerotia formed under conditions that were unfavourable for conidium formation and for epidemics of the disease caused by the fungi. These results support the ecological role of sclerotia as a resting structure that serves as an organ for survival under adverse conditions. Nair and Nodtotchei (1987) reported that repeated germination of sclerotia produce conidia over a relatively long period. A common source of sclerotia in vineyards is grape mummy clusters from previous seasons (Flaherty *et al.*, 1992; Hewitt, 1974).

Chlamydospores

The chlamydospores of *B. cinerea* are hyaline cells of extremely variable form and size (Urbasch, 1983, 1986). It is generally found in aging cultures and commonly occur in the stromatic sectors of cultures of the fungus which are contaminated by other organisms, and in association with sclerotia. Chlamydospores are formed as terminal or intercalary cells by transformation of vegetative mycelium parts and are liberated by hyphal disintegration. They

were observed on and in tissue of naturally and artificially infected tomato and *Fuchsia hybrida* leaves and their numbers increased in older lesions (Urbasch, 1983, 1986). Under moist conditions and without added nutrients, the chlamydospores germinated on the leaves by microconidia which stayed dormant. When fresh nutrients were supplied to the chlamydospores, they germinated with hyphae which penetrated the host, or they produced a new crop of macroconidia. Histological studies of the infection process by *B. elliptica* describe the formation of corresponding structures after conidium germination on oriental lily leaves (Hsieh *et al.*, 2001). On tomato fruit, unsuccessful penetration was often associated with germ tubes which, after attachment to the host, differentiates into several cells (chlamydospores) at the point of attachment (Rijkenberg *et al.*, 1980). On fruit of nectarine, plum and pear, germlings produced from dry airborne *B. cinerea* conidia formed chlamydospores on short germ tubes when fruits were subjected to intermitted dry periods, or were kept for 48 h at 5°C (Holz, 1999). Chlamydospores can therefore serve as short term survival structures which may help the fungus overcome short unfavourable periods encountered on plant surfaces (Urbasch 1983, 1986).

Conidia

Conidia of *B. cinerea* are in general regarded as short-lived propagules in the field and their survival will largely be influenced by temperature extremes, moisture availability, microbial activity and sunlight exposure. In the soil, *Botrytis* species are not particularly good competitors and their conidia are subjected to fungistasis (Coley-Smith, 1980). Conidia of *B. cinerea* were able to survive on fruit surfaces of kiwifruit, remaining viable and infectious throughout the growing season (Walter *et al.*, 1999). However, on the surface of Anjou pears the viability of *B. cinerea* conidia after seven weeks had declined to 10% germination (Spotts, 1985). When *B. cinerea* conidia were exposed to direct sunlight at midday in an Israeli summer, survival was only minutes but conidial survival was considerable longer when conidia were protected from direct sunlight by host tissues (Rotem & Aust, 1991). In a New Zealand vineyard, mean percentages of conidia germinating after exposure to 4 h of sunlight ranged between 81% and 91% and between 49 and 50 % after 8 h of sunlight exposure. Upon re-exposure on the second day, just 10 min of exposure to sunlight caused germination to drop between 26 and 27 % for all isolates tested (Seyb, 2003). The UV spectrum of sunlight appeared to be the most important environmental factor influencing mortality of conidia (Rotem & Aust, 1991; Seyb, 2003).

Microconidia offer a second conidium type to the fungus when placed under adverse conditions. In general it is found in cultures of the fungus which are contaminated by other organisms, and in association with sclerotia. It develops from germ tubes produced by macroconidia, more mature hyphae, inside empty hyphal cells, and from appressoria and sclerotia (Jarvis, 1980a). Germlings of *B. cinerea* form microconidia and chlamydospores in a corresponding manner on plant surfaces. On tomato plants, the differentiation of *B. cinerea* appressoria proceeded by production of microconidia directly on appressoria, or by terminally and laterally outgrowing hyphae and their subsequent formation of microconidia (Urbasch, 1985). The appressoria lost their function and the infection process at the site of interaction was interrupted. Although their sole function is believed to be one of spermatization, they may help the fungus to survive adverse conditions. The unicellular structures are in general produced in chains, but Urbasch (1984) noted that after prolonged adverse conditions, *B. cinerea* grouped clusters of microconidia bearing phialides together and then embedded aggregates of these conidia in mucilage, which is then enclosed with a hull. Due to protection by the hull, the enclosed microconidia aggregates survived without degeneration for up to six months on dry agar plates and formed new mycelia when placed on fresh media.

Mycelium

The survival of mycelium under natural conditions has not been investigated thoroughly and, unless particular care is taken, it is often difficult in practice to decide whether survival is by mycelium or whether microsclerotia or chlamydospores might be involved. *B. cinerea* is considered to be a characteristic component of aerial surfaces of some species of plants whilst being absent or infrequently isolated from others. The frequency of isolation of the fungus tends to increase as the season progresses, reflecting an increasing ability to enter plant tissue as a weak parasite or as a saprophyte during senesce (Blakeman, 1980). Kobayashi (1984) observed that *B. cinerea* conidial masses developed throughout the year from mycelium in the fallen petals of 28 plant species belonging to 19 genera of 14 families. Floral debris bearing mycelia are dispersed by wind and rain and provide a large saprophytically based inoculum that may stick to plant surfaces when wet (Jarvis, 1980). Fourie and Holz (1994) found that infected floral parts of nectarines and plums did not remain attached to the young developing fruit.

INOCULUM PRODUCTION AND DISPERSAL

Occurrence as an endophyte in plant tissue

Vegetative organs are not normally classified as susceptible, but heavy infection during periods of prolonged wetness, may lead to colonisation of leaf tissue. The phenomenon that asymptomatic grape leaf blades carry high amounts of *B. cinerea* suggests that leaf infection is an important primary infection event, and plays an important role in the epidemiology of the pathogen on grapevine (Holz *et al.*, 2003). Young leaves are highly susceptible to infection, that become latent and as the leaves senesce and die, the fungus colonises the tissues and sporulates (Braun & Sutton, 1987, 1988).

The role of weeds as alternative hosts in the survival of pathogens like *Sclerotinia sclerotiorum* (Phillips, 1992) are not a new concept and may be of great significance when considering overwintering possibilities of *B. cinerea*. The presence of weeds are positively correlated with *Botrytis* sp. (Davies *et al.*, 1997). The incidence and severity of *Botrytis* infection during research done by Guery *et al.* (1996) and Nieddu *et al.* (2000) relied on the intensity of grass cover treatments, thus emphasizing the importance of *Botrytis* as a possible endophyte in nearby plant growth (weeds or even commercial plantations). In Japan *B. cinerea* was identified on Malabar nightshade (*Basella rubra* L.), parsley (*Petroselinum crispum* Num.), bishop's weed (*Ammi majus* L.) and blue lace flower (*Didiscus caeruleus* DC.) (Kanno & Honkura, 1998).

Occurrence as a saprophyte on plant tissue

Botrytis cinerea is able to infect a wide range of living and dead tissues (vine trash) within the vineyard (Seyb *et al.*, 2000) and rapidly colonises senescing or moribund tissue including stamens, loose or adhering calyptra, shed pollen and pistils, immature aborted berries, dead flowers and miscellaneous leaf, stem and tendril pieces (Jarvis, 1980; Northover, 1987; Nair *et al.*, 1988). Grape flowers that did not set fruit (10%) within an inflorescence may act as a source of infection (Nair & Parker, 1985; Northover, 1987). Infected dead floral parts trapped in grape clusters are highly infectious and served as potent inocula for cluster infection prominent during grape berry ripening (Marais, 1985; Northover, 1987). Du Plessis (1937) found that grapes from more or less ill-kept vineyards definitely showed much more *Botrytis* rots than did grapes from those vineyards where sanitary measures were applied. *Botrytis cinerea* may sporulate on over-ripe unpicked fruit in the vineyard (Jarvis, 1980).

Other sources of inoculum included obvious diseased tissue of trunk and wood from diseased spurs and cankers on canes, leaf scars on canes and pieces of cluster stem left on the grapevine (Hewitt, 1974). Wrong cultural practices, such as the shredding or disking of pruning into the soil may contribute to inoculum density (Hewitt, 1974).

Effect of the environment. Thomas *et al.* (1988) stated that temperature, relative humidity and wind speed could affect the development of *B. cinerea* on the surfaces of inoculated grape berries. Thicker cuticles, more wax, high radiation and low relative humidity may inhibit germinated conidia in penetrating the cuticle of the host during late spring and summer (Kerssies, 1992).

Temperature. Nair and Nadtotchei (1987) showed that the optimum temperature for *B. cinerea* germination and infection was between 20 and 25°C, while Hyre (1972) found that sporulation reaches its peak at 25°C with no sporulation at 30°C. Nelson (1951a) found that the temperature range of *B. cinerea* infections are wide (Eden *et al.*, 1996). Optimum temperatures for infection of several hosts by *B. cinerea* have been reported to be 21°C (Bulger *et al.*, 1987; Jarvis, 1980; Thomas *et al.*, 1988), while 15°C and 20°C also favour infection (Jarvis, 1980b; Molot, 1987). Infection may also occur at 2°C (Jarvis, 1980), even though low temperatures inhibit (Elad & Evensen, 1995) development of *B. cinerea*. Elad and Yunis (1993) found that non-optimal temperatures, too high or too low, are a factor that predisposes cucumbers to disease development. The rate of activity of the host defence mechanisms at low temperatures is none to very low which means the tissues are predisposed to *Botrytis* spp. infection (Jarvis, 1980). Low temperatures may damage grape inflorescences (Ciccarone, 1959). Free water may change the time required for infection at lower temperatures significantly (Marais, 1985).

Humidity. Botrytis rots are associated with periods of successive rains or high rainfall (Hewitt, 1974). *B. cinerea* needs a high (95%) relative humidity for sporulation (Blakeman, 1980) and the development of external mycelium (Blakeman, 1980; Eden *et al.*, 1996; Harrison *et al.*, 1994; Nelson, 1951a). Flaherty *et al.* (1992) found that after rains or irrigation in spring, sclerotia germinate and produce grey spores (conidia). Cool, wet and humid weather conditions favoured infection and sporulation of *B. cinerea* (Agrios, 1997; Jarvis, 1980; Kerssies, 1993). The incidence of *B. cinerea* in custard apple-tree (*Annona cherimola* Mill.) orchards in Chile started with the rainy period in May (Veronica & Ximena, 1998). Jarvis (1962a) found that rainfall induces dispersal of airborne conidia and that the

maximum airborne spore concentrations above a raspberry plantation occurred when the relative humidity was rising or falling quickly between 65-85%. When there is no rain before harvest, grapes with early *Botrytis* rot usually dry up and mummify (Hewitt, 1974). Kerssies (1994) found no lesion formation at a relative humidity of 93%; germination of conidia and lesion formation occurred between 4 and 25°C; at 30°C germination and lesion formation did not occur, while between 18 and 25°C many lesions occurred. Marais (1985) demonstrated that in the absence of water, germination occurs if the relative humidity is at least 90%. Nair and Allen (1993) found that 63% of the flowers were infected by *B. cinerea* at an optimum temperature for grape flower infection at 23.7°C with a wetness period of at least 13 hours. Laboratory studies done by Flaherty *et al.* (1992) showed that at the optimum temperature of 22°C germination and infection can occur within 15 hours of free moisture. Broome *et al.* (1995) demonstrated that the incidence of berry infection increased with increasing wetness duration at all temperatures. A wet period of 12-24 h at 16°C is required for infection of harvested grape berries, whereas at 3°C a 72-84 h period was needed (Nelson, 1951a). A constant temperature of 12°C required a shorter wet period for infection than a mean temperature of 12°C (Nelson, 1951a). Broome *et al.* (1995) found that only 4 h of wetness is needed at temperatures of 12-30°C. The wet period is only significant at lower humidity levels (Nelson, 1951b).

Light. As cited by Jarvis (1977), Reidemeister (1909) and Doran (1922) stated that light has different effects on various growth processes, although all species are able to germinate and grow in the dark. According to Reidemeister (1909) the effect of light in enhancing sporulation of *Botrytis* spp. has long been known. Near ultraviolet light induce conidia formation, while unfavorable conditions (light and temperature) for conidia production encouraged sclerotia formation (Honda & Mizumura, 1991). They found that the number of conidia formed under darkness relative to that under near ultraviolet radiation was greatest in *B. allii*, followed by *B. cinerea* and *Sclerotinia squamosa*, while *B. fabae* was completely dependent on near ultraviolet radiation for conidium formation. Harada *et al.* (1972), cited by Jarvis (1977), found that a 12-hour dark cycle or continuous light promoted sporulation and suppressed sclerotial formation.

Dispersal and deposition

Air currents. Concentrations of conidia in the air increase as the grapevine matures (Corbaz, 1972). Nair *et al.* (1995) stated that *B. cinerea* could be inoculum-driven and

demonstrated that quantitative relationships exist between inoculum levels at carry over, flowering and harvest stages of the season. Hewitt (1974) suggested that wind plays an important role in the distribution of *B. cinerea* and other fungi involved in the fungal bunch rot complex (Jarvis, 1962a, 1980; Tronsmo & Raa, 1977). Conidia dispersed by air currents are proven to deposit as single cells on plant tissues (Kerssies, 1990) and occur as single colony forming units in nature (Duncan *et al.*, 1995). Thomas and Marois (1986) found that berries exposed to wind had a four-fold increase in spore-number produced per berry while external mycelium did not develop until wind stopped. Elad and Yunis (1993) showed that constant air movement could prevent the formation of a water film on plant organs so that even at high humidity conidia of *B. cinerea* do not germinate if condensation does not occur. Cultivars with dense canopies favour the development of *B. cinerea* (Gubler *et al.*, 1987; Savage & Sall, 1983). Removal of the leaves around grape clusters significantly increased the wind speed and subsequently the evaporative potential that will reduce growth and reproduction of *B. cinerea* on berry surfaces (Duncan, 1995; English *et al.*, 1989; English *et al.*, 1990; English *et al.*, 1993; Stapleton & Grant, 1992; Thomas *et al.*, 1988). Leaf removal treatments proved to be the most successful means in attempting to reduce *Botrytis* bunch rot severity (Gubler *et al.*, 1987; Percival *et al.*, 1993). Thomas *et al.* (1988) found that aerial mycelia developed best at 21°C, 94% relative humidity and 0 m/sec wind speed, while optimum wind speed for conidium production were 0.6 m/sec at 21°C, 94% relative humidity.

Water droplets. Splashing of water droplets (Cole *et al.*, 1996; Jarvis, 1962b) is other means of dispersal of spores in vineyards. Spotts and Holz (1996) found that conidia of *B. cinerea* adhered more strongly when applied in a water suspension or to the wet surface of grape fruit than when dry conidia were applied to dry surfaces. Jarvis (1962b) showed that very few of the *B. cinerea* conidia dispersed by raindrops become wet enough to enter the droplets and the majorities are carried on the droplet surface as a dry coating. These findings imply that raindrops may deposit conidia carried on their surfaces as single cells onto berry surfaces during runoff which indicates the prominent role single conidia play in the epidemiology of *B. cinerea* on grape berries (Coertze *et al.*, 2001; Jarvis, 1962b). Single conidia are unable to induce disease symptoms on sound grape berries at different phenological stages and remained asymptomatic after extended periods of moist or wet incubation. (Coertze *et al.*, 2001; Coertze & Holz, 1999).

Insects. Insects can play a very important role in dispersal and deposition by ensuring the timely placement of fungal conidia on the susceptible tissue of the host plant (Butler, 1960; Butler & Bracker, 1963; Cole *et al.*, 1996; Fermaud & Gaunt, 1995; Fermaud & Le Menn, 1989, 1992; Jarvis, 1980; Louis *et al.*, 1996; Nair & Hill, 1992; Tronsmo & Raa, 1977). Ripe grapes are considered to be susceptible to decay by clusters of conidia (Broome *et al.*, 1995; Hill *et al.*, 1981; Nair and Allen, 1993; Nelson, 1951a, 1956). Injuries of grape clusters inflicted by insects may be important avenues for *B. cinerea* infections (Hewitt, 1974; Savage & Sall, 1983). Insects promote infection of plant pathogens in three main ways. Firstly, by providing infection sites as a result of wounding the plant by feeding and ovipositioning activities. Secondly, by distributing spores. Thirdly, by increasing the supply of nutrients on the surface of the plant by increased leakage from areas where they wounded the cuticle or by secretion of honeydew by the insect (Blakeman, 1980).

Plant pathogens can be disseminated externally on the insect's body or internally through the digestive tract and deposited with faeces. The habit of fruit flies of regurgitating the contents of the crop when feeding is also an effective way by which they can disseminate plant pathogens (Leach, 1940). In the Western Cape province, *Ceratitis* (Diptera: Tephritidae) (Drew) fruit flies also infest grape, which are usually regarded as a non-host. Species in this genus are known for their polyphagous feeding habits and are pests of a wide range of fruit types, especially stone fruit species (Bateman, 1972; Christenson & Foote, 1960). The females of *C. capitata* Wiedemann (Myburgh, 1962, 1964; Schwartz, 1993; White, 1992) cause direct damage to fruit during oviposition when the fruit skin is pierced in order to lay eggs, which in turn can lead to indirect damage such as decay by fungal pathogens (Grové *et al.*, 1997). In a study done by Louis *et al.* (1996), *Drosophila melanogaster* Meig. is regarded as a plurimodal vector of *B. cinerea* and may play a significant role in the overwintering and primary infection of *B. cinerea*. *B. cinerea* could be carried externally or internally by *D. melanogaster* (Louis *et al.*, 1996). Once *D. melanogaster* is infected they become a potential reservoir of the pathogen through the production of conidia, mycelia and microsclerotia since survival structures are still viable if carried internally through the digestive tract (Louis *et al.*, 1996). According to Hewitt (1974), the fruit fly (*D. melanogaster*) will lay their eggs in injured grape tissue, contaminating it with yeast cells, fungus spores and bacteria. Fermaud and Le Menn (1989, 1992) demonstrated that conidia of *B. cinerea* are carried externally or internally on grape berry moth larvae (*Lobesia botrana* Denis & Schiffermeuller). The disseminated conidia

rapidly infected wounds made by the feeding larvae and gave rise to *Botrytis* epidemics. The passage of conidia through the digestive tract of these larvae did not modify the germination ability of the conidia (Fermaud & Giboulot, 1992). *Thrips obscuratus* Crawford is capable of carrying conidia of *B. cinerea* on the head, thorax, legs and the abdominal distal segments with only a few on the wings (Fermaud & Gaunt, 1995). The distribution pattern suggests that adhesion is mechanical (Fermaud & Gaunt, 1995).

GROWTH ON PLANT SURFACES

Infection of the host by *B. cinerea* is significantly influenced by the availability of nutrients. Blakeman (1980) found that nutrients, available in the form of leakages from flowers and fruit especially, stimulate infection. The inhibitory effect of tissue extracts (McClellan & Hewitt, 1973) found early in the season in several parts of the grape and the development of a greater susceptibility with maturity to *B. cinerea* are influenced by the composition of grape berry exudates (Padgett & Morrison, 1990). Fruit is most susceptible when it is physiologically mature (Fourie & Holz, 1985; Fourie & Holz, 1987; Fourie & Holz, 1995; Nair & Hill, 1992). Padgett and Morrison (1990) found that water extracts of berry exudates, containing sugars (Blakeman, 1975; Flaherty *et al.*, 1992; Kosuge & Hewitt, 1964; Nelson, 1951a; Stalder, 1953; Verhoeff, 1980), malic acid, potassium and sodium promoted mycelial growth of *Botrytis cinerea*, while ethanol and ether extracts containing phenols and lipids inhibited growth of *Botrytis cinerea* (Vercesi *et al.*, 1997). Phenolic compounds and malic acid were present in relatively high concentrations in grape berry exudates after bloom, but were low in exudates from mature fruit. Vercesi *et al.* (1997) found that germ tube growth and colonisation by *B. cinerea* are inhibited during the early stages of the grape berry when organic acids are the main carbon source. Pucheu-Planté and Mercier (1983) reported that the pH of grape berry exudates inhibits germination and growth of *B. cinerea*. An optimum at pH 4.0, with depressed growth at either higher or lower values, and total inhibition at pH extremes occurred.

Adhesion

Spotts and Holz (1996) stated that the adhesion of fungal spores to plant surfaces is an important factor in the infection process and the epidemiology of plant diseases. According to Doss *et al.* (1993), two stages of adhesion of conidia of *B. cinerea* occur. The first stage, immediate adhesion, occurs upon hydration of freshly deposited conidia. Immediate

adhesion is characterized by relatively weak attachment forces and is the strongest with hydrophobic substrata. The second stage, which only occurs with viable conidia and is not influenced by the hydrophobic character of the substratum, indicates a delayed adhesion. Delayed germination occurs after viable conidia have been incubated for several hours under conditions that promote germination. This stage also involves secretion of an ensheathing film, referred to as the fungal sheath that remains attached to the substratum upon physical removal of the germlings (Doss *et al.*, 1995). Extracellular mucilages are common on fungal germlings (Jarvis, 1980; McKeen, 1974). The adhesion of ungerminated conidia from other fungal species may be greatly influenced by spore tip mucilage (Hamer *et al.*, 1988), adhesive knobs (Dijksterhuis *et al.*, 1990) and mucilage covered (Bird & McKay, 1987).

Germination

A specific set of environmental conditions is a prerequisite regarding the process of germination. High humidity or wetness (Duncan *et al.*, 1995; Nelson, 1951b; Rousseau & Doneche, 2001), and low wind speed (Thomas *et al.*, 1988), temperature (Kerssies, 1994) and light infiltration (Duncan *et al.*, 1995; Kerssies, 1992, 1993) promote *B. cinerea* germination. Germination occurs at a temperature range between 1 and 30° with an optimum of 20°C (Kerssies, 1994; Nair & Nadtotchei, 1987; Pearson & Goheen, 1994). The optimal temperature for germ tube elongation is 30°C (Hennebert and Gilles in: Jarvis, 1980). Pollen and amino acids (Chou & Preece, 1968; McClellan & Hewitt, 1973), glucose, sucrose (Rousseau & Doneche, 2001) and fructose, of which fructose is the most effecctive (Blakeman, 1975; Kosuge & Hewitt, 1964), and aqueas extracts of the stigma and style (McClellan & Hewitt, 1973) may all stimulate the germination of conidia and the development of germ tubes outside the cuticle. Sugar concentrations as high as 5 x 10-4 M are taken up by free water on the surface of mature grape berries in which presence conidia germinate to form long germ tubes which are ensheathed by a fibrillar-like matrix (Kosuge & Hewitt, 1964). Dry inoculated conidia produce short germ tubes and attempt to penetrate directly (Coertze *et al.*, 2001; Cole *et al.*, 1996). Coertze *et al.* (2001) found that conidia germinate more extensively on wet berries, but germ tube elongation differs at different phenological stages.

Germ tube orientation and appressorial formation

Germ tubes formed by the conidia of *B. cinerea* developed to lengths up to 150 µm before an appressorium was formed on Tokay grapes (Nelson, 1956). McKeen (1974) found on leaves of *Vicia faba* that the turning down of the tip of the germ tube was the first indication that infection was about to occur. The tips of the germ tubes were held firmly against the cuticle of the leaf by mucilage that spread some distance around the germ tube (McKeen, 1974). *B. cinerea* forms a variety of penetration structures, such as protoappressoria, simple appressoria, multicellular lobate appressoria and infection cushions, before penetration of the cuticle on flower parts of plum and nectarine (Fourie & Holz, 1994). Hanssler and Pontzen (1999) found that during infection of leaves in the absence of water, conidia developed a short germ tube and penetrated into the leaf tissue without forming appressoria.

Survival of conidia and germlings

Conidia, the propagative spores and primary inoculum (Coley-Smith, 1980; Doss *et al.*, 1995; Nair and Nadtotchei, 1987) of *B. cinerea*, survive for a shorter period than mycelium (Van der Berg & Lentz, 1968), but under certain conditions they may express considerable powers of survival (Coley-Smith, 1980). Coley-Smith (1980) showed that as dry conidia aged their viability decreased, while Salinas *et al.* (1989) observed that germination of older conidia was delayed and infection reduced. Pollen, however, can restore the lost germination ability (Coley-Smith, 1980) and infectivity of old conidia (Chou & Preece, 1968). McClellan and Hewitt (1973) found that pollen stimulated germination of conidia and germ-tube growth and thus enhanced colonisation of flower parts by *B. cinerea*. Brown (cited in Kosuge & Hewitt, 1964) observed that conidia from fresh cultures germinated readily in water, while conidia from old cultures only germinated readily if they were supplied with additional nutrients. Mycelium of *B. cinerea* can survive one year or longer at a relative humidity of 90-100% at 0°C (Van den Berg & Lentz, 1968).

Infection pathways on diverse plant organs

Penetration of the pathogen may occur through stomata (Clark & Lorbeer, 1976; Verhoeff, 1980; McClellan and Hewitt, 1973; Nair and Parker, 1985), lenticels (Verhoeff, 1980), minute cracks (Hill *et al.*, 1981; Verhoeff, 1980), pedicels (Holz *et al.*, 1997; Holz *et al.*, 1998; Pezet and Pont, 1986), natural openings (Pucheu-Planté and Mercier, 1983), wounds (Brook, 1991; Edlich *et al.*, 1989; Nair *et al.*, 1988; Savage & Sall, 1983; Sharrock & Hallet,

1991; Verhoeff, 1980), flower parts (De Kock & Holz, 1992; Hunter *et al.*, 1972; Hunter & Rohrbach, 1969; Lavy-Meir *et al.*, 1988; McClellan & Hewitt, 1973; Nair & Parker, 1985; Ogawa & English, 1960; Powelson, 1960) or by direct penetration of the cuticle (Nelson, 1956). Holz *et al.* (2002) stated that the importance of *B. cinerea* occurring superficially at the bases of the berry, and probably the base of the pedicel, is underestimated in the epidemiology of *B. cinerea*, and the development of epiphytotics in grapevine.

Direct penetration. McKeen (1974) observed that a pore developed in the fungal wall in the centre or the contacting germ tube. The infection peg as reported by McKeen (1974), covered by the plasmalemma, was pressed against the host cuticle, while the plasmalemma covered the infection peg as it moved through the cuticle. Backhouse and Willets (1987) observed thin walls around the infection pegs that appear to be different in structure and composition from hyphal walls, while McKeen (1974) reported the absence of walls around the infection pegs. A very thin penetration peg grew from the underside of an appressorium that penetrated directly through the cuticula (Clark & Lorbeer, 1976), after which it enlarged into a subcuticular and intercellular mycelium (Nelson, 1956). An increase in germ tube numbers as a direct result of an increase in inoculum concentrations are correlated with increased enzymatic activity which facilitate direct penetration of the host surface (Eden *et al.*, 1996; Van den Heuvel & Waterreus, 1983). Penetration of clusters of conidia at a single site could alter the host response to infection and hence the estimate of the level of susceptibility (Fourie & Holz, 1995; Holz & Coertze, 1996; Holz *et al.*, 1997, 1998). Louis (cited in Verhoeff, 1980) demonstrated that one conidium of *B. cinerea* produced a small lesion, while several together produced a large lesion.

Natural openings. According to Nelson (1951a) lenticels, insect punctures and microscopic injuries are not essential courts of infection. Müller-Thurgau (cited in Nair & Hill, 1992) observed in 1888 that infection of grapes by *B. cinerea* occurred through lenticels. Clark and Lorbeer (1976) noticed that conidia of *B. cinerea*, when inoculated in a nutrient broth, frequently penetrated stomata without forming appressoria. Bessis (cited in Verhoeff, 1980) found no proof for direct penetration of the berry cuticle, and concluded that the pathogen penetrates grape berries through minute cracks or openings in the cuticle. Marais (1985) demonstrated through scanning electron microscopy that conidial germ tubes penetrate berries through numerous micro fissures that form around non-functional stomata.

Specialised host structures. The grapevine internode bears no external organs. The nodes bear a variety of organs like leaves, buds (produce shoots, leaves, flowers and new buds), lateral shoots and tendrils. In the beginning of the season secondary tissues are rare but become much more common as the season progresses and the shoot ages. The epidermis is covered with an external, wax-containing cuticle. The epidermal cells can sometimes bear long, flexuous, woolly hairs, short, bristly, single or multi-sellular hairs, spindly, hook-like hairs, very large, multi-cellular organs similar to rose thorns, swollen-tipped glandular hairs that turn red in the springtime or club-like hairs. Under high temperature and humidity, small, bright, transparent outgrowths, known as pearl glands, can also be observed. Each gland contains 10 to 20 large, water-filled cells that are covered by an envelope of about 200 small, flat cells. This envelope has a small slit (permanently open) enabling exchange of air and water. The epidermis contains only a few stomata, which are widely dispersed over its surface. Lenticels are found only in *Muscadinia* species and in the *Ampelopsis* and *Parthenocissus* genera. Lenticels are openings in the cork located under the epidermis, where they enable exchange of gasses between the atmosphere and the airy space in the cortex (Winkler, A.J., 1894).

The upper epidermis of the grape leaf may be hairy and is covered by a cuticle (containing highly hydrophilic compounds) with a dark wax at the outmost layer. Palisade tissue is found just below the epidermis and is tightly arranged. The polygonal mesophyll cells are lobed and surrounded by large air spaces. These chambers collect water transpired by the cells, which is directed towards the stomata. The lower epidermis consists of numerous stomata, hairs (woolly, pubescent, thorny, glandular) located around the veins and a very thin cuticle. Each stomata has a stomatal pore surrounded by two reniform cells which can open and close. Under each stomata is a large space known as the sub-stomatal chamber. The upper surface of the petiole facing the shoot has a longitudinal groove of varying depth and is often lined with bristly hairs (Winkler, A.J., 1894).

The calyx of the flower is a small, cup-shaped organ that forms a small, rudimentary crown around the receptacle. It is made of five interwoven sepals. The calyptra consists of five petals that alternate with the sepals. The androecium (male organ) consists of five stamens placed opposite the petals. The stamens move freely. Each has a long thin, pale yellow stalk or filament with a bi-lobed anther at its top. The anthers have an opening

towards the center of the flower by means of a longitudinal slit. Each lobe contains two pollen sacs with a large amount of pollen (Winkler, A.J., 1894).

The behaviour of *B. cinerea* on the host surface does not always correlate with the infection pathway as described in literature (Holz *et al.*, 1998). In grape, ontogenic resistance (Gadoury *et al.*, 2002) and the intact grape berry skin provide effective resistance to penetration of solitary conidia (Coertze & Holz, 1999; Coertze *et al.*, 2001), which means that latent infections and predisposed tissue (Nelson, 1951a) may play a significant role during infection. Spores may germinate on the stigma, style and at the attachment of the flower and flower stalk (Marais, 1985) and invade the stigma and style and then into the stylar-end (Hewitt, 1974; McClellan & Hewitt, 1973), after which it becomes latent (Nair, 1985; Nair & Hill, 1992; Nair & Parker, 1985). Recent infection studies revealed that superficially residing conidia at the pedicel-berry attachment zone (Holz *et al.*, 2003) and latent pedicel infections (Coertze & Holz, 1999; Holz *et al.*, 1997, 1998) are more important in *B. cinerea* bunch rot, than latent infections of the style end (McClellan & Hewitt, 1973; Nair & Parker, 1985) or cheek of berries (Nelson, 1956). Holz *et al.* (1998, 2003) showed that *B. cinerea* in bunches of grape are predominantly associated with the bases of the pedicel and the berry. The next prominent positions occupied by the pathogen were leaf blades, rachisis and laterals, with the cheek and style-end virtually free from *B. cinerea* infections (Holz *et al.*, 2003). Solitary conidia readily established asymptomatic latent infection in the laterals and pedicels of bunches at the pea size to bunch closure stages and developed predominantly in the receptacle part of the pedicel (Holz *et al.*, 2000). Mycelium or conidia, the two inoculum types, are responsible for infecting the pedicel (fruit stem). Infection via the pedicel is symptomless or latent (Holz *et al.*, 1998). Pezet and Pont (1986) defines latency as "infection of a host plant by a parasite without the development of visible symptoms during a certain period". Latency is settled in the pedicel where resistance mechanisms destroy a large proportion of infection. These mechanisms become less effective as bunches develop, which means that the pathogen can grow along the vascular tissue out of the pedicel and into the berry. This type of inoculum therefore reaches the berry from the inside and is not affected by the resistance mechanisms that normally stops it when trying to penetrate the berry skin.

B. cinerea can penetrate immature fruit at any growth stage (Hill *et al.*, 1981) and may act as a possible endophyte in tissue senescence (Gindrat & Pezet, 1994). Unripe grape berries

are less susceptible to rot by *B. cinerea* than ripe berries (De Kock & Holz, 1991; Hill *et al.*, 1981; McClellan & Hewitt, 1973). Holz *et al.* (2000) did a study on the resistance of grapes in different development stages and found that young, immature grapes are highly resistant to *B. cinerea* but susceptibility steadily increases after véraison (Creasy and Coffee, 1988; Hill *et al.*, 1981; Nair & Hill, 1992). The young berry exhibits high resistance based on a preformed system cuticula-structure and tannin-like blockers of fungal enzymes in the skin cell walls of the berries (Hill, 1985). Pezet and Pont (1986) found that a pathogenic relationship in grape clusters infected with *B. cinerea* only develops after the beginning of ripening. Verhoeff (1980) suggested three possibilities to explain the transition from a quiescent to an active pathogenic relationship. Firstly, the immature fruit may contain a substance toxic to the fungus, which disappears at maturity. Secondly, the immature fruit does not contain the nutritive substances required by the fungus for its development. These substances appear at maturity. Thirdly, the fungus may be unable to produce enzymes essential to its development; if, however, it is capable of producing them, these enzymes are deactivated in the immature fruit (Verhoeff, 1980).

Wounds. Intact grape berry skin provides an effective barrier (Elad & Evensen, 1995) to penetration of solitary conidia, which are unable to induce disease symptoms on ripe grape berries (Coertze *et al.,* 2001; Coertze and Holz, 1999; Holz *et al.*, 2000). This confirmed the decisive role of predisposed tissue with emphasis on wounding (Jarvis, 1980; Gessler & Jermini, 1985; Nair *et al.*, 1988; Savage & Sall, 1983; Spotts *et al.*, 1998; Verhoeff, 1980) through mechanical activities (pruning, grafting and rough handling of fruit during harvest) (Pienaar, 1972; Sharrock & Hallet, 1991; Verhoeff, 1980), weather conditions (frost, hail, sun, wind and windblown sand) and biological factors such as pathogens (powdery mildew), insects, (Du Plessis, 1937), birds (Marais, 1985), bunch architecture and the rapid water intake leading to splitting, in both symptom expression and the epidemiology of *B. cinerea* on grapevine (Holz *et al.*, 2000). Wounded grapes are more susceptible to *Botrytis* infection than unwounded grapes (Du Plessis, 1937). Wounds are regarded as the major entry sites for the pathogen on grapes (Coertze & Holz, 1999; Du Plessis, 1937; Hill *et al.*, 1981; Nair *et al.*, 1988). Edlich *et al.* (1989) reported that *B. cinerea* is predominantly a wound pathogen under field conditions. Gärtel (cited in Verhoeff, 1980) reported that hail damage on grape berries led to quick infection of *B. cinerea*. Wounds may be inflicted by insects, frost, hail, windblown sand, sunburn, the rapid uptake of water leading to splitting and the expansion of berries in tight clusters (Jarvis, 1980; Savage & Sall, 1983). Coertze *et al.* (2001) stated that

a combination of fresh wounds and new inoculum is needed for successful wound infection (Coertze & Holz, 1999; Mercier & Wilson, 1994; Spotts *et al.*, 1998). Coertze and Holz (2002) found that conidia and germlings never infected dry wounds, but only fresh wounds by fresh conidia under humid or wet conditions. According to their findings this mode of infection should not contribute to a gradual build-up of secondary inoculum and to *B. cinerea* epiphytotics in the vineyard. Turgid grapes picked early in the morning develop fine cracks around the pedicel attachment area, which may become infected with *B. cinerea* (Pienaar, 1972). Flaherty *et al.* (1992) stated that when moisture is high and wind is low, cracks would form in which the mycelium and spores will produce the characteristic grey mould.

CONCLUSION

Estimations of the amount of *B. cinerea* occurring at different positions on leaves and bunches in vineyards in the Western Cape province (Holz *et al.*, 2003) showed that levels may be 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. On the other hand, *B. cinerea* conidia and germlings may also have different survival periods on tissues of the various positions, as is implicated by the low incidence at which the pathogen was detected at the cheek, and the high incidence of occurrence on the rachis, lateral and pedicel.

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. Little is known about the relationship between the inoculum dosage in air and incidence of Botrytis bunch rot and blight, and how the relationship is influenced by environmental and host factors. To better understand this relationship, information is needed on the period over which conidia have accumulated, the time they are able to survive and remain infectious, time of symptom expression in relation to conidium arrival at the infection court and host surface wetness. The aim of this study was to estimate the amount of viable *B. cinerea* occurring in air in vineyards, and at different positions on leaves, inflorescences and bunches of grape at different phenological stages

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2. THE ECOLOGY OF *BOTRYTIS CINEREA* ON LEAVES, INFLORESCENCES AND BUNCHES OF GRAPE

ABSTRACT

Different techniques were used to detect viable *Botrytis cinerea* in air currents and on plant material obtained from table (cultivars Dauphine and Waltham Cross in Paarl and Worcester district) and wine grape (cultivars Chardonnay, Sauvignon Blanc and Merlot in Stellenbosch and Malmesbury district) vineyards in the Western Cape province during 2001-02 and 2002-03. For four consecutive days during prebloom, bloom, pea-size, bunch closure, véraison and harvest, sets of Petri dishes with freshly prepared Kerssies' *B. cinerea* selective medium (spore traps) were left overnight in the bunch zone of vines. Plant material was collected from the vines on the fourth day. Leaves, inflorescences and bunches were treated with paraquat to terminate host resistance and to promote the development of the pathogen on the tissues. The *B. cinerea* inoculum dosage in the air, and the incidence at which the pathogen was detected at various positions on leaves and in bunches normally differed between vineyards. However, the various tests revealed that the pathogen generally occurred in a consistent pattern in air in the bunch zone of vines, on leaves and in bunches from all vineyards. The inoculum dosage in air in the bunch zone of the vine was generally highest during prebloom or during bloom, it decreased at pea size and mostly remained at a very low level at the later growth stages. The estimations of viable *B. cinerea* residing naturally on leaves and in bunches, showed that their amounts depicted levels occurring in air in the bunch zone of the vine. Necrotic leaves occurring during the early season in vineyards were identified as an important source of secondary inoculum for dispersal to the developing bunches. Latent infections at the various positions in bunches were few at véraison and harvest. However, due to the necrotrophic ability of the pathogen, extensive berry rot (due to berry-to-berry contact) and thus severe bunch rot developed from a single berry that become symptomatic at the base of the pedicel/berry attachment zone. The *B. cinerea* occupation pattern explains why Botrytis bunch rot develops mostly from the inner bunch and why disease management strategies should concentrate on the bloom to pre-bunch closure stage and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season.

INTRODUCTION

Botrytis cinerea Pers.: Fr., a pathogen of grape (*Vitis vinifera* L), moves mainly as conidia carried by air currents in vineyards. Conidia are probably deposited intermittently on the surfaces of vines and infect leaves, buds, canes and bunches to cause gray mould (Nair & Hill, 1992). The only susceptible tissues at the beginning of the season are buds and new shoots, which if infected may turn brown and dry out. Once exposed, young leaves are also susceptible (Nair & Hill, 1992). As the leaves mature they become increasingly resistant to infection due to a thicker cuticle layer and the presence of inhibitory compounds (Langcake, 1981). Berries, on which the most prominent phase of the disease is found (Nair & Hill, 1992), are considered resistant to infection when immature, and susceptible when mature (Nelson, 1956; Hill *et al.*, 1981; Nair & Hill, 1992). Thus, in many regions, Botrytis bunch rot is not seen between fruit set and véraison.

In the Western Cape province, estimations of the amount of *B. cinerea* showed that the pathogen occurs more regularly in grape bunches from bloom to bunch closure than from véraison to harvest (Holz *et al.*, 2003). This suggests that *B. cinerea* inoculum in vineyards is produced, liberated and dispersed predominantly during the early part of the season. On the other hand, *B. cinerea* conidia and germlings may have different survival periods on tissues of the various positions, as is implicated by the low incidence at which the pathogen was detected at the cheek, and the high incidence of occurrence on the rachis, lateral and pedicel. In this context it was previously shown that single conidia of the pathogen did not survive for extended periods on berry surfaces (Coertze & Holz, 1999, 2002; Coertze *et al.*, 2001; Gütschow, 2001). 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 site and appropriate developmental stage. Knowledge of the ecology of *B. cinerea* at different positions on bunches and leaves of grapevine is needed to plan effective disease control strategies, for example in devising disease prediction models, timing fungicide application, biological control and resistance breeding.

Little is known about the relationship between the inoculum dosage in air and incidence of Botrytis bunch rot, and how the relationship is influenced by environmental and host factors. To better understand this relationship, information is needed on the period over which conidia have accumulated, the time they are able to survive and remain infectious, time of symptom expression in relation to conidium arrival at the infection court and host surface wetness. The

aims of this study were (i) to estimate the amount of viable *B. cinerea* occurring in air in vineyards, and at different positions on leaves, inflorescences and bunches of grape at different phenological stages, and (ii) to determine the relationships between the number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines and the incidence of *B. cinerea* recorded from the different tissues.

MATERIALS AND METHODS

Vineyards. The investigation was conducted in vineyards in the Worcester, Paarl, Stellenbosch and Malmesbury regions, Western Cape province (Tables 1,2). The vineyards in the Worcester and Paarl regions are approximately 80 km apart and separated by a series of mountain ranges. The vineyards in the Malmesbury and Stellenbosch regions are approximately 30 km from those in the Paarl region. All regions have a moderate Mediterranean climate. Malmesbury is drier than De Doorns. De Doorns is marginally drier than Paarl, and Stellenbosch is marginally wetter than Paarl. Table grape vineyard blocks ranged from 1 to 5 ha and the vines were trained to a slanting trellis at 3 by 1.5 m spacings. Canopy management and bunch preparation were done according to the guidelines of Van der Merwe *et al.* (1991). Wine grape vineyard blocks ranged from 1 to 5 ha and the vines were trained to a two wire trellis system or goblet vines. All vines were trickle irrigated. In most of the vineyards a recommended programme (De Klerk, 1985) for the control of downy mildew, powdery mildew, and *B. cinerea* was generally followed. Sprays against downy mildew started at 10-15 cm shoot length and were applied until pea size. Fungicides used were folpet (Folpan 50 WP, Agrihold), fosetyl-Al/mancozeb (Mikal M 44/26 WP, MayBaker), mancozeb (Dithane M45 80 WP, FBC Holdings) and mancozeb/oxadixyl (Recoil 56/8 WP, Bayer). Applications against powdery mildew started at 2-5 cm shoot length and were applied until 3 wk before harvest. Fungicides used were penconazole (Topaz 10 EC, Syngenta), pyrifenox (Dorado 48 EC, Maybaker) and triadimenol (Bayfidan 25 EC, Bayer). Sprays against *B. cinerea* were applied at flowering, bunch closure, véraison and 2 weeks before harvest. Fungicides used were iprodione (Rovral Flo 25 EC, Aventis) and pyrimethanil (Scala 40 EC, Aventis). Estimates of the amount of viable *B. cinerea* occurring in air and on grapevine material was made at set phenological stages (Tables 1,2) from a different arbitrarily chosen vine before fungicide applications against *B. cinerea* were made.

Spore traps. For four consecutive days at each phenological stage, the bottom section of small Petri dishes (65 mm diameter) containing freshly prepared Kerssies' *B. cinerea* selective medium (Kerssies, 1990) were placed at approximately 17h00 in the afternoon in open, larger Petri dishes (90 mm diameter). For each vine, two larger holder dishes were suspended approximately 1.5 m apart with wire in a horisontal position in the bunch zone of the vine. For each vine, one dish was allocated the treatment code NST (untreated), the other dish was coded ST (surface sterilised). The dishes with the selective medium were collected the following morning at approximately 11h00, their lids were replaced and the dishes were kept at 4°C. After the fourth placement have been collected all dishes were taken to the laboratory, placed on epoxy-coated steel mesh screens (53 x 28 x 2 cm) and 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 de-ionised water to establish high relative humidity (≥93% RH). The chambers were kept at 22°C with a 12-h photoperiod. This treatment prevented the drying out of media during incubation and promoted the development of *B. cinerea*. The dishes were examined daily for the development of sporulating *B. cinerea* colonies, and the number of colonies were recorded after 14 days incubation. For each vine, the total number of viable conidia that had accumulated during the 4-day-period on dishes of each treatment was recorded, and mean number calculated for each treatment.

Grape material. At each sampling, one leaf and one inflorescence or bunch was collected per vine from around the spore traps with the treatment code NST. An equal number of plant parts were collected around the ST coded spore traps. The inflorescences and bunches were cut into short sections bearing approximately 10 laterals with their pedicels and ovaries or berries on a short rachis section. The leaves and sections coded NST were left untreated, those coded ST were surface-sterilised (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried. Both groups of leaves and sections were immersed in paraquat solution (WPK Paraquat, 200 g/l [bipyridyl], WPK Agricultural, Cape Town, South Africa) for 30 seconds, rinsed in sterile de-ionised water and air-dried. The material was placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm) and placed in 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 de-ionised water to establish high relative humidity (≥93% RH). The screens were kept in the chambers at 22°C with a 12-h photoperiod to promote the development of *B. cinerea*. The

treatments provided conditions that discriminate between conidial germination and the development of germlings on the tissue surface, and the development of latent mycelia in the tissues during the incubation period (Holz *et al.*, 2003). The tissues were examined daily for symptom development, and the presence of *B. cinerea* was positively identified by lesion development and the formation of sporulating colonies of the pathogen at specific positions. In the case of leaves, positions were the blades and petioles. Positions in the inflorescences were the rachis, laterals, pedicels and ovaries. Positions on bunches were the rachis, lateral, pedicel-berry attachment zone, and the cheek and stylar end of berries. The presence of *B. cinerea* at each position was recorded, and the incidence calculated for each treatment.

Statistical Procedures. Two trials were conducted, one on wine grapes and the other one on table grapes. The experimental design was a completely randomised design with 15 vines per vineyard as replications. The trials were repeated for two growing seasons (2001/2002 and 2002/2003). The treatment design was a four factor factorial.

Spore traps: The trial on wine grapes was a 2 x 3 x 7 x 2 factorial design. The factors were two locations (Malmesbury and Stellenbosch), three cultivars (Merlot, Chardonnay and Sauvignon Blanc), seven development stages (prebloom 1, prebloom 2, bloom, pea-size, bunch closure, véraison and harvest) and two treatments (untreated and surface-sterilised). An experimental unit was one spore trap. The trail on table grapes was a 2 x 2 x 7 x 2 factorial design. The factors were two locations (Worcester and Paarl), two cultivars (Dauphine and Waltham Cross), seven development stages (prebloom 1, prebloom 2, bloom, pea-size, bunch closure, véraison and harvest) and two treatments (untreated and surface-sterilised). One Petri Dish was an experimental unit. The counts were recorded as binomial type data and were transformed to percentages and logits before subjected to analysis of variance.

Leaves. The trial on wine grapes was a 2 x 3 x 7 x 2 factorial design. The factors were two locations (Malmesbury and Stellenbosch), three cultivars (Merlot, Chardonnay and Sauvignon Blanc), seven development stages (prebloom 1, prebloom 2, bloom, pea-size, bunch closure, véraison and harvest) and two treatments (untreated and surface-sterilised). One leaf was an experimental unit. The trail on table grapes was a 2 x 2 x 7 x 2 factorial design. The factors were two locations (Worcester and Paarl), two cultivars (Dauphine and Waltham Cross), seven development stages (prebloom 1, prebloom 2, bloom, pea-size, bunch closure, véraison and harvest) and two treatments (sterile and non-sterile). One leaf was an

experimental unit. Incidences of *B. cinerea* were recorded at the blade and petiole as ordinal type data and transformed into percentages and logits (indices of 0 = 0% infection; 1 = 25% infection; 2 = 50% infection; 3 = 75% infection; 4 = 100% infection).

Inflorescences: The trial on wine grapes was a 2 x 3 x 1 x 2 factorial design. The factors were two locations (Malmesbury and Stellenbosch), three cultivars (Merlot, Chardonnay and Sauvignon Blanc), one development stage (bloom) and two treatments (untreated and surface-sterilised). One section was an experimental unit. The trail on table grapes was a two x 2 x 1 x 2 factorial design. The factors were two locations (Worcester and Paarl), two cultivars (Dauphine and Waltham Cross), one development stage (bloom) and two treatments (untreated and surface-sterilised). One section was an experimental unit. Incidences of sections yielding *B. cinerea* at any of the different positoins were recorded as binomial type data and were transformed to percentages and logits before subjected to analysis of variance.

Bunches: The trial on wine grapes was a 2 x 3 x 4 x 2 factorial design. The factors were two locations (Malmesbury and Stellenbosch), three cultivars (Merlot, Chardonnay and Sauvignon Blanc), four development stages (bloom, pea-size, bunch closure, véraison and harvest) and two treatments (untreated and surface-sterilised). One section was an experimental unit. The trial on table grapes was a 2 x 2 x 4 x 2 factorial design. The factors were two locations (Worcester and Paarl), two cultivars (Dauphine and Waltham Cross), four development stages (bloom, pea-size, bunch closure, véraison and harvest) and two treatments (untreated and surface-sterilised). One section was an experimental unit. Incidences of positions (laterals, the pedicel-berry attachment zone, and the cheek and stylar end of berries) yielding *B. cinerea* were recorded as binomial type data and were transformed to percentages and logits before subjected to analysis of variance.

Levene's test for homogeneity of variance was performed to test if the seasonal variability in observations were of comparable magnitude. Analyses of variance were performed using SAS version 8.2 (SAS 1999). Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). Student's t-Least Significant Difference was calculated at the 5% confidence level to compare treatment means (Ott, 1998). Using data recorded for both seasons from the different vineyards of each cultivar, correlation coefficients were computed to explore the degree of closeness of linear relationships between the number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines and the incidence of *B. cinerea* recorded from the different tissues.

RESULTS

Growth of *B. cinerea* on spore traps and from grape material. Microscopy revealed that *B. cinerea* developed on Petri dishes from all the vineyards in a scattered pattern mainly from single cells. The number of viable conidia that had landed on the dishes could thus be recorded as separate colonies during incubation. In nearly all vineyards during prebloom to fruit set, *B. cinerea* colonies developed regularly from dishes suspended during the 4-day period following a wet period. Following a dry period, their development from dishes was mostly erratic. From bunch closure onwards, individual colonies occasionaly developed from a few dishes collected after both wet or dry periods. However, based on colony development per treatment dish per day, no clear pattern of production, liberation and dispersal of *B. cinerea* inoculum could be found in any of the vineyards. Colony numbers on dishes suspended 1.5 m apart in the bunch zone of a vine varied greatly. The numbers also differed between days at a vine in the same vineyard.

Symptom expression occurred in a consistent pattern on plant material collected in the different vineyards. On leaves, browning of the tissue followed by the development of sporulating *B. cinerea* colonies, was first noticeable on leaf blades approximately 3 to 4 days after the paraquat treatment. The pathogen developed first alongside the veins and from the leaf basis. On some leaves, one to more separate lesions formed, or the pathogen developed scattered over the entire leaf blade. On some leaves a lesion developed only at the leaf basis. On inflorescences at full bloom, symptoms were expressed within 2 days as small brown lesions on laterals and rachises, and within 3 to 4 days on pedicels. On some inflorescences lesions expanded very rapidly, spread within 5 to 7 days to the entire rachis and most of the laterals and pedicels which were later profusely covered with sporulating *B. cinerea* colonies. However, ovaries remained asymptomatic. On the structural bunch parts, brown lesions appeared on the rachis, laterals and pedicel after 4 to 7 days and sporulation occurred after 5 to 8 days. On berries, symptom expression was noticed first at the base. This was characterised 3 to 5 days after the paraquat treatment by a browning of the skin at the base, which gradually extended to the central portion of the berry. Sporulating colonies of the pathogen generally occurred after 5 to 7 days, first at the pedicel-berry attachment zone, and later on the discolored cheek. Individual lesions seldom occurred on the berry cheek, and the pathogen developed from a few berries initially from this position. On some of the berries that yielded the pathogen at the pedicel-berry attachment zone, sporulation remained confined

to this position. For both treatments the stylar end of the berries remained at all samplings mostly asymptomatic and virtually free from *B. cinerea.*

Levene's test for homogeneity of variance (P>0.05) indicates that for all experiments the seasonal variability in data of the observations were of comparable magnitude and, hence, a combined analysis was validated. In cases where there were significant evidence for non-normality it was due to high kurtosis and not skewness, which was an indication of lots of zeros and thus non-normality was due to kurtosis. The data was therefore further interpretated (Glass *et al.*, 1972). Data of the stylar end was included in the analysis, but are not further discussed.

Detection of *B. cinerea* in wine grape vineyards. *Spore traps.* The analysis of variance (ANOVA) for the effect of day, locality, season, cultivar, growth stage and treatment showed that locality, season, cultivar, growth stage, and their interaction had a highly significant effect ($P < 0.01$) on *B. cinerea* occurrence in the air around the bunch zone of vines (Table 3). In both regions, some similarities in the pattern of *B. cinerea* occurrence in vineyards were found between the two seasons (Table 4). In the 2001/2002 season, the number of colonies recorded on the spore traps were high during bloom, it decreased significantly at pea size and remained at a low level at the later growth stages. In the season 2002-2003, colony numbers peaked during the prebloom and bloom period, and were mostly very low from pea size to the harvest stages.

Leaves. The pathogen occurred in distinct patterns on the blades and petioles. The analysis of variance (ANOVA) for the effect of locality, season, cultivar, growth stage and treatment showed that locality, season, cultivar, growth stage and treatment, and their interaction, had a highly significant effect ($P < 0.01$) on *B. cinerea* occurrence on leaf blades (Table 5). For clarity, the effects for the lower order interaction locality x cultivar x growth stage x treatment ($P < 0.0001$) are discussed (Table 6) using the mean values for the two seasons. Leaf blades mostly displayed the highest incidence of *B. cinerea* from prebloom to fruit set in both the NST and ST treatments. During this period, in some vineyards but not in all, significantly more leaf blades yielded the pathogen in NST than the ST treatment. Based on the incidence of blades yielding *B. cinerea* in the NST treatment, amounts of the pathogen in all vineyards declined significantly from bloom to bunch closure stage. Incidences then gradually declined from pea size stage to véraison, and the pathogen was not found on the leaf blades during harvest. The pathogen developed in nearly a similar trend from leaf blades

in the ST treatment. Petioles occasionally yielded *B. cinerea* (Table 7). In the 2001/2002 season, the pathogen developed from this position mainly at bloom and bunch closure. In the 2002/2003 seasons, *B. cinerea* was associated with petioles only at prebloom 2 and bunch closure.

The relationship between the number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines and the incidence of *B. cinerea* recorded from leaf tissue is given in Table 8 (Malmesbury) and Table 9 (Stellenbosch) for the blades, and Table 10 (both regions) for petioles, respectively. For the blade, the incidence of *B. cinerea* tended to correlate positively with the number of *B. cinerea* colonies recorded on spore traps during bloom. For the petiole, no distinct relationship was found.

Inflorescences. The analysis of variance (ANOVA) for the effect of locality, season, cultivar, and treatment showed that locality, season, cultivar, treatment, and their interaction, had a significant effect ($P < 0.05$) on *B. cinerea* occurrence on inflorescences at bloom (Table 11). Important responses can be derived from the significant interaction. For the 2001/2002 season in the NST treatment, incidences of *B. cinerea* were mostly high (Table 12). Inflorescences in the ST treatment yielded the pathogen at significantly lower incidences. However, an exception was found on Merlot. For Stellenbosch, incidences of *B. cinerea* for both treatments were at an equal, high level. For Malmesbury, incidences of *B. cinerea* were low, but significantly higher in the ST treatment. For the 2002/2003 season, the occurrence of *B. cinerea* was generally at a low level, except for the Malmesbury Sauvignon blanc NST treatment, which showed a 100% incidence. *B. cinerea* did also not develop in the ST treatment from inflorecences collected from Stellenbosch. For both seasons, there was no significant relationship between the number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines at bloom and the incidence of *B. cinerea* recorded on inflorescences (Tables 13, 14).

Bunches. The analysis of variance (ANOVA) for the effect of locality, season, cultivar, growth stage and treatment showed that season, cultivar, growth stage, treatment, and their interaction, had a highly significant effect ($P < 0.01$) on *B. cinerea* occurrence at the laterals, pedicel-berry attachment base and the cheek of berries (Table 15). Several general responses can be derived from the significant effect of the interaction. Based on the incidence of *B. cinerea* found in both the NST and ST treatments, the pathogen predominantly occurred at laterals and the pedicel-berry attachment zone of bunches (Table 16), and rarely at the cheek

(Table 19). For both treatments, *B. cinerea* incidences at the lateral and pedicel-berry attachment zone were mostly significantly higher at pea size stage than at bunch closure, and were generally low at véraison and harvest.

The relationship between the number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines and the incidence of *B. cinerea* recorded at the different positions in bunches is given in Tables 17, 18, and 20. The incidence of of *B. cinerea* tended to correlate positively only for the lateral and pedicel-berry attachment zone, and the correlation was significant positive for the NST of only Sauvignon blanc and Merlot at bunch closure (Table 17), and for the ST treatment of Chardonnay at pea size stage (Table 18). There was no significant relationship for the cheek (Table 20).

Detection of *B. cinerea* in table grape vineyards. *Spore traps.* The analysis of variance (ANOVA) for the effect of day, locality, season, cultivar, growth stage, and treatment showed that the locality x season x cultivar x growth stage interaction had a highly significant effect ($P < 0.01$) on *B. cinerea* occurrence in the air around the bunch zone of vines (Table 21). The significant interaction was due mainly to data obtained during the different seasons and growth stages. In the 2001/2002 season in both regions and both cultivars, *B. cinerea* colony numbers were significantly higher during bloom than at the other growth stages (Table 22). The number of colonies decreased during the growing season, but were significantly higher during pea size and bunch closure than during véraison and harvest. *B. cinerea* colonies seldom developed on the dishes during the latter two stages. In the 2002/2003 season from bloom to harvest, *B. cinerea* colony numbers were only significantly higher during bloom at Worcester for Waltham Cross, and at Paarl for Dauphine (Table 22). *B. cinerea* colonies seldom developed on the dishes during pea size, bunch closure, véraison and harvest. However, in both regions, *B. cinerea* colony numbers in Dauphine vineyards were significantly higher during prebloom 2 than bloom. This was also the case for Waltham Cross in the Worcester region.

Leaves. The analysis of variance (ANOVA) for the effect of locality, season, cultivar, growth stage and treatment showed that locality, season, cultivar, growth stage and treatment, and their interaction, had a highly significant effect ($P < 0.01$) on *B. cinerea* occurrence on leaf blades (Table 23). Based on the leaf blade data, the pathogen occurred at a significantly high level during prebloom 2 and during bloom in all vineyards (Table 24). During these stages in some vineyards, but not in all, significantly more leaf blades yielded the pathogen in

NST than the ST treatment. On the other hand, with the exception of Waltham Cross, the pathogen rarely developed in both treatments from leaf blades obtained from pea size to harvest. Petioles seldom yielded *B. cinerea*, (Table 26), except for the prebloom and bloom stages, when the pathogen developed from the tissue during the 2001/2002 and 2002/2003 seasons, respectively. The number of *B. cinerea* colonies recorded on spore traps placed in the bunch zone of vines and the incidence of *B. cinerea* recorded from leaf tissue tended to correlate positive only during the prebloom and bloom period (Table 25). There was no significant relationship for the petiole (Table 27).

Inflorescences. The analysis of variance (ANOVA) for the effect of locality, season, cultivar, and treatment showed that locality, season, cultivar, treatment, and their interaction, had a significant effect ($P < 0.05$) on *B. cinerea* occurrence on inflorescences at bloom (Table 28). According to the data for the NST treatment, *B. cinerea* occurred for both seasons at bloom at exceptionally high levels on inflorescences of vines at both regions (Table 29). There was no significant relationship between the number of *B. cinerea* colonies recorded on spore traps and the incidence of *B. cinerea* recorded on NST inflorescences (Table 30).

Bunches. The analysis of variance (ANOVA) for the effect of locality, season, cultivar, growth stage and treatment showed that season, cultivar, growth stage, treatment, and their interaction, had a highly significant effect ($P < 0.01$) on *B. cinerea* occurrence at the laterals (Table 31). For the pedicel-berry attachment base, *B. cinerea* occurrence was significantly effected ($P < 0.01$) by locality, season, growth stage, treatment. For the stylar end and the berry cheek, *B. cinerea* occurrence was significantly effected ($P < 0.01$) by season, growth stage and treatment. According to the significant interactions, *B. cinerea* resided primarily at positions in the inner bunch at pea size stage (Tables 32, 34). Furthermore, at this growth stage, the incidence of *B. cinerea* tended to correlate positive only during 2002/2003 for the lateral and pedicel-berry attachment zone of Dauphine (Tables 33, 35).

DISCUSSION

The data on the occurrence of *B. cinerea* in air and on grape material gives a new perspective to the relationship between the inoculum dosage in air, conidial densities on leaf and bunch surfaces and incidence of Botrytis bunch rot and blight. It is generally assumed that for *B. cinerea*, inoculum is always present in the field and that production, liberation and dispersal of inoculum is an ongoing process (Jarvis, 1980). In French vineyards, a fluctuation

was found in the concentration of *B. cinerea* conidia in the air during the growing season; the highest numbers occurred from véraison to vintage (Corbaz, 1972; Bulit & Verdu, 1973). *B. cinerea* is thus considered difficult to control on grape largely because of its ubiquitous nature in vineyards, and its complex life cycle (Broome *et al.*, 1995; Nair & Balasubramaniam, 1995). This study showed that inoculum of the pathogen is not always present in air and that production, liberation and dispersal of inoculum is not an ongoing process in vineyards in the Western Cape province. The *B. cinerea* inoculum dosage in air differed between seasons, regions, and vineyards. Inoculum dosages also frequently differed between the two positions selected for placement of dishes for the NST and ST treatments of a single vine. These differences can be ascribed to the influence exerted in each vineyard on *B. cinerea* by different sets of environmental and climatical conditions, cultivation practices (Jarvis, 1980) and the location of vineyard trash colonised by the pathogen. For example, trellis systems used for wine grape vineyards differed from that used in table grape vineyards. Furthermore, for all the regions, weather conditions were more conducive for the development of *B. cinerea* during the 2001/2002 than 2002/2003 season (Haasbroek, P.D., LNR Infruitec-Nietvoorbij, Privaatsak X5013, Stellenbosch, 7599). The number of conidia that occur in a canopy depends largely on the balance between two competing forces, deposition and turbulent transport, and the vertical position of the inoculum source. In general, conidia produced on a source on the ground and lower in the canopy are exposed to slower wind speeds, less turbulence and rapid rates of sedimentation. They are thus transported over a short range (Fitt *et al.*, 1985). In vineyards, 95% of *B. cinerea* conidia are deposited within 1 m from the ground source (Seyb, 2003). A similar pattern has been reported for *B. cinerea* dispersal in snap bean fields in which few conidia were detected beyond 2.5 m from the source (Johnson & Powelson, 1983).

The various tests revealed that the pathogen generally occurred in a corresponding pattern in air in the bunch zone of vines, on leaves and in bunches from all vineyards. Based on the combined data for the various tests, the inoculum dosage in air in the bunch zone of the vine was generally highest during prebloom to fruit set, it decreased at pea size and mostly remained at a very low level at the later growth stages. The estimations of viable *B. cinerea* residing naturally on leaves and in bunches, showed that their amounts depicted levels occurring in air in the bunch zone of the vine. The different tests also revealed that for leaf blades and inflorescences, amounts of inoculum occurring on the tissue surface and in the tissues may fluctuated largely during the period bloom to fruit set. The data of the paraquat

treatments on untreated and surface-sterilized bunch sections confirmed the findings of Holz and co-workers (Holz, 1999; Holz *et al.*, 2000) who showed that *B. cinerea* is largely associated with the pedicel-berry attachment base, that it seldom develops from the berry cheek, and that the pathogen is absent in the stylar end. The data also confirmed their findings (Holz, 1999; Holz *et al.*, 2000) that latent infections in the berry base were few at véraison and harvest. However, due to the necrotrophic (spreading) ability of the pathogen, extensive berry rot (due to berry-to-berry contact) and thus severe bunch rot can develop from a single berry that become symptomatic at the base of the pedicel/berry attachment base. The *B. cinerea* occupation pattern explains why pre- and postharvest Botrytis bunch rot develops mostly from the inner bunch (Nair, 1985; Nair & Hill, 1992; Nair & Parker, 1985) and why disease management strategies should concentrate on the bloom to pre-bunch closure stage and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season.

My investigation on the relationship between inoculum dosage in air, conidial densities on bunch surfaces and incidences of decay recorded at the various positions in bunches, confirmed the hypothesis (Coertze *et al*, 2001; Holz *et al*, 2003) that for *B. cinerea*, bunch infection by airborne conidia should not contribute to a gradual built-up of secondary inoculum, and to *B. cinerea* epiphytotics. It also substantiates the recommendation (Holz *et al*, 2003) that *B. cinerea* studies on timing of fungicide application, biological control, host resistance and disease prediction models should place more emphasis on the behaviour of the pathogen on structures of the inner bunch. For these structures no clear relationship was found between inoculum dosage in air, conidial densities on leaf and bunch surfaces and subsequent symptom expression. Disease expression only developed when host resistance was terminated by applying paraquat as a stress factor. The data also confirmed that the different bunch parts differ in resistance to natural *B. cinerea* infection. *B. cinerea* conidia and germlings therefore have different survival periods on tissues of the various positions, as is implicated by the low incidence at which the pathogen was detected at the cheek, and the high incidence of occurrence on the rachis, lateral and pedicel. In this context it was previously shown that single conidia of the pathogen did not survive for extended periods on berry surfaces (Coertze & Holz, 1999, 2002; Coertze *et al.*, 2001; Gütschow, 2001). Passive defence (proanthocyanidins [Hill *et al.*, 1981], substances in exudates [Coertze *et al.*, 2001; Kosuge & Hewitt, 1964; McClellan & Hewitt, 1973; Padgett & Morrison, 1990; Pezet & Pont, 1984) and active defence mechanisms (lignification-like reactions [Hoos & Blaich,

1988], phytoalexins [Coertze *et al.*, 2001; Langcake, 1981] and suberin [Coertze *et al.*, 2001; Hill, 1985) may play a differential role in the resistance of the different tissues to infection by *B. cinerea*, and in the survival of conidia, germlings and latent mycelia of the pathogen.

The phenomenon that the *B. cinerea* inoculum dosage in air was highest during prebloom or bloom, and that grape leaf blades carry high amounts of *B. cinerea* during this period, suggests that leaf infection is an important primary infection event, and plays an important role in the epidemiology of the pathogen on grape. There are various factors essential for high propagule numbers in the air: a viable, productive inoculum source, conditions favourable for propagule production, and for their dispersal at the source site. Correlations have been found between dispersal and conditions favourable for sporulation (usually surface wetness with moderate temperature) in many *Botrytis* species (Jarvis, 1980). The frequency and duration of wetness events, and temperature, vary greatly during a growing season. It is anticipated that interrupted wetness periods, and temperature, will affect the number of propagules produced (Rotem *et al.*, 1978). A complicated relationship thus exists in the field between environmental conditions and propagule production and dispersal. In grapevine, sclerotia are a source of conidia that result in primary infection of young tissue (Nair & Nadtotchei, 1987). Thomas *et al.* (1981) showed that the bulk of sclerotia recovered from vineyard soils in the Western Cape province developed on vine leaves and shredded prunings. Under laborary conditions, *B. cinerea* sclerotia continue to sporulate for about 12 weeks after the production of the first crop of conidia (Nair and Nadtotchei, 1987). Primary leaf infection that become latent is therefore likely during prebloom when daily air temperature during spring reaches 20°C (Nair and Nadtotchei, 1987). Necrotic leaves in the canopy, which are commonly generated from canopy trimming typically after flowering, were identified as an important source of secondary inoculum (Seyb, 2003). Necrotic leaves in the canopy were found to have high tissue specific sporulation ability and were colonised rapidly under a range of temperature conditions, giving the fungus the potential to produce cycles of secondary inoculum in rapid succession. In addition, necrotic leaves in the canopy are closer to the target tissues than ground trash. The quantity of necrotic leaves is highly dependent on vineyard management practices such as the frequency and timing of trimming. The timing of leaf trimming and exactly when the fungus can colonise the leaves after trimming relative to environmental conditions will dictate the timing of potential inoculum production. All of these factors mean that the primary infection cycles occur early season on leaves, and that

necrotic leaves are an important source of secondary inoculum for dispersal to the developing bunches.

In conclusion, the findings that the *B. cinerea* inoculum dosage in air was highest during prebloom or bloom, 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 and inoculum levels in bunches are the 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, preventative applications are recommended to reduce two primary infection events: (a) between budding and pre-bloom to counteract primary leaf infection; (b) during late bloom or early pea size stage, to reduce the amount of the pathogen on leaves and infloresences and to prevent colonisation of floral debris. A third spray can be applied at bunch closure to reduce the amount of *B. cinerea* at various positions of the inner bunch, especially for cultivars with tight bunches.

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Table 1. Table grape vineyards and growth stages used for the detection of *Botrytis cinerea* in air and on plant tissue during 2001-2003

Region and growth stage	Cultivar, % Brix and collection date					
	Dauphine			Waltham Cross		
	Sporetraps	Plant tissue	% Brix	Sporetraps	Plant tissue	% Brix
Prebloom 1						
Worcester	---	---	---	---	---	---
	23-27/09/2002	27/09/2002	---	23-27/09/2002	27/09/2002	---
Paarl	---	---	---	---	---	---
	23-27/09/2002	27/09/2002	---	23-27/09/2002	27/09/2002	---
Prebloom 2						
Worcester	---	---	---	---	---	---
	7-11/10/2002	11/10/2002	---	7-11/10/2002	11/10/2002	---
Paarl	---	---	---	---	---	---
	7-11/10/2002	11/10/2002	---	7-11/10/2002	11/10/2002	---
Bloom						
Worcester	22-26/10/2001	26/10/2001	---	22-26/10/2001	26/10/2001	---
	21-25/10/2002	25/10/2002	---	21-25/10/2002	25/10/2002	---
Paarl	22-26/10/2001	26/10/2001	---	22-26/10/2001	26/10/2001	---
	21-25/10/2002	25/10/2002	---	21-25/10/2002	25/10/2002	---
Pea size						
Worcester	19-23/11/2001	23/11/2001	---	19-23/11/2001	23/11/2001	---
	18-22/11/2002	22/11/2002	---	18-22/11/2002	22/11/2002	---
Paarl	19-23/11/2001	23/11/2001	---	19-23/11/2001	23/11/2001	---
	18-22/11/2002	22/11/2002	---	18-22/11/2002	22/11/2002	---
Bunch closure						
Worcester	10-14/12/2001	14/12/2001	5.2	10-14/12/2001	14/12/2001	6.5
	9-13/12/2002	13/12/2002	5.1	9-13/12/2002	13/12/2002	6.2
Paarl	10-14/12/2001	14/12/2001	6.6	10-14/12/2001	14/12/2001	6.5
	9-13/12/2002	13/12/2002	6.2	9-13/12/2002	13/12/2002	6.2
Véraison						
Worcester	7-11/01/2002	11/01/2002	5.2	7-11/01/2002	11/01/2002	8.1
	6-10/01/2003	10/01/2003	5.9	6-10/01/2003	10/01/2003	8.4
Paarl	7-11/01/2002	11/01/2002	6	7-11/01/2002	11/01/2002	7.1
	6-10/01/2003	10/01/2003	6.3	6-10/01/2003	10/01/2003	7.2
Harvest						
Worcester	18-22/02/2002	22/02/2002	14.1	28-01/02/2002	01/02/2002	11.8
	17-21/02/2003	21/02/2003	14.3	27-31/01/2003	31/01/2003	11.7
Paarl	18-22/02/2002	22/02/2002	16.7	28-01/02/2002	01/02/2002	18.4
	17-21/02/2003	21/02/2003	16.9	27-31/01/2003	31/01/2003	18.9

Table 2. Wine grape vineyards and growth stages used for the detection of *Botrytis cinerea* in air and on plant tissue during 2001-2003

Region and growth stage	Cultivar, % Brix and collection date								
	Merlot			Chardonnay			Sauvignon blanc		
	Sporetraps	Plant tissue	% Brix	Sporetraps	Plant tissue	% Brix	Sporetraps	Plant tissue	% Brix
Prebloom 1									
Stellenbosch	---	---	---	---	---	---	---	---	---
	23-27/09/2002	27/09/2002	---	23-27/09/2002	27/09/2002	---	23-27/09/2002	27/09/2002	---
Malmesbury	---	---	---	---	---	---	---	---	---
	23-27/09/2002	27/09/2002	---	23-27/09/2002	27/09/2002	---	23-27/09/2002	27/09/2002	---
Prebloom 2									
Stellenbosch	---	---	---	---	---	---	---	---	---
	7-11/10/2002	11/10/2002	---	7-11/10/2002	11/10/2002	---	7-11/10/2002	11/10/2002	---
Malmesbury	---	---	---	---	---	---	---	---	---
	7-11/10/2002	11/10/2002	---	7-11/10/2002	11/10/2002	---	7-11/10/2002	11/10/2002	---
Bloom									
Stellenbosch	22-26/10/2001	26/10/2001	---	22-26/10/2001	26/10/2001	---	22-26/10/2001	26/10/2001	---
	21-25/10/2002	25/10/2002	---	21-25/10/2002	25/10/2002	---	21-25/10/2002	25/10/2002	---
Malmesbury	22-26/10/2001	26/10/2001	---	22-26/10/2001	26/10/2001	---	22-26/10/2001	26/10/2001	---
	21-25/10/2002	25/10/2002	---	21-25/10/2002	25/10/2002	---	21-25/10/2002	25/10/2002	---
Pea size									
Stellenbosch	19-23/11/2001	23/11/2001	---	19-23/11/2001	23/11/2001	---	19-23/11/2001	23/11/2001	---
	18-22/11/2002	22/11/2002	---	18-22/11/2002	22/11/2002	---	18-22/11/2002	22/11/2002	---
Malmesbury	19-23/11/2001	23/11/2001	---	19-23/11/2001	23/11/2001	---	19-23/11/2001	23/11/2001	---
	18-22/11/2002	22/11/2002	---	18-22/11/2002	22/11/2002	---	18-22/11/2002	22/11/2002	---
Bunch closure									
Stellenbosch	10-14/12/2001	14/12/2001	5	10-14/12/2001	14/12/2001	6	10-14/12/2001	14/12/2001	6.2
	9-13/12/2002	13/12/2002	5.2	9-13/12/2002	13/12/2002	6.3	9-13/12/2002	13/12/2002	6
Malmesbury	10-14/12/2001	14/12/2001	---	10-14/12/2001	14/12/2001	6.4	10-14/12/2001	14/12/2001	5.5
	9-13/12/2002	13/12/2002	---	9-13/12/2002	13/12/2002	6.5	9-13/12/2002	13/12/2002	5.6

(Continued on next page)

Table 2. *(Continued from previous page)*

Region and growth stage	Cultivar, % Brix and collection date								
	Merlot			Chardonnay			Sauvignon blanc		
	Sporetraps	Plant tissue	% Brix	Sporetraps	Plant tissue	% Brix	Sporetraps	Plant tissue	% Brix
Véraison									
Stellenbosch	7-11/01/2002	11/01/2002	5.2	7-11/01/2002	11/01/2002	16.9	7-11/01/2002	11/01/2002	12.3
	6-10/01/2003	10/01/2003	5.4	6-10/01/2003	10/01/2003	16.4	6-10/01/2003	10/01/2003	12.1
Malmesbury	7-11/01/2002	11/01/2002	---	7-11/01/2002	11/01/2002	18.2	7-11/01/2002	11/01/2002	16.5
	6-10/01/2003	10/01/2003	---	6-10/01/2003	10/01/2003	18.1	6-10/01/2003	10/01/2003	16.9
Harvest									
Stellenbosch	28-01/02/2002	01/02/2002	17	28-01/02/2002	01/02/2002	21	28-01/02/2002	01/02/2002	12.3
	27-31/01/2003	31/01/2003	17.5	27-31/01/2003	31/01/2003	21.3	27-31/01/2003	31/01/2003	12.8
Malmesbury	28-01/02/2002	01/02/2002	---	28-01/02/2002	01/02/2002	22.6	28-01/02/2002	01/02/2002	17.1
	27-31/01/2003	31/01/2003	---	27-31/01/2003	31/01/2003	22.8	27-31/01/2003	31/01/2003	17.8

Table 3. Analysis of variance on the number of *Botrytis cinerea* colonies recorded on spore traps placed in the bunch zone of wine grape (cultivars Merlot, Chardonnay and Sauvignon blanc) during 2001-02 and 2002-03

Source of Variation	df	MS	P>F
Day (D)	35	12.906	0.0980
Location (L)	1	86.600	0.0029
Season (S)	1	17863.125	<.0001
L x S	1	0.667	0.7935
Cultivar (C)	2	644.576	<.0001
L x C	2	37.169	0.0222
S x C	2	284.688	<.0001
L x S x C	2	857.495	<.0001
Growth Stage (G)	6	16694.909	<.0001
L x G	6	221.022	<.0001
S x G	4	18667.090	<.0001
L x S x G	4	29.606	0.0165
C x G	12	413.408	<.0001
L x C x G	12	333.266	<.0001
S x C x G	8	195.874	<.0001
L x S x C x G	8	469.340	<.0001
Treatment (T)	1	57.200	0.0155
L x T	1	3.000	0.5787
S x T	1	111.512	0.0007
L x S x T	1	200.006	<.0001
C x T	2	10.120	0.3536
L x C x T	2	5.726	0.5552
S x C x T	2	18.669	0.1472
L x S x C x T	2	8.914	0.4002
G x T	6	54.929	<.0001
L x G x T	6	44.833	0.0001
S x G x T	4	28.793	0.0190
L x S x G x T	4	29.169	0.0178
C x G x T	12	24.802	0.0025
L x C x G x T	12	7.039	0.7292
S x C x G x T	8	16.180	0.1030
L x S x C x G x T	8	2.317	0.9837
D (L x S x C x G x T)	1144	9.726	
Error	7317	8.478	
Corrected Total	8639		

Table 4. Means of the effect for the interaction locality - cultivar -season - growth stage (GS) on *Botrytis cinerea* incidences recorded on spore traps in wine grape vineyards (cultivars Merlot, Chardonnay and Sauvignon blanc)

	Stellenbosch						Malmesbury					
	Merlot		Chardonnay		Sauvignon blanc		Merlot		Chardonnay		Sauvignon blanc	
GS	1[y]	2[z]	1	2	1	2	1	2	1	2	1	2
Prebloom 1		0.9 n-q		1.9 j-m		1.7 k-m		0.1 p-s		0.6 n-s		1.1 m-o
Prebloom 2		3.4 h		2.6 h-j		3.2 hi		6.4 g		0.1 rs		8.2 f
Bloom	15.1 d	0.4 o-s	13.3 e	3.2 hi	24.4 a	2.1 j-l	13.2 e	0.3 p-s	21.5 b	0.2 p-s	17.2 c	0.3 p-s
Pea size	0.7 n-s	0.0 s	0.7 n-s	0.0 s	1.1 m-o	0.0 s	0.7 n-s	0.0 rs	0.8 n-s	0.2 p-s	2.4 i-k	0.1 q-s
Bunch closure	0.2 p-s	0.1 q-s	0.3 p-s	0.0 s	0.5 o-s	0.2 p-s	0.0 rs	0.0 s	0.4 o-s	0.0 s	0.2 p-s	0.0 s
Véraison	0.5 o-s	0.5 o-s	1.3 l-n	1.3 l-n	0.9 n-p	0.9 n-p	0.1 rs	0.1 rs	0.1 p-s	0.1 p-s	0.0 rs	0.0 rs
Harvest	0.1 rs	0.0 s	0.2 p-s	0.1 p-s	0.2 p-s	0.1 rs	0.1 rs	0.0 s	0.1 p-s	0.0 s	0.0 rs	0.1 rs

Average values of the number of colonies recorded on spore traps obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Season 2001-02

[z] Season 2002-03

Table 5. Analysis of variance on the incidence of wine grape leaves (cultivars Merlot, Chardonnay and Sauvignon blanc) yielding *Botrytis cinerea* at the blade and petiole during 2001-02 and 2002-03

		Blade		Petiole	
Source of Variation	**df**	**MS**	**P>F**	**MS**	**P>F**
Location (L)	1	52018.519	<.0001	315.104	0.1638
Season (S)	1	12588.922	<.0001	13062.574	<.0001
L x S	1	12064.021	<.0001	46.503	0.5926
Cultivar (C)	2	3029.803	0.0016	1974.826	<.0001
L x C	2	4180.845	0.0001	127.604	0.4559
S x C	2	2170.693	0.0097	1085.590	0.0013
L x S x C	2	2854.969	0.0023	9.598	0.9426
Growth Stage (G)	6	100622.950	<.0001	7017.841	<.0001
L x G	6	3203.836	<.0001	238.575	0.1849
S x G	4	3598.611	<.0001	9893.576	<.0001
L x S x G	4	6100.694	<.0001	280.035	0.1419
C x G	12	1258.887	0.0013	1252.141	<.0001
L x C x G	12	4753.464	<.0001	217.816	0.1881
S x C x G	8	2091.059	<.0001	457.378	0.0042
L x S x C x G	8	767.101	0.1080	109.462	0.7149
Treatment (T)	1	126806.713	<.0001	1458.623	0.0028
L x T	1	6685.185	0.0002	1377.604	0.0036
S x T	1	23948.446	<.0001	2564.889	<.0001
L x S x T	1	1486.243	0.0746	150.670	0.3356
C x T	2	10951.678	<.0001	925.637	0.0034
L x C x T	2	1727.720	0.0249	115.451	0.4913
S x C x T	2	4665.683	<.0001	1902.042	<.0001
L x S x C x T	2	2688.054	0.0032	89.013	0.5781
G x T	6	29701.918	<.0001	2027.331	<.0001
L x G x T	6	1687.632	0.0014	1253.158	<.0001
S x G x T	4	11511.806	<.0001	2230.035	<.0001
L x S x G x T	4	1988.194	0.0020	226.910	0.2325
C x G x T	12	4726.794	<.0001	1536.880	<.0001
L x C x G x T	12	6153.679	<.0001	579.175	<.0001
S x C x G x T	8	4076.649	<.0001	1829.774	<.0001
L x S x C x G x T	8	3019.705	<.0001	264.149	0.1122
Error	2016	467.138		162.409	
Corrected Total	2159				

Table 6. Means of the effect for the interaction locality - cultivar -growth stage (GS) - treatment on *Botrytis cinerea* incidences recorded on leaf blades from wine grape vineyards (cultivars Merlot, Chardonnay and Sauvignon blanc)

	Stellenbosch						Malmesbury					
	Merlot		Chardonnay		Sauvignon blanc		Merlot		Chardonnay		Sauvignon blanc	
GS	NST[y]	ST[z]	NST	ST	NST	ST	NST	ST	NST	ST	NST	ST
Prebloom 1	100.0 a	3.3 p-r	46.7 gh	15.0 m-p	33.3 h-k	3.3 p-r	0.0 r	0.0 r	25.0 i-n	0.0 r	58.3 fg	0.0 r
Prebloom 2	100.0 a	36.7 hi	80.0 b-d	35.0 h-j	36.7 hi	26.7 i-m	26.7 i-m	13.3 n-q	45.0 h	33.3 h-k	73.3 c-e	13.3 n-q
Bloom	74.2 c-e	43.3 h	69.2 d-f	28.3 i-l	91.7 ab	10.8 o-r	25.0 i-n	35.8 h-j	62.5 ef	5.8 p-r	82.5 bc	4.2 p-r
Pea size	1.7 qr	21.7 k-o	10.8 o-r	6.7 p-r	20.0 l-o	21.7 k-o	15.0 m-p	4.2 p-r	0.0 r	7.5 p-r	0.8 r	0.0 r
Bunch closure	11.7 o-r	6.7 p-r	21.7 k-o	0.0 r	35.0 h-j	13.3 n-q	1.7 qr	0.0 r	0.0 r	1.7 qr	11.7 o-r	10.0 o-r
Véraison	0.0 r	6.7 p-r	0.0 r	0.0 r	24.2 j-m	0.0 r	0.0 r	0.0 r	2.5 qr	2.5 qr	2.5 qr	4.2 p-r
Harvest	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r

Average values of data recorded on leaf blades obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Leaves left untreated

[z] Leaves surface-sterilised

Table 7. Means of the effect for the interaction season - growth stage (GS) - treatment on *Botrytis cinerea* incidences recorded on leaf petioles from wine grape vineyards (cultivars Merlot, Chardonnay and Sauvignon blanc)

GS	Treatment	2001-2002	2002-2003
Prebloom 1	NST[y]	0.0 e	0.0 e
	ST[z]	0.0 e	0.0 e
Prebloom 2	NST	0.0 e	5.3 d
	ST	0.0 e	2.8 de
Bloom	NST	35.3 a	0.0 e
	ST	12.5 b	0.0 e
Pea size	NST	2.2 de	0.0 e
	ST	0.8 e	0.0 e
Bunch closure	NST	5.0 d	5.0 d
	ST	8.9 bc	8.9 bc
Véraison	NST	0.0 e	0.0 e
	ST	0.0 e	0.0 e
Harvest	NST	0.8 e	0.0 e
	ST	0.0 e	0.0 e

Average values of data recorded on leaf petioles obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Leaves left untreated
[z] Leaves surface-sterilised

Table 8. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on leaf blades left untreated (NST) , or surface sterilised (ST)

	Malmesbury					
	Merlot		Chardonnay		Sauvignon blanc	
GS	NST	ST	NST	ST	NST	ST
Prebloom 1	---	---	-0.1375 (0.4241)	---	0.0262 (0.8797)	---
Prebloom 2	-0.0613 (0.7225)	-0.0465 (0.7878)	---	0.1212 (0.4812)	0.1647 (0.3372)	0.0105 (0.9516)
Bloom	-0.2944 (0.0121*)	0.6858 (<.0001*)	0.5476 (<.0001*)	0.4371 (0.0001*)	0.7194 (<.0001*)	0.3272 (0.0050*)
Pea size	0.1644 (0.1677)	0.1729 (0.1465)	---	-0.0443 (0.7118)	0.1929 (0.1045)	---
Bunch closure	0.1107 (0.3544	---	---	-0.0286 (0.8117)	-0.1115 (0.3512)	0.0892 (0.4561)
Véraison	---	---	0.2546 (0.0309*)	---	-0.0271 (0.8214)	---
Harvest	---	---	---	---	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 9. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on leaf blades left untreated (NST) , or surface sterilised (ST)

	Stellenbosch					
	Merlot		Chardonnay		Sauvignon blanc	
GS	NST	ST	NST	ST	NST	ST
Prebloom 1	---	0.1121 (0.5151)	-0.2451 (0.1497)	-0.1533 (0.3720)	0.3476 (0.0378*)	-0.1496 (0.3838)
Prebloom 2	---	0.0213 (0.9020)	0.1243 (0.4702)	-0.0048 (0.9780)	-0.0090 (0.9584)	-0.0730 (0.6724)
Bloom	0.2068 (0.0813)	0.5568 (<.0001*)	-0.4860 (<.0001*)	0.4962 (<.0001*)	0.6095 (<.0001*)	0.6102 (<.0001*)
Pea size	-0.0310 (0.7962)	---	-0.0763 (0.5240)	-0.0309 (0.7967)	-0.1694 (0.1549)	-0.1072 (0.3702)
Bunch closure	-0.0062 (0.9591)	0.1089 (0.3624)	-0.0872 (0.4662)	---	0.0417 (0.7279)	0.0579 (0.6288)
Véraison	---	0.1276 (0.2855)	---	---	0.0763 (0.5239)	---
Harvest	---	---	---	---	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 10. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on leaf petioles left untreated (NST) , or surface sterilised (ST)

GS	Treatment	2001-2002	2002-2003
Prebloom 1	NST	---	---
	ST	---	---
Prebloom 2	NST	---	-0.1019 (0.1355)
	ST	---	0.0161 (0.8138)
Bloom	NST	0.2027 (0.0028*)	---
	ST	-0.0745 (0.2759)	---
Pea size	NST	-0.0602 (0.3788)	---
	ST	-0.1618 (0.0173*)	---
Bunch closure	NST	0.0712 (0.2973)	-0.0285 (0.6775)
	ST	0.0574 (0.4016)	0.1600 (0.0186*)
Véraison	NST	---	---
	ST	---	---
Harvest	NST	-0.1101 (0.1065)	---
	ST	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 11. Analysis of variance on the incidence of wine grape (cultivars Merlot, Chardonnay and Sauvignon blanc) inflorescences yielding *Botrytis cinerea* at bloom during 2001-02 and 2002-03

Source of Variation	df	MS	P>F
Location (L)	1	7143.026	<.0001
Season (S)	1	134456.261	<.0001
L x S	1	65707.814	<.0001
Cultivar (C)	2	10593.910	<.0001
L x C	2	32532.338	<.0001
S x C	2	2263.545	0.0051
L x S x C	2	25054.296	<.0001
Treatment (T)	1	62424.046	<.0001
L x T	1	15482.458	<.0001
S x T	1	214.636	0.4766
L x S x T	1	2203.166	0.0231
C x T	2	29239.617	<.0001
L x C x T	2	4820.561	<.0001
S x C x T	2	2898.733	0.0012
L x S x C x T	2	1432.364	0.0349
Error	336	422.699	
Corrected Total	359		

Table 12. Means of the effect for the interaction locality - season - cultivar - treatment on *Botrytis cinerea* incidences recorded on inflorescences (cultivars Merlot, Chardonnay and Sauvignon blanc)

	Stellenbosch						Malmesbury					
	Merlot		Chardonnay		Sauvignon blanc		Merlot		Chardonnay		Sauvignon blanc	
Season	NST[y]	ST[z]	NST	ST	NST	ST	NST	ST	NST	ST	NST	ST
2001-2002	98.5 a	98.0 a	58.7 b	21.1 de	100.0 a	65.9 b	1.2 f	8.1 e	93.8 a	41.6 c	65.9 b	3.1 f
2002-2003	7.5 ef	0.0 f	7.7 e	0.0 f	20.0 de	0.0 f	1.7 f	4.3 f	34.5 cd	3.3 f	100.0 a	0.0 f

Average values of data recorded on inflorescences obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Inflorescences left untreated

[z] Inflorescences surface-sterilised

Table 13. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on inflorescences left untreated (NST) , or surface sterilised (ST)

	Malmesbury					
	Merlot		Chardonnay		Sauvignon blanc	
Season	NST	ST	NST	ST	NST	ST
2001-2002	0.0178 (0.9178)	0.0607 (0.7252)	0.0981 (0.5690)	0.0853 (0.6210)	0.0556 (0.7474)	-0.0767 (0.6567)
2002-2003	-0.0470 (0.7854)	-0.1106 (0.5208)	0.0560 (0.7455)	-0.0689 (0.6897)	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 14. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on inflorescences left untreated (NST) , or surface sterilised (ST)

	Stellenbosch					
	Merlot		Chardonnay		Sauvignon blanc	
Season	NST	ST	NST	ST	NST	ST
2001-2002	-0.0127 (0.9413)	-0.2435 (0.1524)	-0.0651 (0.7061)	0.0057 (0.9739)	---	-0.0124 (0.9430)
2002-2003	---	-0.0622 (0.7187)	0.1149 (0.5048)	---	0.1025 (0.5520)	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 15. Analysis of variance on the incidence of *Botrytis cinerea* recorded at different positions in bunches of wine grape (cultivars Merlot, Chardonnay and Sauvignon blanc) during 2001-02 and 2002-03

				Berry					
		Lateral		Base		Cheek		Stylar-end	
Source of Variation	**df**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**
Location (L)	1	52.682	0.4734	37.179	0.2004	2.672	0.4623	0.213	0.3416
Season (S)	1	56005.017	<.0001	2381.052	<.0001	112.101	<.0001	0.734	0.0778
L x S	1	66.550	0.4204	0.194	0.9264	33.986	0.0089	0.456	0.1644
Cultivar (C)	2	1073.576	<.0001	354.680	<.0001	33.940	0.0011	0.192	0.4421
L x C	2	944.158	0.0001	244.790	<.0001	25.703	0.0057	0.203	0.4236
S x C	2	858.500	0.0002	405.689	<.0001	8.055	0.1964	0.156	0.5160
L x S x C	1	3282.925	<.0001	462.863	<.0001	220.453	<.0001	0.561	0.1233
Growth Stage (G)	3	16966.093	<.0001	8244.863	<.0001	263.607	<.0001	1.606	0.0001
L x G	3	780.489	<.0001	182.596	<.0001	5.628	0.3323	0.317	0.2588
S x G	3	8580.834	<.0001	1521.322	<.0001	83.297	<.0001	0.766	0.0212
L x S x G	3	690.872	0.0002	41.633	0.1384	50.283	<.0001	0.666	0.0375
C x G	6	1026.274	<.0001	579.951	<.0001	32.055	<.0001	0.195	0.5481
L x C x G	3	4063.505	<.0001	424.874	<.0001	43.049	<.0001	0.105	0.7212
S x C x G	5	1468.836	<.0001	501.609	<.0001	36.334	<.0001	0.213	0.4772
L x S x C x G	1	23.874	0.6293	391.512	<.0001	420.278	<.0001	1.164	0.0265
Treatment (T)	1	16057.232	<.0001	750.051	<.0001	126.912	<.0001	0.251	0.3018
L x T	1	712.658	0.0085	181.308	0.0048	21.374	0.0378	0.129	0.4594
S x T	1	10601.092	<.0001	66.970	0.0858	32.246	0.0108	0.014	0.8072
L x S x T	1	1542.337	0.0001	379.468	<.0001	0.185	0.8466	0.001	0.9434
C x T	2	1378.850	<.0001	16.504	0.4827	6.106	0.2911	0.001	0.9963
L x C x T	2	502.339	0.0076	113.291	0.0069	0.044	0.9911	0.061	0.7712
S x C x T	2	1575.241	<.0001	23.482	0.3549	36.165	0.0007	0.219	0.3944
L x S x C x T	1	583.373	0.0172	1.011	0.8327	55.885	0.0008	0.028	0.7309
G x T	3	1309.636	<.0001	373.710	<.0001	92.381	<.0001	0.220	0.4233
L x G x T	3	2177.550	<.0001	242.675	<.0001	15.847	0.0225	0.094	0.7548
S x G x T	3	684.314	0.0002	16.112	0.5452	14.567	0.0319	0.003	0.9981

(Continued on next page)

Table 15. *(Continued from previous page)*

				Berry					
		Lateral		Base		Cheek		Stylar-end	
Source of Variation	**df**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**
L x S x G x T	2	1019.655	<.0001	460.537	<.0001	0.144	0.9713	0.001	0.9945
C x G x T	6	518.645	<.0001	52.779	0.0306	5.654	0.3344	0.005	1.0000
L x C x G x T	3	3672.559	<.0001	309.014	<.0001	2.593	0.6653	0.138	0.6237
S x C x G x T	5	413.740	0.0012	65.366	0.0135	38.320	<.0001	0.227	0.4396
L x S x C x G x T	1	1006.647	0.0018	13.325	0.4432	100.963	<.0001	0.043	0.6698
Error	1036	102.426		22.644		4.942		0.236	
Corrected	1110								

Table 16. Means of the effect for the interaction season - cultivar -growth stage (GS) - position (P) - treatment on *Botrytis cinerea* incidences recorded in bunches of wine grape (cultivars Merlot, Chardonnay and Sauvignon blanc)

	2001-2002						2002-2003					
	Merlot		Chardonnay		Sauvignon blanc		Merlot		Chardonnay		Sauvignon blanc	
GS, P	NST[y]	ST[z]	NST	ST	NST	ST	NST	ST	NST	ST	NST	ST
Lateral												
Pea size	30.1 bc	28.0 bc	29.9 bc	19.7 d	56.1 a	30.2 bc	6.3 f-i	0.4 ij	5.8 f-j	2.1 ij	8.2 f-h	4.6 g-j
Bunch closure	14.8 de	11.6 ef	27.0 bc	9.2 e-g	32.7 b	1.9 ij	0.0 j	0.0 j	3.0 h-j	1.6 ij	1.1 ij	1.0 ij
Véraison	1.4 ij	2.9 h-j	14.6 de	3.1 h-j	26.0 c	0.2 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.2 j
Harvest	1.1 ij	0.0 j	5.2 g-j	2.0 ij	5.0 g-j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j
Pedicel-berry base												
Pea size	17.8 c	21.4 b	9.3 d	5.1 ef	27.9 a	16.3 c	4.1 fg	0.2 j	7.7 de	5.0 ef	9.2 d	4.9 ef
Bunch closure	3.1 f-i	2.5 f-j	2.6 f-j	0.8 ij	1.4 g-j	0.4 ij	0.0 j	0.0 j	1.2 h-j	0.5 ij	0.3 ij	0.1 j
Véraison	3.7 f-h	0.0 j	0.1 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j
Harvest	0.2 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j

Average values of data recorded on laterals and pedicel-berry bases obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Bunches left untreated

[z] Bunches surface-sterilised

Table 17. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on laterals and pedicel-berry bases left untreated (NST) , or surface sterilised (ST)

	2001-2002					
	Merlot		Chardonnay		Sauvignon blanc	
GS	NST	ST	NST	ST	NST	ST
Lateral						
Pea size	-0.0854 (0.6203)	---	0.0705 (0.5562)	0.0978 (0.4138)	-0.1293 (0.2792)	-0.2930 (0.0125*)
Bunch closure	0.6579 (<.0001)	---	0.0756 (0.5277)	0.0883 (0.4610)	0.3301 (0.0046*)	0.0694 (0.5627)
Véraison	0.1708 (0.3192)	-0.2411 (0.1566)	-0.0180 (0.9169)	-0.0718 (0.6773)	-0.2754 (0.0192*)	-0.0506 (0.6732)
Harvest	-0.0639 (0.7113)	---	---	---	-0.0878 (0.4635)	---
Pedicel-berry base						
Pea size	-0.0018 (0.9915)	---	0.0442 (0.7122)	0.1360 (0.2546)	-0.0519 (0.6650)	-0.2155 (0.0691)
Bunch closure	0.4878 (0.0025*)	---	0.0047 (0.9685)	0.0315 (0.7929)	0.2716 (0.0210*)	-0.0312 (0.7949)
Véraison	-0.1954 (0.2535)	---	0.0367 (0.8319)	---	---	---
Harvest	-0.0639 (0.7113)	---	---	---	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 18. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on laterals and pedicel-berry bases left untreated (NST) , or surface sterilised (ST)

	2002-2003					
	Merlot		Chardonnay		Sauvignon blanc	
GS	NST	ST	NST	ST	NST	ST
Lateral						
Pea size	---	-0.0452 (0.7062)	---	0.5121 (<.0001*)	---	0.0201 (0.8669)
Bunch closure	---	---	-0.0286 (0.8686)	-0.2317 (0.1739)	---	-0.0724 (0.5456)
Véraison	---	---	---	---	---	---
Harvest	---	---	---	---	---	---
Pedicel-berry base						
Pea size	---	-0.0321 (0.7888)	---	0.2520 (0.0327*)	---	0.0215 (0.8581)
Bunch closure	---	---	-0.0286 (0.8686)	-0.1234 (0.4736)	---	-0.0632 (0.5982)
Véraison	---	---	---	---	---	---
Harvest	---	---	---	---	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 19. Means of the effect for the interaction season - growth stage (GS) - position (P) - treatment on *Botrytis cinerea* incidences recorded on berry cheeks from wine grape vineyards (cultivars Merlot, Chardonnay and Sauvignon blanc)

	2001-2002		2002-2003	
GS, P	**NST[y]**	**ST[z]**	**NST**	**ST**
Berry cheek				
Pea size	1.5 ab	0.2 b	3.9 a	1.2 ab
Bunch closure	0.1 b	0.0 b	0.7 ab	0.1 b
Véraison	0.0 b	0.0 b	0.0 b	0.0 b
Harvest	0.0 b	0.0 b	0.0 b	0.0 b

Average values of data recorded on berry cheeks obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Bunches left untreated

[z] Bunches surface-sterilised

Table 20. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on berry cheeks left untreated (NST) , or surface sterilised (ST)

	2001-2002		2002-2003	
GS	**NST**	**ST**	**NST**	**ST**
Berry cheek	---	---	---	---
Pea size	-0.0291 (0.6978)	-0.0180 (0.8106)	---	0.0099 (0.8850)
Bunch closure	0.0332 (0.6587)	-0.0191 (0.7995)	-0.0183 (0.8787)	-0.0435 (0.6548)
Véraison	---	---	---	---
Harvest	---	---	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 21. Analysis of variance on the number of *Botrytis cinerea* colonies recorded on spore traps placed in the bunch zone of table grape (cultivars Dauphine and Waltham Cross) during 2001-02 and 2002-03

		Colonies	
Source of Variation	**df**	**MS**	**P>F**
Day (D)	59	16.389	0.4872
Location (L)	1	17.009	0.3097
Season (S)	1	7399.485	<.0001
L x S	1	14.897	0.3417
Cultivar (C)	1	30.189	0.1760
L x C	1	2.063	0.7235
S x C	1	169.929	0.0013
L x S x C	1	78.061	0.0296
Growth Stage (G)	6	6289.741	<.0001
L x G	6	79.777	<.0001
S x G	4	4446.099	<.0001
L x S x G	4	11.778	0.5815
C x G	6	314.294	<.0001
L x C x G	6	168.291	<.0001
S x C x G	4	81.458	0.0006
L x S x C x G	4	306.638	<.0001
Treatment (T)	1	257.134	<.0001
L x T	1	12.750	0.3791
S x T	1	907.101	<.0001
L x S x T	1	0.057	0.9532
C x T	1	92.771	0.0178
L x C x T	1	5.688	0.5568
S x C x T	1	26.172	0.2076
L x S x C x T	1	15.947	0.3253
G x T	6	124.656	<.0001
L x G x T	6	3.142	0.9795
S x G x T	4	226.371	<.0001
L x S x G x T	4	11.318	0.6008
C x G x T	6	34.488	0.0512
L x C x G x T	6	17.284	0.3913
S x C x G x T	4	29.952	0.1227
L x S x C x G x T	4	7.684	0.7603
D (L x S x C x G x T)	1330	16.467	
Error	4275	8.687	
Corrected Total	5759		

Table 22. Means of the effect for the interaction locality - cultivar -season - growth stage (GS) on *Botrytis cinerea* incidences recorded on spore traps in table grape vineyards (cultivars Dauphine and Waltham Cross)

	Worcester				Paarl			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
GS	1[y]	2[z]	1	2	1	2	1	2
Prebloom 1		0.2 n-p		0.0 p		0.0 p		0.0 p
Prebloom 2		6.8 e		2.6 gh		4.8 f	2.4 g-j	3.0 g
Bloom	8.6 d	1.1 k-p	15.1 a	1.3 j-m	13.0 b	2.1 g-k	11.8 c	0.0 p
Pea size	1.7 h-k	0.0 p	1.2 k-n	0.0 p	1.3 k-m	0.5 m-p	1.5 h-l	0.5 l-p
Bunch closure	4.6 f	0.1 op	1.5 i-l	0.1 op	1.7 h-k	0.2 nop	2.5 g-i	0.3 n-p
Véraison	0.1 n-p	0.1 n-p	0.1 n-p	0.1 n-p	0.5 m-p	0.5 m-p	0.4 m-p	0.4 m-p
Harvest	0.0 p	0.0 p	0.0 p	0.0 p	0.0 p	0.4 m-p	0.0 p	0.0 p

Average values of the number of colonies recorded on spore traps obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Season 2001-02
[z] Season 2002-03

Table 23. Analysis of variance on the incidence of table grape leaves (cultivars Dauphine and Waltham Cross) yielding *Botrytis cinerea* at the blade and petiole during 2001-02 and 2002-03

		Blade		Petiole	
Source of Variation	**df**	**MS**	**P>F**	**MS**	**P>F**
Location (L)	1	22168.403	<.0001	62.500	0.4466
Season (S)	1	17738.294	<.0001	13724.008	<.0001
L x S	1	9559.573	<.0001	64.286	0.4402
Cultivar (C)	1	8751.736	<.0001	3210.069	<.0001
L x C	1	6250.000	<.0001	293.403	0.0993
S x C	1	1809.573	0.0303	3327.431	<.0001
L x S x C	1	301.786	0.3760	877.431	0.0044
Growth Stage (G)	6	94298.884	<.0001	10054.911	<.0001
L x G	6	6168.080	<.0001	139.980	0.2548
S x G	4	1104.948	0.0220	14143.750	<.0001
L x S x G	4	6065.885	<.0001	21.354	0.9394
C x G	6	4785.442	<.0001	2420.486	<.0001
L x C x G	6	5940.154	<.0001	289.583	0.0135
S x C x G	4	7280.469	<.0001	3576.042	<.0001
L x S x C x G	4	4222.656	<.0001	379.688	0.0072
Treatment (T)	1	37515.625	<.0001	2918.403	<.0001
L x T	1	1777.778	0.0318	501.736	0.0312
S x T	1	13054.018	<.0001	1484.573	0.0002
L x S x T	1	104.960	0.6016	17.907	0.6837
C x T	1	250.000	0.4204	6.944	0.7997
L x C x T	1	3210.069	0.0039	27.778	0.6118
S x C x T	1	1501.786	0.0484	79.365	0.3911
L x S x C x T	1	931.002	0.1201	166.865	0.2137
G x T	6	15928.497	<.0001	1391.518	<.0001
L x G x T	6	1709.301	0.0002	418.254	0.0008
S x G x T	4	355.469	0.4492	1684.375	<.0001
L x S x G x T	4	132.031	0.8489	36.979	0.8490
C x G x T	6	485.293	0.2724	67.212	0.7118
L x C x G x T	6	15852.009	<.0001	39.087	0.9028
S x C x G x T	4	412.240	0.3692	10.417	0.9836
L x S x C x G x T	4	3915.885	<.0001	30.729	0.8878
Error	1344	384.797		107.825	
Corrected Total	1439				

Table 24. Means of the effect for the interaction locality - cultivar -growth stage (GS) - treatment on *Botrytis cinerea* incidences recorded on leaf blades from table grape vineyards (cultivars Dauphine and Waltham Cross)

	Worcester				Paarl			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
GS	NST[y]	ST[z]	NST	ST	NST	ST	NST	ST
Prebloom 1	75.0 bc	1.7 l	0.0 l	0.0 l	46.7 de	0.0 l	100.0 a	0.0 l
Prebloom 2	95.0 a	21.7 ij	26.7 hi	33.3 gh	73.3 bc	66.7 c	100.0 a	26.7 hi
Bloom	40.8 d-g	36.7 e-h	35.0 f-h	3.3 kl	83.3 b	45.8 d-f	38.3 e-g	50.8 d
Pea size	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	0.8 l	0.0 l	0.0 l
Bunch closure	0.0 l	6.7 kl	0.0 l	3.3 kl	0.0 l	1.7 l	0.0 l	0.0 l
Véraison	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l
Harvest	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	13.3 jk	0.0 l

Average values of data recorded on leaf blades obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Leaves left untreated

[z] Leaves surface-sterilised

Table 25. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on leaf blades left untreated (NST) , or surface sterilised (ST)

	Worcester				Paarl			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
GS	NST	ST	NST	ST	NST	ST	NST	ST
Prebloom 1	0.4226 (0.1166)	-0.6814 (0.0052*)	---	---	---	---	---	---
Prebloom 2	-0.4320 (0.1079)	0.0284 (0.9200)	-0.5130 (0.0505)	0.6271 (0.0123*)	0.5634 (0.0288*)	0.7059 (0.0033*)	---	0.0998 (0.7234)
Bloom	0.7746 (<.0001*)	0.5398 (0.0021*)	-0.3850 (0.0357*)	0.1784 (0.3456)	-0.6299 (0.0002*)	0.2051 (0.2770)	0.2636 (0.1593)	-0.2384 (0.2045)
Pea size	---	---	---	---	---	-0.0941 (0.6210)	---	---
Bunch closure	---	-0.0621 (0.7444)	---	-0.1048 (0.5814)	---	-0.0297 (0.8761)	---	---
Véraison	---	---	---	---	---	---	---	---
Harvest	---	---	---	---	---	---	-0.0823 (0.6656)	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 26. Means of the effect for the interaction season - growth stage (GS) - treatment on *Botrytis cinerea* incidences recorded on leaf petioles of Dauphine and Waltham Cross grapes

GS	Treatment	2001-2002	2002-2003
Prebloom 1	NST[y]	0.0 d	0.0 d
	ST[z]	0.0 d	0.0 d
Prebloom 2	NST	0.0 d	10.0 c
	ST	0.0 d	2.1 d
Bloom	NST	46.6 a	0.0 d
	ST	22.5 b	0.0 d
Pea size	NST	0.4 d	0.0 d
	ST	0.0 d	0.0 d
Bunch closure	NST	0.0 d	0.0 d
	ST	0.0 d	0.0 d
Véraison	NST	0.0 d	0.0 d
	ST	0.0 d	0.0 d
Harvest	NST	1.7 d	0.0 d
	ST	0.0 d	0.0 d

Average values of data recorded on leaf petioles obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Leaves left untreated
[z] Leaves surface-sterilised

Table 27. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on leaf petioles left untreated (NST) , or surface sterilised (ST)

GS	Treatment	2001-2002	2002-2003
Prebloom 1	NST	---	---
	ST	---	---
Prebloom 2	NST	---	0.1115 (0.3964)
	ST	---	0.1138 (0.3865)
Bloom	NST	-0.0923 (0.4830)	---
	ST	-0.0319 (0.8089)	---
Pea size	NST	-0.1197 (0.3623)	---
	ST	---	---
Bunch closure	NST	---	---
	ST	---	---
Véraison	NST	---	---
	ST	---	---
Harvest	NST	-0.0337 (0.7982)	---
	ST	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 28. Analysis of variance on the incidence of laterals in table grape (cultivars Dauphine and Waltham Cross) inflorescences yielding *Botrytis cinerea* at bloom during 2001-02 and 2002-03

Source of Variation	df	MS	P>F
Location (L)	1	13935.128	<.0001
Season (S)	1	25073.802	<.0001
L x S	1	364.208	0.3079
Cultivar (C)	1	18070.783	<.0001
L x C	1	3398.214	0.0020
S x C	1	8045.114	<.0001
L x S x C	1	5061.014	0.0002
Treatment (T)	1	147418.325	<.0001
L x T	1	3124.660	0.0031
S x T	1	39634.789	<.0001
L x S x T	1	10769.406	<.0001
C x T	1	5006.705	0.0002
L x C x T	1	2433.358	0.0088
S x C x T	1	12.124	0.8523
L x S x C x T	1	1699.651	0.0283
Error	224	348.739	
Corrected Total	239		

Table 29. Means of the effect for the interaction locality - season - cultivar - treatment on *Botrytis cinerea* incidences recorded on inflorescences (Cultivars Dauphine and Waltham Cross)

	Worcester				Paarl			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
Season	NST[y]	ST[z]	NST	ST	NST	ST	NST	ST
2001-2002	98.8 a	89.7 ab	61.7 c	57.7 c	100.0 a	80.6 b	98.4 a	57.7 c
2002-2003	100.0 a	1.1 e	92.9 ab	0.0 e	87.1 ab	52.8 c	100.0 a	25.0 d

Average values of data recorded in inflorescences obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Inflorescences left untreated

[z] Inflorescences surface-sterilised

Table 30. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on inflorescences left untreated (NST) , or surface sterilised (ST)

	Worcester				Paarl			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
Season	NST	ST	NST	ST	NST	ST	NST	ST
2001-2002	0.3018 (0.2743)	-0.1921 (0.4928)	-0.0095 (0.9732)	0.0249 (0.9299)	---	0.29819 (0.2804)	0.0973 (0.7301)	-0.0087 (0.9754)
2002-2003	---	0.1544 (0.5827)	---	---	-0.1289 (0.6471)	0.5722 (0.0258*)	---	-0.660 (0.0074*)

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 31. Analysis of variance on the incidence of *Botrytis cinerea* recorded at different positions in bunches of table grape (cultivars Dauphine and Waltham Cross) during 2001-02 and 2002-03

				Berry					
		Lateral		Base		Cheek		Stylar-end	
Source of Variation	**df**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**
Location (L)	1	1939.916	<.0001	1556.070	<.0001	1.887	0.5763	0.079	0.3499
Season (S)	1	6626.393	<.0001	1717.821	<.0001	229.633	<.0001	0.193	0.1444
L x S	1	835.464	<.0001	364.680	<.0001	11.736	0.1636	0.008	0.7685
Cultivar (C)	1	1115.694	<.0001	1.714	0.7328	17.186	0.0919	0.268	0.0851
L x C	1	397.366	0.0002	38.892	0.1042	0.040	0.9356	0.000	0.9644
S x C	1	1264.889	<.0001	94.602	0.0114	89.814	0.0001	0.098	0.2981
L x S x C	1	1813.869	<.0001	690.730	<.0001	198.425	<.0001	0.067	0.3892
Growth Stage (G)	3	8149.586	<.0001	4300.643	<.0001	953.375	<.0001	0.881	<.0001
L x G	3	2201.876	<.0001	1555.355	<.0001	0.978	0.9220	0.047	0.6654
S x G	3	2977.261	<.0001	1284.555	<.0001	210.548	<.0001	0.260	0.0349
L x S x G	3	1166.638	<.0001	332.840	<.0001	10.791	0.1480	0.027	0.8248
C x G	3	304.146	<.0001	55.486	0.0104	36.361	0.0005	0.204	0.0801
L x C x G	3	638.505	<.0001	46.671	0.0235	0.095	0.9973	0.005	0.9805
S x C x G	3	419.955	<.0001	24.820	0.1679	114.918	<.0001	0.148	0.1784
L x S x C x G	3	2297.767	<.0001	742.373	<.0001	198.905	<.0001	0.038	0.7345
Treatment (T)	1	4322.737	<.0001	1304.914	<.0001	253.535	<.0001	0.286	0.0751
L x T	1	1245.125	<.0001	764.025	<.0001	31.230	0.0232	0.058	0.4246
S x T	1	1556.147	<.0001	333.536	<.0001	83.103	0.0002	0.001	0.9137
L x S x T	1	199.167	0.0084	5.545	0.5393	0.283	0.8285	0.002	0.8745
C x T	1	272.659	0.0020	35.737	0.1193	11.174	0.1741	0.002	0.8772
L x C x T	1	49.225	0.1892	15.495	0.3048	0.005	0.9764	0.001	0.9268
S x C x T	1	434.151	0.0001	91.190	0.0129	4.040	0.4136	0.025	0.5976
L x S x C x T	1	413.663	0.0001	108.063	0.0068	6.484	0.3004	0.090	0.3186
G x T	3	1676.372	<.0001	1000.279	<.0001	190.566	<.0001	0.220	0.0635
L x G x T	3	1085.969	<.0001	626.009	<.0001	24.995	0.0063	0.032	0.7887

(Continued on next page)

Table 31. *(Continued from previous page)*

		Berry							
		Lateral		Base		Cheek		Stylar-end	
Source of Variation	**df**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**	**MS**	**P>F**
S x G x T	3	353.379	<.0001	234.232	<.0001	76.065	<.0001	0.004	0.9889
L x S x G x T	3	209.280	<.0001	1.804	0.9467	0.218	0.9908	0.016	0.9124
C x G x T	3	84.584	0.0312	84.383	0.0007	24.622	0.0069	0.003	0.9923
L x C x G x T	3	64.939	0.0782	5.085	0.7921	0.367	0.9804	0.012	0.9425
S x C x G x T	3	137.423	0.0025	56.021	0.0099	5.900	0.4028	0.010	0.9510
L x S x C x G x T	3	435.373	<.0001	77.979	0.0013	5.722	0.4170	0.056	0.6041
Error	896	28.514		14.700		6.038		0.090	
Corrected	959								

Table 32. Means of the effect for the interaction season - cultivar -growth stage (GS) and treatment on *Botrytis cinerea* incidences recorded in bunches of table grapes (cultivars Dauphine and Waltham Cross)

	2001-2002				2002-2003			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
GS, P	NST[y]	ST[z]	NST	ST	NST	ST	NST	ST
Lateral								
Pea size	22.3 b	8.5 d	36.3 a	14.4 c	9.0 d	1.4 g	6.7 de	2.0 g
Bunch closure	0.2 g	0.0 g	5.1 ef	0.2 g	2.3 g	0.0 g	1.0 g	0.0 g
Véraison	2.5 fg	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g
Harvest	1.0 g	0.0 g	11.8 c	2.4 g	0.0 g	0.0 g	0.0 g	0.0 g
Pedicel-berry base								
Pea size	19.8 a	7.2 c	19.8 a	7.3 c	9.6 b	1.1 d-g	2.4 de	2.5 d
Bunch closure	0.0 g	0.0 fg	2.0 d-f	0.1 fg	0.3 fg	0.1 fg	0.4 e-g	0.0 g
Véraison	0.4 fg	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g	0.0 g
Harvest	0.0 g	0.0 g	1.7 d-g	1.1 d-g	0.0 g	0.0 g	0.0 g	0.0 g

Average values of data recorded on laterals and pedicel-berry bases obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Bunches left untreated

[z] Bunches surface-sterilised

Table 33. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on laterals and pedicel-berry bases left untreated (NST) , or surface sterilised (ST)

	2001-2002				2002-2003			
	Dauphine		Waltham Cross		Dauphine		Waltham Cross	
GS	NST	ST	NST	ST	NST	ST	NST	ST
Lateral								
Pea size	-0.2903 (0.1197)	0.2799 (0.1342)	0.3280 (0.0768)	-0.0524 (0.7832)	0.8425 (<.0001*)	0.0562 (0.7680)	---	-0.2849 (0.1270)
Bunch closure	-0.2470 (0.1882)	---	0.1049 (0.5814)	-0.0990 (0.6027)	-0.1033 (0.5869)	---	-0.0957 (0.6148)	---
Véraison	0.0693 (0.7161)	---	---	---	---	---	---	---
Harvest	-0.0757 (0.6910)	---	-0.1455 (0.4430)	---	---	---	---	---
Pedicel-berry base								
Pea size	-0.2553 (0.1732)	0.2479 (0.1865)	0.3207 (0.0840)	-0.2063 (0.2741)	0.9214 (<.0001*)	0.1852 (0.3273)	---	-0.2870 (0.1241)
Bunch closure	-0.2470 (0.1882)	---	0.1227 (0.5184)	-0.0990 (0.6027)	-0.0835 (0.6610)	0.0332 (0.8616)	-0.0049 (0.9796)	---
Véraison	0.0681 (0.7206)	---	---	---	---	---	---	---
Harvest	---	---	-0.1150 (0.5451)	---	---	---	---	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

Table 34. Means of the effect for the interaction season - growth stage (GS) - position (P) - treatment on *Botrytis cinerea* incidences recorded on berry cheeks from table grape vineyards (cultivars Dauphine and Waltham Cross)

	2001-2002		2002-2003	
GS, P	**NST[y]**	**ST[z]**	**NST**	**ST**
Berry cheek				
Pea size	9.0 a	3.0 b	2.9 b	1.5 c
Bunch closure	0.6 d	0.1 d	0.4 d	0.1 d
Véraison	0.0 d	0.0 d	0.0 d	0.0 d
Harvest	0.0 d	0.0 d	0.0 d	0.0 d

Average values of data recorded on berry cheeks obtained from 15 vines per vineyard. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Bunches left untreated

[z] Bunches surface-sterilised

Table 35. Correlation coefficients between the number of *Botrytis cinerea* colonies recorded at different growth stages (GS) on spore traps placed in the bunch zone of vines and *B. cinerea* incidences on berry cheeks left untreated (NST) , or surface sterilised (ST)

	2001-2002		2002-2003	
GS	**NST**	**ST**	**NST**	**ST**
Berry cheek				
Pea size	0.1421 (0.2788)	0.1486 (0.2573)	0.2391 (0.0658)	-0.1738 (0.1844)
Bunch closure	-0.1067 (0.4173)	-0.1344 (0.3059)	-0.0517 (0.6949)	0.0257 (0.8456)
Véraison	-0.0897 (0.4953)	---	---	---
Harvest	---	---	-0.0242 (0.8545)	---

Total of experiments carried out between 2000 and 2003 with material from 15 vines per vineyard. Values are correlation coefficients and corresponding P values (in parentheses); * = significant at P = 0.05.

3. FENHEXAMID EFFICACY AGAINST *BOTRYTIS CINEREA* ON LEAVES AND INFLORESCENCES OF GRAPE AT BLOOM

ABSTRACT

The efficacy of fenhexamid on leaves and inflorescences carrying natural *B. cinerea* inoculum was compared with those inoculated with dry, airborne conidia. Shoots were obtained during late bloom from a vineyard (wine grape cultivar Merlot) in the Stellenbosch region. The shoots were divided into two main groups. One group of shoots were left uninoculated, the other shoots were inoculated by dusting with dry *B. cinerea* conidia in a settling tower. Before inoculation, equal numbers of shoots in each main group was sprayed with fenhexamid, or left unsprayed. Following inoculation and incubation, shoots of each treatment were divided in two equal groups. The one lot of shoots were rinsed in water. The other lot of shoots were immersed in paraquat solution to terminate host resistance and to promote the development of the pathogen from the tissues. For both uninoculated and inoculated shoots, irrespective of fungicide treatment, leaves remained asymptomatic at both the blade and petiole position for the water rinse treatment. No symptom of *B. cinerea* decay developed at any of the positions on leaves from shoots sprayed with fenhexamid. Spraying of shoots with fenhexamid completely suppressed *B. cinerea* infection and symptom expression on both uninoculated and inoculated inflorescences. For inoculated shoots, *B. cinerea* developed from approximately 50% of the laterals in the water rinse treatment. However, inflorescences rinsed in water remained asymptomatic.

INTRODUCTION

Botrytis cinerea Pers.: Fr., a pathogen of grapevine (*Vitis vinifera* L.), moves mainly through conidia by air currents in vineyards which are deposited intermittently on the surfaces of leaves, inflorescences and bunches (Part 2). Studies with *B. cinerea* on various aspects such as timing of fungicide application, biological control, host resistance and disease prediction models usually comprise 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). Berries are considered resistant to infection when immature, and susceptible when mature (Nelson, 1956; Hill *et al.*, 1981; Nair & Hill, 1992). Incidence of

disease severity is thus 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 (bloom, pea size, bunch closure, véraison) for the control of *B. cinerea* in bunches (Pearson & Riegel, 1983; Nair *et al.*, 1987; Northover, 1987; De Kock & Holz, 1991,1994; LeRoux, 1995).

It is generally assumed that for *B. cinerea*, inoculum is always present in the field and that production, liberation and dispersal of inoculum is an ongoing process (Jarvis, 1980). In French vineyards, a fluctuation was found in the concentration of *B. cinerea* conidia in the air during the growing season; the highest numbers occurred from véraison to vintage (Corbaz, 1972; Bulit & Verdu, 1973). However, recent studies showed that inoculum of the pathogen is not always present in air and that production, liberation and dispersal of inoculum is not an ongoing process in vineyards in the Western Cape province. The inoculum dosage, in air in the bunch zone of the vine, was generally highest during prebloom to fruitset, it decreased at pea size and mostly remained at a very low level at the later growth stages (Part 2). The estimations of viable *B. cinerea* residing naturally on leaves and in bunches, showed that their amounts depicted levels occurring in air in the bunch zone of the vine (Part 2, Holz *et al.*, 2003). Very high occupation incidences occurred on leaves and inflorescences during bloom, and the pathogen developed predominantly from the leaf blade, and in the case of inflorescences, from the rachis, lateral and pedicel. Different tests (Part 2) also revealed that for leaf blades and inflorescescens, amounts of inoculum occurring on the tissue surface and in the tissues may have fluctuated largely during the period bloom to fruit set. From the pea size stage in bunches, the pathogen was mostly associated with the pedicel-berry attachment base. The pathogen occupied the berry cheek infrequently and the stylar end of the berries was virtually free of the pathogen (Part 2; Holz *et al.*, 2003). Collectively, these findings indicate that Botrytis bunch rot is unlikely to be caused by colonisation of the pistil, and subsequent latency in the stylar end, as was observed elsewhere (McClellan & Hewitt, 1973; Nair & Parker, 1985). It is also unlikely for berry rot to be caused by the very low amounts of *B. cinerea* occurring on the skin surface, or in the skin tissue, as was suggested by Holz and co-workers (Coertze & Holz, 1999; 2002; Coertze *et al.*, 2001; Holz *et al.*, 2003). Instead, berry rot developed primarily from the berry-pedicel attachment base. The investigations also showed that latent infections in the berry base were high early in the season, and few at véraison and harvest. Thus, due to the necrotrophic (spreading) ability of the pathogen, extensive berry rot (due to berry-to-berry contact) and severe bunch rot can

develop from a single berry that become symptomatic at the base of the pedicel/berry attachment base. The *B. cinerea* occupation pattern explains why pre- and postharvest Botrytis bunch rot develops mostly from the inner bunch (Nair, 1985; Nair & Hill, 1992; Nair & Parker, 1985).

The findings on the inoculum dosage of *B. cinerea* in air in vineyards (Part 2), and on the ecology of the pathogen on leaves, inflorescences and bunches (Part 2, Holz *et al.*, 2003) showed that disease management strategies should concentrate on the prebloom to pre-bunch closure stage, and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season. Thus, to effectively reduce *B. cinerea* in grapevine, preventative applications are recommended (Part 2; Van Rooi & Holz, 2003) to reduce two primary infection events: (a) between budding and pre-bloom to counteract primary leaf infection; (b) during late bloom or early pea size stage, to reduce the amount of the pathogen on leaves and infloresences and to prevent colonisation of floral debris. A third spray can be applied at bunch closure to reduce the amount of *B. cinerea* at various positions of the inner bunch, especially for cultivars with tight bunches.

Van Rooi and Holz (2003) determined fungicide efficacy by observing artificially inoculated intact vinelets for symptom expression at nodes, internodes, leaf blades, petioles and inflorescences, and by determining surface colonisation and penetration by isolation studies. The study showed that the fungicides, if applied properly to shoots at the prebloom stage, should effectively reduce the amount of *B. cinerea* in leaves, and completely prevent the infection of nodes, internodes and inflorescences. Laboratory tests (Part 2) on shoots obtained from different vineyards showed that, for leaf blades and inflorescescens, amounts of *B. cinerea* occurring on (surface inoculum) and in the tissues (latent infections) may fluctuate during the period bloom to fruit set. The aim of this study was to compare the efficacy of fenhexamid on leaves and inflorescences carrying natural *B. cinerea* inoculum with those inoculated with dry, airborne conidia.

MATERIALS AND METHODS

Grapevine material. Shoots with sound, unblemished leaves and infloresences were obtained on 11 November 2002 (experiment one) and on 18 November 2002 (experiment two) during late bloom from a vineyard (wine grape cultivar Merlot) in the Stellenbosch region. After removal, the shoots were placed in flasks containing 20% sucrose solution to

maintain turgidity, and transported to the laboratory. The shoots were cut back to approximately 20 cm, bearing three to five inflorescences and two to three leaves. The shoots were then inserted into sterile aluminium foil-wrapped "oases" (florist's sponge), soaked with a 20% sucrose solution to maintain turgidity and placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm). The shoots were divided into two main groups. One group of shoots was left uninoculated, the other shoots were inoculated. Before inoculation, equal numbers of shoots in each main group was left unsprayed, or were sprayed with fenhexamid.

Fungicide Treatment. Shoots selected for fungicide treatment were placed in a spray chamber and sprayed with fenhexamid (Teldor 500 SC, Bayer) at the recommended dosage (75ml Fenhexamid/100L H_2O) (Nel *et al.*, 1999). 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 pre-runoff 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. This system ensured proper fungicide coverage (Van Rooi, 2002; Van Rooi & Holz, 2003), thereby allowing uniform evaluation of fungicide efficacy at the different positions on shoots. 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; 12 g Biolab agar, 200 g potatoes, 20 g sucrose, 1000 ml H_2O) 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 both experiments. For inoculation, 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 shoots that were positioned on the floor of the tower. 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 de-ionised water to establish high relative humidity (≥93% RH). The chambers were kept for 24 h at 22°C with a 12 h photoperiod daily. 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, shoots of each treatment were divided in two equal groups. The one lot of shoots was rinsed in water and air dried. The other lot of shoots were immersed in paraquat solution (WPK Paraquat, 200 g/l [bipyridyl], WPK Agricultural, Cape Town, South Africa) for 30 seconds, rinsed in sterile de-ionised water and air-dried. The shoots were then replaced in the moist chambers and kept for 14 days at 22°C with a 12 h photoperiod. The different treatments provided conditions that facilitated the development of conidia occurring on the surface of the shoots, or by mycelia in the tissue during incubation (Holz *et al.*, 2003). Leaves and inflorescences on the shoots were monitored daily for symptom expression and the development of *B. cinerea*. Positions monitored on the leaves were at the blades and petioles. Positions on the inflorescences were the rachis, laterals, pedicels and ovaries. After 9 days the number of segments yielding sporulating *B. cinerea* colonies were recorded and the incidences calculated. The incidences were used to quantify the amount of *B. cinerea* occurring superficially or in the tissue at the various positions on leaves and in inflorescences.

Statistical Analysis. The experimental design was a completely randomized design with 9 treatment combinations and 15 shoots, each from a different, arbitrarily chosen vine, as replications. The trial was repeated twice. In the case of leaves, one leaf was an experimental unit. Incidences of *B. cinerea* were recorded at the blade or petiole as ordinal type data and transformed into percentages and logits (index 0 = 0% infection; index 1 = 25% infection; index 2 = 50% infection; index 3 = 75% infection; index 4 = 100% infection). In the case of inflorescences, one inflorescence was an individual unit. Incidences of *B. cinerea*

were recorded at the laterals as binomial type data and were transformed to percentages and logits before subjected to analysis of variance. Levene's test for homogeneity of variance was performed to test if the experiments were of comparable magnitude. Analyses of variance were performed using SAS version 8.2 (SAS 1999). Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Difference was calculated at the 5% confidence level to compare treatment means (Ott, 1998).

RESULTS

Levene's test for homogeneity of variance ($P<0.05$) indicated that for both leaves and inflorescences, the experiment variability in observations was not of comparable magnitude and hence each experiment was analysed separately (Tables 2 and 3). In cases where there were significant evidence for non-normality it was due to high kurtosis and not skewness, which was an indication of lots of zeros and thus non-normality was due to kurtosis. The data was therefore further interpreted (Glass *et al.*, 1972).

For both uninoculated and inoculated shoots, irrespective of fungicide treatment, leaves remained asymptomatic at both the blade and petiole position for the water rinse treatment (Table 3). The only exception was found with inoculated leaves, which yielded 1.7% infected blades. On the other hand, when the shoots were left unsprayed, symptoms were expressed on leaves from both uninoculated and inoculated shoots after the paraquat treatment. Incidences of blades yielding *B. cinerea* were high in the first experiment, but were substantially lower in the second experiment. However, no symptom of *B. cinerea* decay developed at any of the positions on leaves from shoots sprayed with fenhexamid.

Spraying of shoots with fenhexamid completely suppressed *B. cinerea* infection and symptom expression on both uninoculated and inoculated inflorescences (Table 2). For both uninoculated and inoculated inflorescences on unsprayed shoots, incidences of laterals yielding *B. cinerea* in the paraquat treatment were high in the first experiment, but were substantially lower in the second experiment. For inoculated shoots, *B. cinerea* developed from approximately 50% of the laterals in the water rinse treatment. However, inflorescences rinsed in water remained asymptomatic.

DISCUSSION

Data obtained in this study confirmed the finding of Van Rooi and Holz (2003) on the effective reduction by fungicides of the amount of *B. cinerea* on leaves and inflorescences, and the complete prevention of infection at bloom. The findings also emphasize the neccessity of fungicide application during bloom in the implementation of an effective Botrytis bunch rot control programme in vineyards. In South African (Part 2) and New Zealand (Seyb, 2003) vineyards, the dosage of viable *B. cinerea* inoculum in air is high during early season, and low during late season. Laboratory studies showed that *B. cinerea* resided in a similar pattern in asymptomatic vegetative and generative grape material. The relationship between *B. cinerea* inoculum dosage in air and on grapevine tissue tended to correlate positive only during bloom, and then only in case of the leaf blades (Part 2). In spite of the regular occurrence of high amounts of inoculum in air and on vegetative and generative parts, Botrytis bunch rot is not seen between fruit set and véraison in vineyards of many regions. Laboratory studies showed that disease expression only developed when host resistance was terminated by applying paraquat or freezing as stress factors (Coertze *et al.*, 2001; Holz *et al.*, 2003; Van Rooi & Holz, 2003). This implies that the pathogen requires assistance to enable the infection cycle to run its full course, and to generate a symptom. There are strong indications that the timing of leaf trimming and exactly when the fungus can colonise the leaves after trimming relative to environmental conditions will dictate the inoculum production. Primary leaf infection that become latent is therefore likely during prebloom when daily air temperature during spring reaches 20°C. Necrotic leaves in the canopy, which are commonly generated from canopy trimming typically after flowering, were identified as an important source of secondary inoculum (Seyb, 2003). Necrotic leaves in the canopy were found to have high tissue specific sporulation ability and were colonised rapidly under a range of temperature conditions, giving the fungus the potential to produce cycles of secondary inoculum in rapid succession. In addition, necrotic leaves in the canopy are closer to the target tissues than ground trash. All of these factors mean that necrotic leaves are an important source of secondary inoculum for dispersal to the developing bunches, and that primary leaf infection should be prevented.

Laboratory studies with asymptomatic leaves and inflorescences obtained from vineyards showed (Part 2, Gütschow, 2001; Holz *et al.*, 2003) 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; Van Rooi, 2002). My laboratory studies and those of others (Van Rooi & Holz, 2003) showed that fungicides, if applied properly to shoots and bunches under controlled conditions, effectively reduced the amount of *B. cinerea* at the various positions on leaves and inflorescence, and prevented infection and symptom expression at bloom. However, these goals are not achieved in vineyards where the fungicides are applied by conventional spraying methods. Therefore, more work is needed to evaluate fungicide application techniques by conventional spraying methods for proper fungicide coverage, and the reduction of *B. cinerea* in bunches.

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Table 1. Analysis of variance of data for the effect of fenhexamid treatment on the incidence of *Botrytis cinerea* recorded at different positions on Merlot leaves and inflorescences

Source of Variation	df	Experiment 1						Experiment 2					
		Laterals		Blade		Petiole		Laterals		Blade		Petiole	
		MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Treatment	8	29381.25	<.0001	22458.33	<.0001	16435.19	<.0001	11999.16	<.0001	8822.917	<.0001	375	<.0001
Error	126	257.5161		148.1481		16.5344		650.2879		351.1905		67.46032	
Corrected Total	134												

Table 2. Means of the effect of fenhexamid treatment on the incidence of *Botrytis cinerea* recorded at the laterals of Merlot inflorescences at bloom

Experiment	Uninoculated				Inoculated			
	Untreated		Fenhexamid		Untreated		Fenhexamid	
	W[y]	P[z]	W	P	W	P	W	P
1	0.0 c	100.0 a	0.0 c	0.0 c	52.5 b	100.0 a	0.0 c	0.0 c
2	0.0 c	66.7 a	0.0 c	0.0 c	50.1 b	50.6 b	0.0 c	0.0 c

Average values of data recorded on shoots obtained from 15 vines. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Water
[z] Paraquat

Table 3. Means of the effect of fenhexamid treatment on the incidence of *Botrytis cinerea* recorded on leaves of Merlot at bloom

Position and experiment	Uninoculated				Inoculated			
	Untreated		Fenhexamid		Untreated		Fenhexamid	
	W[y]	P[z]	W	P	W	P	W	P
Blade								
1	0.0 d	100.0 a	0.0 d	0.0 d	1.7 c	73.3 b	0.0 d	0.0 d
2	0.0 b	55.0 a	0.0 b	0.0 b	0.0 b	55.0 a	0.0 b	0.0 b
Petiole								
1	0.0 c	100.0 a	0.0 c	0.0 c	0.0 c	8.3 b	0.0 c	0.0 c
2	0.0 b	15.0 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b

Average values of data recorded on shoots obtained from 15 vines. Means with the same letter do not differ significantly at the 5% level according to student's least significant difference test.

[y] Water
[z] Paraquat