INFECTION PATHWAYS OF *BOTRYTIS CINEREA* ON SELECTED WINE GRAPE CULTIVARS

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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

INFECTION PATHWAYS OF *BOTRYTIS CINEREA* ON SELECTED WINE GRAPE CULTIVARS

An understanding of the infection pathways of *Botrytis cinerea* in grape bunches will help to combat this devastating pathogen of grape. Many studies have been done to determine the possible infection pathways of *B. cinerea*. Most of these studies made use of artificial inoculations that deposit groups of conidia on the plant surface. The deposition of clusters of conidia is not a common phenomenon in nature. The aim of this study was to investigate the infection pathways of (i) naturally- as well as (ii) artificially inoculated *B. cinerea* conidia during all the phenological stages of three wine grape cultivars, and to compare the (iii) pathogenicity and virulence, on grape and nectarine fruit, of isolates obtained from different host plants.

In the natural infection study the occurrence of *Botrytis cinerea* and subsequent disease expression at different positions in bunches of wine grapes (cultivars Chenin Blanc, Shiraz and Chardonnay) was determined from 1999 to 2001. Different techniques were used to detect viable inoculum at different positions (rachises, laterals, pedicels, and the peicel end, cheek and style end of berries) in bunches. Isolations were made on Kerssies' *B. cinerea* selective medium, or bunches were used untreated, or treated with paraquat. Paraquat was used to terminate host resistance and to promote the development of the pathogen from the tissues. The material was used untreated to detect the pathogen on the surface, or were surface-sterilized to detect mycelia (latent infection) in the tissue. In the artificial inoculation study, bunches of wine grapes (cultivars Chenin Blanc, Chardonnay and Shiraz) at pea size, bunch closure, and harvest were dusted with dry conidia of *Botrytis cinerea* in a settling tower and incubated for 24 h at high relative humidity (±93%). Following incubation, the bunches were divided in two groups. The one group was surface-sterilised in 70% ethanol for 5 s, the other group was left untreated. Bunches of the sterile group, and from the untreated group were used for isolation. From each bunch rachis segments, laterals, pedicels and berry skin segments (from the pedicel-end and cheek) were removed. The sections were placed in Petri dishes on Kerssies' *B. cinerea* selective medium and on a water agar medium

supplemented with paraquat, and incubated at 22°C under diurnal light. Occupation by the pathogen was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues. Lastly, in the virulence and pathogenicity experiment on grape and nectarine fruit *Botrytis cinerea* isolates, which were obtained from different host plants, were compared by simulating natural infection. Cold-stored fruit, considered highly susceptible to *B. cinerea* were therefore inoculated with single, airborne conidia of the pathogen. Different tests were conducted to assess surface penetration and lesion formation. Isolations were made from fruit skins on Kerssies' *B. cinerea* selective medium. Nectarine fruit were treated with paraquat, and grape berries were frozen for 1 h at –12°C. Paraquat and freezing were used to terminate host resistance and to promote the development of the pathogen from the tissues.

In the natural infection studies *B. cinerea* occurred in a consistent pattern in bunches of the three cultivars. *B. cinerea* consistently developed from the tissue of the rachis, laterals, pedicel and pedicel-end, but not from the berry cheek. The rachis, lateral and pedicel contained much higher levels of *B. cinerea* than any position on the berry. Furthermore, the pathogen consistenly occurred at relatively high levels on the rachises throughout the season. Collectively, the data showed that in the Western Cape province, *B. cinerea* occured more regularly in wine grape bunches during the early part of the season, than later in the season.

The data of the artificial studies confirmed the findings made with the natural infection studies. In these experiments the pathogen resided more often on the structural bunch parts than on the berries. Overall, the isolation studies revealed that conidia occurred predominantly on the rachis. The incidence of *B. cinerea* was furthermore constantly high in the inner bunch after each inoculation, and in bunches of different maturities. The data therefore indicated that, when available, conidia penetrated loose and tight clustered bunches in a similar way. Finally, in the virulence and pathogenicity experiments the results showed clearly that no host specialisation exists in the *B. cinerea* isolates used in this study.

From these studies it is clear that in the Western Cape province *B. cinerea* occurs more readily in the inner structural parts of the bunches and more so during the earlier parts of the season. These findings should be considered when planning and implementing disease control programmes.

OPSOMMING

INFEKSIEWEË VAN *BOTRYTIS CINEREA* OP GESELEKTEERDE WYNDRUIF KULTIVARS

Indiepte kennis van die infeksieweë van *Botrytis cinerea* op druiwetrosse word benodig vir die beheer van dié vernietigende patogeen van druiwe. Vele studies is al gedoen om die moontlike infeksieweë van die swam op druiwe trosse te ondersoek. Die meeste van die studies het gebruik gemaak van kunsmatige inokulasie tegnieke waar die konidia van die swam in groepe op die korreloppervlak gedeponeer is. In die natuur is dit 'n rare verskynsel dat konidia in groepe op die korreloppervlak land. Die doel van die studie was om die infeksieweë van *B. cinerea* op drie wyndruif kultivars te ondersoek wat (i) natuurlik- en (ii) kunsmatig geïnokuleer is met konidia gedurende al die fenologiese stadia, en om die (iii) virulensie en patogenisisteit van isolate wat van verskillende gashere verkry is, op druiwe en nektariens te vergelyk.

In die natuurlik-geïnokuleerde druiwe is die voorkoms van *B. cinerea* en die gevolglike siektevoorkoms op verkillende posisies in trosse van wyndruiwe (Chenin Blanc, Chardonnay, Shiraz) gedurende 1999 tot 2001 bepaal. Verskillende tegnieke is gebruik om lewensvatbare inokulum by verskillende posisies (ragis, lateraal, pedisel en pedisel-end van die korrel) in die tros waar te neem. Isolasies is op Kerssies' *B. cinerea* selektiewe medium gemaak, of trosse is onbehandeld gebruik, of behandel met paraquat. Paraquat is gebruik om die gasheer se natuurlike weerstand te verlaag en om die ontwikkeling van die patogeen te bevorder. Die plantmateriaal is onbehandeld gelaat om die patogeen op die oppervlak waar te neem, of die oppervlak is gesteriliseer om die latente myselium in die weefsel waar te neem. In die kunsmatige inokulasiestudies is trosse, van wyndruiwe (Chenin Blanc, Chardonnay, Shiraz), geïnokuleer met droë spore, van *B. cinerea,* in 'n inokulasietoring en die plantmateriaal is dan geinkubeer vir 24 h by 'n hoë relatiewe humiditeit (93%). Na die inkubasie proses is die trosse in twee groepe verdeel. Die een groep druiwe het oppervlak sterilisasie ondergaan in 70% etanol vir 5 s, en die ander groep was onbehandeld gelaat. Trosse van die onbehandelde en gesteriliseerde groep druiwe is gebruik vir isolasies. Vanuit elke tros is daar segmente van die ragis, laterale, pediselle en korrels (van die pedisel-end en wang gedeeltes) geïsoleer. Die

segmente is in Petri bakkies met Kerssies' *B. cinerea* selektiewe medium en op water agar medium, wat paraquat bevat het, geïsoleer en geïnkubeer onder 'n 12 h dagligperiode teen 22°C. Die patogeen is positief geïdentifiseer deur sporulerende kolonies op die onderskeie weefseltipes. Laastens, in die virulensie- en patogenisiteitsproewe op druiwe en nektariens is verskillende isolate van *B. cinerea*, verkry vanaf verskillende gasheerplante, vergelyk deur natuurlike inokulasie toestande na te boots. Koue opgebergde vrugte, wat beskou word as hoogs vatbaar vir die infeksie van *B. cinerea*, is geïnokuleer met droë, enkel luggedraagde spore van die patogeen. Verskillende toetse is gedoen om die oppervlak penetrerende en letselvormende vermoëns van die onderskeie isolate te toets. Isolasies is van die skille van die vrugte gemaak en op Kerssies' *B. cinerea* selektiewe medium geplaas. Die nektarienvrugte is met paraquat behandel en die druifkorrels is gevries vir 1 h teen –12°C. Paraquat en bevriesing is gebruik om die gasheer se weerstand te verlaag en om die ontwikkeling van die patogeen te bevorder.

In die natuurlik-geïnokuleerde studies het *B. cinerea* 'n konstante patroon getoon in die trosse van die drie verskillende wyndruif kultivars. *B. cinerea* het konstant ontwikkel uit die ragis, laterale, pedisel en pedisel-end, maar selde uit die korrelwang. Die ragis, lateral en pedisel dele het baie hoër vlakke van van die swam bevat as enige deel op die korrel. Die patogeen het ook konstant volop deur die hele seisoen op die ragis voorgekom. Gesamentlik wys die data dat, *B. cinerea* in wyndruiwe, in die Wes Kaap provinsie, meer geredelik vroeër in die seisoen voorkom, eerder as later.

Data van die kunsmatige inokulasiestudies het die bevindinge van die natuurlike inokulasiestudies tot 'n groot mate bevestig. In dié studies het die patogeen meer geredelik die strukturele dele van die tros, eerder as op die korrels, bewoon. Oor die algemeen het die isolasieproewe gewys dat die konidia meer op die ragis voorkom as op enige ander deel. Die voorkoms van *B. cinerea* was ook oor die algemeen baie hoër in die strukturele dele van die tros, as op die korrel self. Die verskynsel het onder trosse van verskillende ontwikkelingsvlakke voorgekom. Die data het dus ook gewys dat konidia, wanner dit beskikbaar is, minder- sowel as meer kompakte trosse op 'n soortgelyke manier penetreer. Laastens, in die virulensie en patogenisiteitseksperimente het die resultate duidelik gewys dat daar geen gasheer spesifieke gedrag onder *B. cinerea* isolate is nie.

In die studies het dit duidelik na vore gekom dat, *B. cinerea* meer geredelik in die strukturele binne dele van die wyndruif tros, in die Wes Kaap provinsie voorkom. En so ook

eerder aan die begin van die seisoen, as later in die seisoen. Dié kennis moet in aanmerking geneem word by die beplanning en implementering van siektebeheerprogramme.

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1. THE BIOLOGY, DISEASE ETIOLOGY AND VIRULENCE OF *BOTRYTIS CINEREA* ON GRAPEVINE

INTRODUCTION

Grey mould is a disease caused by *Botrytis cinerea* Pers: Fr. and it is found on a wide variety of crops including tomato, cucumber, gerbera, rose, onions, strawberry and grapes (Coley-Smith *et al.*, 1980). On grapes, the fungus causes bunch rot under a wide range of climatic conditions at pre- and post harvest stage. (Agrios, 1997; Coley-Smith, 1980). *B. cinerea* bunch rot is a major decay of stored grapes in the Western Cape province of South Africa and is responsible for annual losses of more than five million rand (Marais, 1985; Lourens, 1986).

An epidemic of grey mould disease is dependent on a complex of biological events, such as the production and dispersal of various inocula, infection and pathogen survival. The events are greatly affected by temperature, rainfall, humidity and crop phenology (Jarvis, 1980). During the last few years the development of disease prediction models has helped with combating *B. cinerea* bunch rot on grape by providing strategic fungicide spray programmes (Molot, 1987; Nair & Allen, 1993; Broome *et al.*, 1995). The use of these models is dependent on factors like wetness duration, temperature, relative humidity and crop phenology. *B. cinerea* studies on timing of fungicide application, biological control, host resistance is therefore based on an understanding of the amount of conidia occurring naturally on the surface of grape berries, of the amount of mycelia occurring in grape berry tissue, and the ecology of this inocula.

DISPERSAL

According to Jarvis (1980), there are four important types of dispersal propagules: conidia, sclerotia, ascospores and mycelia. In South Africa the teliomorph of *B. cinerea, Botryotinia fuckeliana* (de Bary) Whetzel, has not been reported and ascospores will probably not play a significant role in the epidemiology of the disease. Coley-Smith (1980) furthermore suggests that in most species of *Botrytis* the apothecial stage is very rare or absent.

Sclerotia

Sclerotia are considered the most important survival structure in the life of *Botrytis* species (Coley-Smith, 1980). Mostly the sclerotia will not be directly infective, but will be a good source of conidia, which will then infect the grapevine (Jarvis, 1980). Sclerotia can germinate myceliogenic, sporogenic or carpogenic (Coley-Smith, 1980). Sclerotia mostly germinate forming conidiophores (Coley-Smith, 1980), though Nair and Nadtotchei (1987) found that in Australia sporogenic germination led to the infection of grape flowers and berries. A high portion of sclerotia can survive for a year from –70 °C to +25 °C and at 30 °C there is a more rapid decline in the viability of the sclerotia than at lower temperatures (Coley-Smith, 1980). The ultrastructure of the sclerotia suggests that they are well adapted for long periods of survival (Nair & Martin, 1987). Sclerotia will remain on the canes left on the vines up to two months after harvest (Nair & Nadtotchei, 1987). However, no evidence was found of sclerotia on canes that were left on the ground, or on bunches left on vines after harvest. According to Nair and Nadtotchei (1987), repeated germination of sclerotia produced conidia over a long period. The best temperatures for the sporogenic germination of the sclerotia and the rotting of the berries are between 20 °C and 25 °C (Nair & Nadtotchei, 1987). Rainfall and splashing water would be expected to dislodge conidia from germinating sclerotia and initiate conidial production by removing the suppression on sporulation. On apple, sclerotia also play an important role in causing dry eye rot. Sclerotia are dispersed as they are moved with cull piles and through the soil in tillage operations (Ellerbrock & Lorbeer, 1977). The sclerotia are formed in late autumn on the apple skin, and this will remain on the ground. When the temperature increases, the sclerotia will start to germinate to form macroconidia (Tronsmo & Raa, 1977).

Conidia

The conidia of most *Botrytis* spp. are dry and are dispersed in large quantities in air currents and in, or on, water droplets (Jarvis, 1980). *B. cinerea* dispersed its spores into the air in a circardian pattern during two periods (Jarvis, 1962a). These two periods correspond with a drastic change in relative humidity during the day. Furthermore, the most striking deviation from the normal pattern, in otherwise unsuitable conditions, occurred during rainfall (Jarvis, 1980). The hygroscopic movement of the conidiophores does not eject many spores into the air, but it is only dislodged so that the spores mostly lie in masses between the hyphae (Jarvis, 1962b).

The association of *B. cinerea* with the grape berry moth larvae suggests that this insect plays an important role in dissemination of the pathogen (Fermaud & Le Menn, 1989). On vineyard berries, the emergence of the conidiophores of *B. cinerea* is favoured at the entrance of larval galleries (Fermaud & Le Menn, 1992). The conidia germinated and mycelial growth took place inside the galleries. On immature berries, the artificial supply of viable conidia on the cuticle of second-generation larvae caused a significant increase in the percentage of larval injuries infected by *B. cinerea* (Fermaud & Le Menn, 1992). Kovacs (cited in Jarvis, 1980) found that bees could carry conidia between strawberry flowers. *Thrips obscuratus*, a New Zealand flower thrip, was capable of carrying *B. cinerea* conidia on its body (Fermaund & Gaunt, 1995). Conidia were also carried externally on the cuticle of *Drosophila melanogaster* and through the intestinal tract (Louis *et al.*, 1996). During the life cycle of the fly, *B. cinerea* can germinate, develop into mycelium and differentiate into microsclerotia. *Drosophila* flies, once infected, therefore become a potential reservoir of the pathogen in three ways namely, conidia, mycelia and microsclerotia (Louis *et al.*, 1996).

Mycelia

B. cinerea can also exist in the vineyard as mycelia (Gessler & Jermini, 1985; Northover, 1987). Windblown and rain-splashed pieces of plant debris containing mycelia are almost certainly under-estimated as dispersal propogules in most crops. Petals and completely senescent flowers, which are readily infected by *B. cinerea*, are dispersed by wind and rain and provide a large saprophytically based inoculum (Jarvis, 1980). According to Taylor (cited in Jarvis, 1980), mycelia can also be dispersed as latent infection in most stored products. Conidia and sclerotia readily germinate during storage to form mycelia, so the pathogens are mostly present in the form of mycelia (Van den Berg & Lentz, 1968). The survival of the mycelium, under stored conditions without nutrients, varied between 1 month and a year depending on the temperature and the relative humidity. Survival decreased with decreasing relative humidity and was significantly less at 20 °C than at 0 °C (Van den Berg & Lentz, 1968). The mycelium of *B. cinerea* could be in some of the floral debris, due to earlier infection (McClellen & Hewitt, 1973; Pezet & Pont, 1986; Holz *et al.*, 1997). According to Northover (1987), these infected floral debris remained in the clusters or attached to the ripening berries and later in the season when the conditions are favorable, conidia will be produced and it will cause infection of the berries.

Dispersal

Sclerotia are dispersal propagules in the sense that they are moved with cull piles and through soil tillage (Ellerbrock & Lorbeer, 1977). The sclerotia can also remain on fruit skins lying on the ground through the winter and conidia produced by these sclerotia can be dispersed by insects or wind (Tronsmo & Raa, 1977). Conidia-bearing sclerotia were detected in canes left on the vines and on the ground (Nair & Nadtotchei, 1987). These sclerotia will be a source of conidia, which will then be dispersed, in a more active fashion. The conidia of most *Botrytis* species are dry and dispersed in air currents and water droplets (Jarvis, 1980). There seem to be two ways in which rain disperses conidia; dry spores are dispersed on air shock waves and turbulent currents; and composite projectiles of dry conidia coating water droplets are splashed for 1-2 m. According to Jarvis (1962b), the spores sitting on the droplet will not get wet. It will only stay on the outside of the drop and as the drop roles over the plant surface; the spores will be stuck to surface. A few spores might enter the drop of water and germinate. Petals and completely senescent flowers, which are readily infected by *B. cinerea*, are dispersed by wind and rain. This provides a saprophytically based inoculum, sticking to plant surfaces when wet (Jarvis, 1980).

The larvae of *Lobesia botrana* (grape berry moth) cause wounds to the grapes, which gives a passage for the conidia of *Botrytis* to enter the berry easy. These conidia are carried in the insect (Fermaud & Le Menn, 1992). According to Fermaud and Gaunt (1995) *Thrips obscuratus,* the New Zealand flower thrip, is capable of carrying *B. cinerea* conidia on its body. They found that between full bloom and complete petal fall up to 17% of the adult thrips were naturally contaminated. They also found up to 12 propagules per contaminated thrip. Conidia of *B. cinerea* are carried externally on the cuticle of *Drosophila melanogaster* (fruit fly) and might be carried internally through the digestive tract (Louis *et al.,* 1996). Conidia germinated in the insect crop and developed into mycelia. *Drosophila* must be seen as a plurimodal vector of *B. cinerea*, supporting non-persistent, semi persistent and possibly persistent transmission of *B. cinerea* (Louis *et al.,* 1996).

THE ROLE OF BUNCH ARCHITECTURE

According to Marois *et al.* (1986), the effect of cluster tightness on the development of *B. cinerea* disease on grape is more complex than thought before. A correlation between cluster tightness and Botrytis bunch rot may be partly due to the reduced deposition of cuticle and

epicuticular wax at the berry-to-berry contact areas. Small holes (0.5-2.0μm) were also observed where berries were in contact with other berries during their growth (Marois *et al.*, 1986). Both Muscat and Cabernet Sauvignon have a loose cluster architecture and are only slightly affected by bunch rot in the field, but they have highly susceptible berries (Vail & Marois, 1991). Cultivars that have berries that are highly susceptible to *B. cinerea* and a tight cluster architecture may be predisposed to the development of more severe bunch rot epidemics than cultivars with higher susceptible berries and a loose cluster architecture. Tighter clusters also retain water for a longer period than looser clusters and water is essential for the germination of the pathogen (Vail & Marois, 1991). Grape cultivars with looser bunch architecture, berries less compressed and better aerated had lower infection (Nair & Parker, 1985). Nair and Parker (1985) also found that berries would rot from the inside of the bunch (where water will be typically retained longer) to the outside. Compression between berries in the bunch causing splitting and partial severance of the grape from its pedicel makes it more susceptible to infection by *B. cinerea* (Jarvis, 1980). Percival *et al.* (1993) showed that Riesling had a thick cuticle, but due to a compact cluster, there were many contact areas between the berries, which contributed to a flawed epicuticular wax and cutin polymer structure, and longer periods of wetness. These factors contributed to the fact that Riesling is susceptible to bunch rot in spite of its thick cuticle. Northover (1987) on the other hand, found that the loose-cluster Chardonnay had a seasonal mean of 92% infected clusters compared to 46% for the tight-clustered clone, and only 22% for Aurore and Seyval, both which had tight clusters at maturity. Lastly, some workers found that berries, which occur in tight clusters, are more susceptible to *B. cinerea* than those in loose clusters (Savage & Sall, 1983; Marois *et al.*, 1986; Gubler *et al.*, 1987; Vail & Marois, 1991).

INFECTION

Different infection pathways have been described for *B. cinerea* on grape berries, namely through stigmata (McClellen & Hewitt, 1973; Nair & Parker, 1985), wounds (Nair *et al.*, 1988), natural openings (Pucheu-Panté & Mercier, 1983), pedicels (Pezet & Pont, 1986; Holz *et al.*, 1997), or by direct penetration of the cuticle (Nelson, 1956).

Adhesion of conidia

The attachment to plant surfaces of conidia of *B. cinerea* occurs in two stages (Doss *et al.*, 1993). Immediate adhesion occurs upon the hydration of freshly deposited conidia. Adhesion of the hydrated conidia occurred even when they had been killed prior to deposition by treatment with propylene oxide. The forces characterising immediate adhesion are relatively weak and are the strongest with hydrophobic substrata. The second stage of adhesion only occurs with viable conidia and is not influenced by the hydrophobic character of the substratum. The second stage of adhesion occurs after viable conidia have been incubated for several hours under conditions that promote germination (Doss *et al.*, 1995). This stage involves the secretion of an ensheathing film, which remains attached to the substratum despite the attempts of physical removal (Doss *et al.*, 1995).

Germination

Different isolates of *B. cinerea* can be classified as being nutrient dependent or independent (Blakeman, 1975). Blakeman (1975) suggested that isolates of *B. cinerea*, which germinate independently of nutrients *in vitro*, may have a nutrient requirement for germination on plant leaves to overcome the action of inhibitors and microbial antagonists. Simple sugars such as fructose and sucrose, and amino acids, dissolve in the water that is present on the berry surface (Kosuge & Hewitt, 1964). Kosuge and Hewitt (1964) also found that these nutrients would become more available as the berries mature. Blakeman (1975) found that simple sugars alone do not improve germination profoundly and that fructose was the best sugar. Amino acids, in the absence of simple sugars, were the best nitrogen source (Blakeman, 1975). According to Blakeman (1975), the reason for *B. cinerea* not germinating satisfactory on leave surfaces is due to competition with bacteria. With the exception of a slight stimulation by inositol, none of the growth factors tested had any effect on germination of the conidia (Blakeman, 1975). Benlioğlu and Yilmaz (1992) found that plant growth regulators (PGR) had no effect on the germination, except for PGR 4-CPA, which had a negative correlation with germination. Spores harvested from 7- to 10- day -old cultures showed only 10% germination after 2 h of incubation in distilled water and 17% after 6 h incubation (Chou & Preece, 1968). The addition of pollen diffusate diluted 1:10 increased the germination to 98% after 2 h and to 100% after 6 h of incubation (Chou & Preece, 1968). Chou and Preece (1968) also observed that the percentage germination would decrease, as the culture gets older. Borecka and Millikan (1973) found that the addition of pollen extract

promoted the germination of conidia in water, and noted that germ tubes of *B. cinerea* were considerably longer when pollen extract were added to the water droplets. Hill *et al.* (1981) found no significant difference in the germination of conidia on immature and mature grapes. McClellen and Hewitt (1973), on the other hand, found that conidia germinated and grew poorly on the extract of immature grapes. They also showed that extracts of the pollen, stigma and style stimulated germination and germ tube growth of conidia (McClellen & Hewitt, 1973).

Inoculum density

The number of *B. cinerea* on the surface of grape berries are very low throughout the season and occurred as single colony forming units (Duncan *et al.*, 1995; G. Holz, Department of Plant Pathology, University of Stellenbosch; unpublished data). Louis (cited in Verhoeff, 1980) showed that a single conidium produced a small lesion and a group produced a large lesion. When Verhoeff (1980) inoculated tomato fruit with *B. cinerea*, using high or low inoculum concentrations, he found that large numbers led to spreading lesions and small numbers led to non-spreading lesions. Jarvis (1962b) found that the conidia, which are dispersed by raindrops, are carried on the drop, as a dry coating so very few will get wet. It was also found by Coertze and Holz (1999) that increasing the densities of single dry conidia does not necessarily lead to higher number of infections and lesion formation on grape berries under moist and wet conditions. Nair *et al.* (1995) said that bunch rot by *B. cinerea* can be inoculum-driven and they demonstrated that quantitative relationships exists between inoculum levels at carry over, flowering and harvest stages of the season.

Germ tube behaviour

According to Dey, and Mercure *et al.* (cited in Islam, 1998), it is essential for infection structure formation that close contact, or adhesion of the germ tube to the host surface be facilitated. Germ tubes of *B. cinerea* showed negative phototropism to near ultraviolet (NUV) and blue (300-520 nm) light followed by far-red (700-810 nm), whereas red light (600-700 nm) induced positive phototropism significantly (Islam *et al.*, 1998). Minimum germ tube growth occurred during exposure to negative phototropism-inducing wavelengths, whereas it was maximum under positive phototropism-inducing wavelengths (Islam *et al.*, 1998). Islam *et al.* (1998) also found that red light suppressed the formation of infection

hyphae, which resulted in a high proportion of germ tubes without infection hyphae. According to Fromme, and Islam and Honda (cited in Islam, 1998), negative phototropism facilitates the contact of the tips of conidium germ tubes with the host surface that subsequently promotes infection structure formation and favours the infection process. McKeen (1974) observed that the tip of the germ tube is held firmly against the cuticle by mucilage, which spread some distance around the germ tube, after the tip has turned down for infection. Clark and Lorbeer (1976) noticed that the mucilage was deposited around the appressoria of *B. cinerea* on onion leaves. *B. cinerea* can form quite a few penetration structures before penetration. On flower parts of plums and nectarines, the fungus can form protoappressoria, simple appressoria, multicellular lobate appressoria and infection cushion (Fourie & Holz, 1994). Backhouse and Willetts (1987) observed infection cushions on mung bean hypocotyls, while Sharman and Heale (1977) observed dome-shaped infection cushions only in the presence of exogenous nutrients on the surface of carrot roots.

Dry inoculated conidia (onto glass) germinate rapidly to produce short germ tubes, where conidia inoculated in the presence of an aqueous glucose mixture germinated to produce long germ tubes (Cole *et al.*, 1996). These germ tubes were also enclosed by an extensive sheath of fibrillar-like material. Antibody binding was also located throughout the fibrillar-like matrix material associated with the conidia and germ tubes. Cole *et al.* (1996) also found traces of amorphous matrix material at the short germ tubes, of dry inoculated conidia, at the site of penetration of the leaf surface. According to Nelson (1956) conidia of *B. cinerea* formed germ tubes with lengths up to 150 μm before an appressoria was formed on Tokay grapes. Coertze *et al* (2001) found that the germtube behaviour varied under different wetness regimes. They found that on wet berries the conidia germinated and usually formed more than one germ tube, that occationally branched, and on moist berries the conidia formed a single, unbranched germ tube.

Penetration through natural openings and wounds

For some host-pathogen combinations, penetration through stomata is the usual way of establishing a parasitic relationship (Verhoeff, 1980). However, in *Botrytis* species penetration through stomata seems to be the exception rather than the rule (Verhoeff, 1980). In the case of *B. squamosa,* Clark and Lorbeer (1976) observed penetration through the stomata of onion leaves. They found that no appressoria were formed. Bessis (cited in

Verhoeff, 1980) found no proof for direct penetration of the grape berry cuticle by *B. cinerea*, and concluded that the pathogen penetrates grape berries through minute cracks in the cuticle. Direct penetration of grape berries by *B. cinerea* was however observed by Nelson (1951a).

Eldich *et al.* (1989) concluded that *B. cinerea* was predominantly a wound pathogen, under field conditions. Kiwifruit can be wounded when picked from the plant and these wounds can be a place where *B. cinerea* can penetrate the fruit (Brook, 1991). Wounds will also form during the packaging process, which will then also be ideal for the pathogen to enter the fruit (Sharrock & Hallett, 1991). Injuries of grape clusters, which may be the result of insect feeding or from the expansion of berries in tight clusters, may be important avenues for the pathogen to infect (Savage & Sall, 1983).

Penetration through flower parts

According to Nair and Parker (1985) and McClellen and Hewitt (1973), *B. cinerea* invades the flower of grapes through the stigma and the style during bloom. Nair (1985) isolated *B. cinerea* from healthy and surface sterilised flowers. Powelson (1960) showed that *B. cinerea* expanded from latent infections of floral parts into the receptacle and concluded that the calyx offers the primary pathway for *B. cinerea* on strawberries. Raspberry fruit, of which the flowers were inoculated with dry conidia of *B. cinerea*, decayed faster than the fruit of flowers that were not inoculated (Williamson *et al.*, 1987). Raspberry flower buds are rarely infected, but open flowers are quickly colonised and necrotic stamens and styles were an important source of infection for the developing fruit (Dashwood & Fox, 1988). Dashwood and Fox (1988) also found that deep-seated infection of the flowers was infrequent until fruits from the first flush of flowers had formed and airborne spores were released. Lavy-Meir *et al.* (1988) indicated that floral organs served as a pathway for stem-end infection, by *B. cinerea,* in both normal and non-ripening tomato fruit. According to Bristow *et al.* (1986) the stamens may be more important that the styles as a source of latent infection in strawberries. In pear flowers, hyphal growth ceased in the upper portion of the styles at the onset of stylar senescence (De Kock & Holz, 1992). Unlike the styles, hyphae in the filaments grew without restriction and progressed within 4 days, via vascular tissue, through sepals into tissues of the upper end of the flower receptacle, or of the mesocarp adjoining the sepals, without causing symptoms. *B. cinerea* entered filaments and spread into the receptacle or mesocarp at any time between blossom and harvest and then became latent in these tissues (De Kock & Holz, 1992). The sepals of apple flowers are infected by conidia of *Botrytis* and it stays latent in

the growing fruit, until the fruit matures, then dry eye rot will develop (Tronsmo & Raa, 1977). Fourie and Holz (1994) on the other hand found that infected floral parts of nectarines and plums did not remain attached to the young developing fruit. Therefore, floral parts of nectarines and plums do not serve as infection pathways to the fruit. *B. cinerea* also infects rose flowers (Volpin & Elad, 1991). According to Hunter *et al.* (1972), the flower parts of macadamias are the main source of nutrients for the fungus.

Penetration of the cuticle

The cuticle is composed of an insoluble polyester (cutin), which is embedded in a complex mixture of nonpolar lipids (wax) (Kolattukudy & Crawford, 1987). Cutin is composed of interesterified hydroxy- and hydroxyepoxy- fatty acids (Kolattukudy & Crawford, 1987). The epicuticular wax provides a constitutive defense mechanism against the pathogen (Marois *et al.*, 1986). According to Aist (cited in Percival *et al.*, 1993), the epicuticular wax develops after bloom in the form of overlapping platelets. As the fruit matures, the wax layer will thicken. Kolattukudy (cited in Percival *et al.*, 1993) postulated that penetration of *B. cinerea* through the cutin polymer is facilitated by the use of fungal cutinases. It was also found by Aist (cited in Percival *et al.*, 1993) that although production of the cutin polymer is predominantly genetically controlled, it is also influenced by climatic conditions.

In *B. cinerea*, a pore develops in the fungal wall in the center of the contacting germ tube (McKeen, 1974). The infection peg, covered by the plasmalemma, was pressed against the host cuticle, and the plasmalemma covered the infection peg, as it was moving through the cuticle (McKeen, 1974). Backhouse and Willetts (1987) observed thin walls around the infection peg that appear to be different from hyphal walls. Clark and Lorbeer (1976) and Nelson (1956) both observed that a penetration peg grew from the tip of the germ tube or an appressoria. Whether direct penetration is enzymatic or mechanical is still unclear, but it seems that there is more evidence pointing at enzymatic degradation. It was found on bean leaves that cutin esterase was prominent during the early phases of incubation when it allowed the pathogen to penetrate the outer layer of the host quickly (Kapat *et al.*, 1998). Kapat *et al.* (1998) also found that the presence of cutin esterase was constant throughout the incubation period. Endopolygalacturonase, which is a cell-wall-degrading enzyme, is present in conidia of *B. cinerea* (Verhoeff & Liem, 1978). According to Rijkenberg *et al.* (1980), the cuticle of immature tomato fruit is dissolved enzymatically rather than ruptured mechanically. Rijkenberg *et al.* (1980) found that the cutinolytic activity seem to be present

in the infection structure rather than in the conidia. As mentioned above, the endopolygalacturonase were also found in the conidia. McKeen (1974) observed that the cuticle of *Vicia faba* leaves were enzymatically dissolved. Sharman and Heale (1977) suggested that initial penetration is enzymatic and subsequent inward depression of the periderm wall, mechanical. Backhouse and Willetts (1987) also supported the statement that *B. cinerea* does not penetrate mechanically. On the contrary, Salinas and Verhoeff (1995) observed the mechanical penetration of the cuticle of ray florets and suggested that no degradation of the cuticle occurred. According to Verhoeff (1980), Blackman and Welsford concluded that the cuticle of the leaves of *Vicia faba* is ruptured mechanically by pressure of the tip of the germ tube. The expression of the cutinase gene (*cut*A) during infection of tomato leaves is low during early phases of infection, but high when the fungus has colonised the leaf and starts to sporulate (Van der Vlugt-Bergmans *et al.*, 1977).

Percival *et al.* (1993) observed that the thickness and quality of the cuticle influenced the rate of fungal penetration. Riesling grape berries have a thick cuticle and compact cluster architecture so that there was a lot of berry contact in the bunch. The cuticle at the area of contact had a flawed epicuticular wax and cutin polymer so that penetration was easier in these areas.

Latent infection

Botrytis cinerea can penetrate the flower parts or the tissues of the developing fruit of pear (De Kock & Holz, 1992), strawberries (Bristow *et al.*, 1986), grape (McClellen & Hewitt, 1973; Pezet & Pont, 1986), red raspberries (Dashwood & Fox, 1988), apples (Tronsmo & Raa, 1977) and seeds of safflower and sunflower (Sackston, 1960) to establish latent infections. A pathogenic relationship, will, however not be established until the fruit ripens. The mycelium of the pathogen stays latent in the infected tissue (Verhoeff, 1980). Pezet and Pont (1986) found that grape berries stay asymptomatic between the flowering period and ripening; hereafter *Botrytis* will resume its growth. Nair and Parker (1985) showed that 10% of the grape flowers in an inflorescence did not set fruit. These flowers seem to be a good source of infection when the bunches will start to develop. De Kock and Holz (1991) did not find any evidence to show a relationship between early infection and subsequent disease development in grape. There is also no relationship between post-harvest decay and flower infection of plums or nectarines (Fourie & Holz, 1994). Holz *et al.* (1997) showed that latent infections in the grape pedicel are very important for it leads to postharvest decay.

Verhoeff (1980) suggested possibilities to explain the transition from a latent to an active pathogenic relationship: In unripe fruit, fungitoxic compounds will first disappear as the fruit ripens. According to Verhoeff (1980), there are phenols in young grape berries, which could be, according to Boubals (cited in Verhoeff, 1980), detrimental to the pathogen's development. The development of the fungus may be affected by the nutritional differences between unripe and ripe fruit. Ripe fruit has more sugar that unripe fruit and *B. cinerea* grows well on simple carbohydrates. Grape berry exudates contain sugars (Kosuge & Hewitt, 1964) and it has been reported that grape berries with higher sugar content are more susceptible to infection (Nelson, 1951a; Kosuge & Hewitt, 1964). The composition of berry exudates changes throughout the season (Padgett & Morrison, 1990). Water extracts of berry exudates contained sugars, malic acid, potassium and sodium. Ethanol and ether extracts contained phenols and lipids. After bloom, the concentration of phenolic compounds and malic acid was high and by maturity, it was low again. Sugar and potassium were low at the earlier stages and became higher later (Padgett & Morrison, 1990). Vercesi *et al.* (1997) found that an increasing sugar concentration always promoted hyphal growth better than any concentration of tartaric or malic acid. From setting to the onset of ripening, when the main carbon source is organic acids, fungal growth will be poor. Sugar will be formed as the fruit starts to ripen and sugar is a better carbon source so the growth of the fungus will be enhanced (Vercesi *et al.*, 1997).

The pathogen does not have the enzymes that are required to invade the unripe fruit. According to Verhoeff (1980), it may be possible that the solubility of calcium and magnesium must reach a certain level before the enzymes of the pathogen are able to hydrolyze the cell wall. Tronsmo *et al.* (1977) found that the level of these compounds decreases during maturation. During the ripening process, the pectic material in the middle lamella becomes more soluble (Verhoeff, 1980). Chardonnet *et al.* (1997) found that calcium might be translocated out of the berry during ripening.

Infection by mycelium

Hyphal growth from senescent or necrotic flower parts can cause fruit infections during or after bloom (Verhoeff, 1980). Powelson (1960) found that the mycelium of *B. cinerea* grows into the receptacle from necrotic flower parts, so the mycelium could invade the strawberry fruit. According to Fourie and Holz (1994), flower infection of nectarine and plum may play an indirect role in fruit decay by serving as secondary inoculum. Loose floral debris in the

grape cluster is being colonised by the pathogen and probably serves as loci for infection at véraison (Gessler & Jermini, 1985; Nair & Parker, 1985; Northover, 1987).

Infection of green tissue

Pezet and Pont (1986) found that if the climatic conditions were favourable *B. cinerea* would infect green parts of the grapevine like leaves and young clusters. Kamoen *et al.* (1985) observed that, on green leaves, germination is often poor and infection rarely occurs in healthy tissue. In comparison to petal infection the infection of green tissue is very unsuccessful due to more resistance on the green tissue (Kamoen *et al.*, 1985). Kamoen *et al.* (1985) also observed that on green leaves the spores develop short germ tubes. This phenomenon was ascribed to inhibition by phytoalexins, inhibition by preformed germination inhibitors on the leave surface, lack of nutritional food, inhibition due to antagonism by leave microflora (Kamoen *et al.*, 1985). It was also found that if pectin was included in the infection droplet infection that was more successful. Kamoen *et al.* (1985) concluded that this behaviour could be ascribed to the stimulation of pectin degrading enzymes synthesis by the pectin.

Unripe berries are less susceptible to rot than ripe berries (McClellen & Hewitt, 1973; Hill *et al.*, 1981; De Kock & Holz, 1991). However, Hill *et al.* (1981) found that the pathogen can penetrate immature fruit at any stage. *B. cinerea* was unable to breach the cuticle of green nectarines and plum fruit (Fourie & Holz, 1995). Bulger *et al.* (1986) found that no infection occurred from inoculation of green strawberries at any wetness duration. Infection of green tissue will only occur in the field through the direct contact of green leaves with infected senescent leaves or the falling of infected flowers on the green leaves of strawberries (Garrett, 1960).

THE ENVIRONMENT

Wind speed

Aerial mycelium will develop best at conditions with no wind, 21°C and 94% humidity (Thomas *et al.*, 1988). The greatest number of conidia was produced at 21°C, 94% humidity and 0.6m/sec wind speed (Thomas *et al.*, 1988). Thomas *et al.* (1988) furthermore found that wind speed and humidity had the greatest effect on evaporative potential than did

temperature. Thomas and Marois (1986) found that mycelia exposed to 94% relative humidity and a wind speed of 0.3m/sec developed four times more spores than mycelia exposed to no wind. At 35% relative humidity, most spores will develop mycelium, but when wind is present, no mycelium will develop.

According to English *et al.* (1989) wind speed was the factor most strongly affected by the removal of the leaves. The removal of leaves from around the grape cluster significantly increased the evaporative potential that was correlated with increase in wind speed within the canopies (English *et al.*, 1989). English *et al.* (1993) found that leaf removal played a far more important role in reducing the incidence of *Botrytis* bunch rot in vineyards during wetter and more humid growing seasons in which rains were infrequent and of short duration. Savage and Sall (1984) also found that the wind penetration through the different canopy designs might have a great impact on disease development.

Temperature

Optimum temperatures near 21°C have been reported for the infection of several hosts by *B. cinerea* (Bulgar *et al.*, 1987; Jarvis, 1980; Thomas *et al.*, 1988). Jarvis (1980) reported that temperature between 15°C and 21°C favour infection of most hosts, but infection could also occur at 2°C. Shoemaker and Lorbeer (1977) found that germination of *B. cinerea* conidia on onion leaves was at its maximum at 15°C and it occurred over the range of 12 to 27°C; growth in culture was maximum at 24°C, and it occurred from 9 to 31°C. Yunis *et al.* (1990) grew cucumbers in a greenhouse and reported that during the first phase of the epidemic infection was correlated with air temperature between 11 and 21°C. They found that during the second phase the temperatures varied between 11 and 16°C. Hunter *et al.* (1969) said that B. *cinerea* incidence was negatively affected by temperatures above 22 °C. Hyre (1972) found that lesion size and sporulation increased with temperature to 25°C. Grape flowers and berries were infected by *B. cinerea* over a wide range of temperatures but the optimum was 23.7 and 20.8 °C, respectively (Nair & Allen, 1993).

Wetness and humidity

Germination of conidia occurred at relative humidities above 92% (Elad & Yunis, 1993). Coertze and co-workers (Coertze & Holz, 1999; Coertze *et al.*, 2001) found that germination on grape berries was not greatly affected, but that subsequent growth was markedly

influenced by wetness regime. On moist berries, conidia formed one unbranched germ tube and on wet berries, most conidia germinated with more than one germ tube. Growth under moist conditions was less than 25% of that under wet conditions. Coertze and Holz (2002) also found that wounds remained uninfected on dry berries, but yielded significantly more infections as the degree of wetness increased. Salinas *et al.* (1989) showed that rotting of ray florets and receptacles by *B. cinerea* occurred when inoculated flowers were kept wet for a few days. Kerssies (1993) found that there was a positive relationship between lesions formed on gerbera flowers and relative humidity. At relative humidities below 93% no growth occurred and mycelium survived only up to one month (Van den Berg & Lentz, 1969). There is an increased incidence of strawberry flower infection with increased wetness duration for temperatures between 5 and 35°C (Bulgar *et al.*, 1987). Rose petals developed lesions within 24 h at or above 94% relative humidity (Williamson *et al.*, 1995). The conidia of *B. cinerea* have a considerable ability to tolerate severe drying and infection may occur after several days of intermitted growth of the germ tube (Good & Zathureczky, 1967). Infection of dusted (with *B. cinerea* conidia) grape berries was moderate at 91% relative humidity, high at 97% and complete at 99% (Nelson, 1951b).

Nutrition

The concentration of phenolic compounds and malic acid was high in grape berry exudates after bloom, but decreased as the fruit ripened (Padgett & Morrison, 1990). Sugar and potassium levels were low at bloom and higher at maturity. Mycelial growth was inhibited by the phenols and lipids in the berry exudates, while the sugar, malic acid, potassium, and sodium promoted mycelial growth (Padgett & Morrison, 1990). Glucose and fructose are taken up by the free water on the berry and it promotes germination (Kosuge & Hewitt, 1964). Hobbs and Waters (1964) found that if *Chrysanthemum morifolium* plants were given more nitrogen it was more susceptible to *Botrytis* attacks. Calcium ions have an inhibitory effect on spore germination (Wisniewski *et al.*, 1995). Shaul *et al.* (1996) found that the addition of hormones like gibberellic acid on the flower buds of roses inhibited the forming of blight on the flowers and that the hormone, abscisic acid, promoted the disease.

HOST RESISTANCE

Pathogen infection is resisted by plants through physical or chemical defenses. These defenses may be preformed, or it can be induced after pathogen penetration (Kombrink & Somssich, 1995). Induced defenses include the production of oxidative species, cell wall strengthening, phytoalexin biosynthesis and the accumulation of defense related proteins such as pathogenesis-related proteins (Kombrink & Somssich, 1995). It was also noted by Hill (1985) that the resistance of immature grape berries is based on two factors. Firstly, in the cell walls of the berry skin there is a preformed defense system that inhibits the hydrolytic enzymes of the pathogen. Secondly, the attacking fungus triggers an active defense system around the infected site. These defense systems comprise of phytoalexin (Langcake, 1981), suberin (Hill, 1985) and lignin formation (Hoos & Blaich, 1988). All these actions tend to become weaker towards ripening (Hill *et al.,* 1981; Creasy & Coffee, 1988; Bavaresco *et al.*, 1997). Many researchers showed that phytoalexin production is positively correlated with resistance of grapevine to *B. cinerea* (Bavaresco *et al.,* 1997; Creasy & Coffee, 1988; Jeandet & Bessis, 1989; Hain *et al.,* 1993; Hoos & Blaich, 1988; Langcake & McCarthy, 1979; Liswidowati *et al.,* 1991). There is also a decline in the inducible stilbene synthase enzyme as the berries ripen (Bais *et al.*, 2000). Sbaghi *et al.* (1996) found that isolates of *B. cinerea* that could degrade stilbene-type phytoalexins had greater virulence. During bloom and near harvest is the time when the berries have the least resistance and this is the period when the phytoalexin production is the least (Creasy & Coffee, 1988). Pezet and Pont (1992) said that Gamaret grapes was relatively resistant to *B. cinerea* due to several factors including phytoalexin, glycolic acid, phenolic compounds, proanthocyanidins and the thickness of the skin. Phytoalexin induction is used as a tool to screen grapevines for resistance to *B. cinerea* (Jeandet *et al.*, 1992; Sbaghi *et al.*, 1995).

Calcium also plays a very important role in the host's resistance to *B. cinerea.* It will reduce the damage to plants like *Ruscus hypoglossum* (Elad & Kirshner, 1992) and reduces the gray mould incidence on greenhouse grown eggplants, pepper and cucumbers (Yunis *et al.*, 1991). In broad beans and tulips, the formation of papillae has been observed as a method of stopping the infection of nonpathogenic *Botrytis* species (Mansfield & Hutson, 1980). It has also been noted that the artificial expression of defense-related proteins like chitinase and β-1, 3-glucanase reduced the rate of lesion development, either alone (Punja & Raharjo, 1996) or in combination (Alexander *et al.*, 1993). At present, it is believed that the

host plant's ability to recognise and respond to one or more stimuli produced by the pathogen during the early stages of pathogenesis, is very important in understanding host resistance and pathogenicity (Hutcheson, 1998).

PATHOGENICITY AND VIRULENCE

B. cinerea has a very wide host range. The pathogen can attack up to 235 plant species (McFarlane, 1968). It causes gray mould on many economically important crops including tomato, cucumber, gerbera, rose, onions, strawberry and grapes (Coley-Smith *et al.*, 1980). The conidia of *B. cinerea* are able to infect almost any plant tissue, if a site is available through injury (Staples & Mayer, 1995). It is though important to note that a combination of fresh wounds and new conidia is needed for this infection process to take place in grapes (Coertze *et al.*, 2001). For a plant pathogen, pathogenicity is seen as having the characteristics to cause disease. Virulence is the observable effects of the pathogen on a plant; it is not synonymous with, but describes degrees of pathogenicity (Agrios, 1997). *B. cinerea* can penetrate plant tissue by forming a variety of penetration structures like appressoria and forcing it through the epidermis, which is then followed by the secretion of cutinase (Salinas *et al.*, 1986). According to Gronover *et al.* (2001), the pathogen does not necessarily need these infection structures to penetrate, for it seems able to penetrate healthy plant tissue directly. This might also prove to be a controversial point of discussion, because Coertze & Holz (1999) suggested that single conidia of *B. cinerea* were unable to infect fresh grape berries. All the mechanisms involved in the infection process and the establishment of a pathogenic relationship on a host is still unclear.

Many researchers have though tried to make sense of the forces at work in the infection process and the establishment of a pathogenic relationship between *B. cinerea* and its host plants. Many enzymes as well as other molecules have been indicated as important in the pathogenicity of *B. cinerea*. Amongst these are β-glucosidase, which is important in the initial part of the infection process, and carboxymethylcellulose, which might become important when infection is already established (Sasaki & Nagayama, 1994, 1996). Cutinase is another cell wall degrading enzyme that is important in the earlier stages of pathogenicity (Kolattukudy & Crawford, 1987; Kapat *et al.*, 1998). Kapat *et al.* (1998) also pointed out that exopolygalacturonase as well as pectin methyl esterase (Wasfy *et al.*, 1978) levels are high during the earlier stages of infection, whereas the levels of pectate lyase and

endopolygalacturonase (endoPG) were lower. The concentration of endoPG did increase during the latter part of infection. Phenol oxidase (Wasfy *et al.*, 1978), acid protease (Zalewska-Sobczak *et al.*, 1981) and hexose oxidase (Edlich *et al.*, 1989) have also been indicated as secretions from *B. cinerea* during the infection process. Recent investigations into the genetics of *B. cinerea* indicated that certain genes might be important in the pathogeneses of this pathogen. Wubben *et al.* (2000) found six genes (*Bcqg* 1-6) involved in the production of endopolygalacturonases, which are the enzymes involved in the break down of pectin in the plant cell wall. The first two genes (*Bcqg* 1& 2) proved to be encoding for the basic isozymes. Ten Have *et al.* (1998) suggested that the gene *Bcqg* 1 is compulsory for full virulence of *B. cinerea.* Zheng *et al.* (2000) showed that the mitogen-activated protein kinase gene is essential for infection in *B. cinerea.* Lastly, Gronover *et al.* (2001) showed that two genes (bcg 1 & 2), which encode for α subunits of heterotrimeric GTP – binding proteins, are important in pathogenicity of *B. cinerea.* Heterotrimeric GTP – binding proteins are proteins involved in the regulation of the transduction of signals from the environment to target genes in eukaryotic cells.

Another part of the pathogenic ability of a pathogen is its capacity to detoxify antifungal molecules from the host (Staples & Mayer, 1995). It is well known that *B. cinerea* produces laccase enzymes, which oxidises grape stilbenic phytoalexins, resveratrol and pterostilbene (Breuil *et al.*, 1999; Pezet, 1998). Bar Nun *et al.* (1988) showed that when the latter ability of *B. cinerea* is inhibited it interferes with the pathogens pathogenic ability. Saponins are preformed fungitoxic compounds, which plays a role in plant defense. Quidde *et al.* (1999) found that *B. cinerea* has the ability to produce more than one saponin-specific enzyme, which give it the capacity to detoxify the antifungal compounds.

The traditional view is that there is no proof of host specialisation in *B. cinerea* (Jarvis, 1980). There are though reports of differences in virulence towards different host plants (Di Lenna *et al.*, 1980; Derckel *et al.*, 1999; Giraud *et al.*, 1999; Zalewska-Sobczak *et al.*, 1981; Coley-Smith *et al.*, 1980). Some researchers (Kerssies *et al.*, 1997; Giraud *et al.*, 1999) found genetic variability amongst *B. cinerea* isolates and suggested a host: pathogen relationship (Thompson & Latorre, 1999). Giraud *et al.* (1999) found variation in the genetic structure of different *B. cinerea* isolates as well as differences in fungicide resistance amongst these isolates and stated that *B. cinerea* is a complex of sibling species, occurring sympatrically on many host plants.

FINAL COMMENTS

Many studies have been done to determine the infection pathway of *B. cinerea*. These pathways include the style end (McClellan & Hewitt, 1973; Nair & Parker, 1985), wounds (Nair *et al*., 1988), natural openings (Pucheu-Panté & Mercier, 1983), pedicels (Pezet & Pont, 1986; Holz *et al*., 1997), or by penetration of the cuticle (Nelson, 1956). Most studies of this nature make use of artificial inoculations that deposit groups of conidia on the plant surface (Nair *et al*., 1995; Broome *et al*., 1995; Marois *et al*., 1986; Avissar & Pesis, 1991; Chardonnet *et al*., 1997). The latter may be a very rare phenomenon in nature, where inoculation might occur as the deposition of single airborne conidia. Coertze *et al*. (2001) noted that the deposition of clusters of conidia on the plant surface most probably masked the true effects of the plants active defense systems as well as the effects of plant exudates. Coertze and co-workers (Coertze & Holz, 1999; Coerze *et al*., 2001) showed that the behaviour of single airborne conidia on the host surface differ from the behaviour of clusters of conidia. In light of the above facts it is clear that conclusions, drown from studies where groups of conidia are deposited on the host, may be misleading. A study was thus conducted with the following aims:

1) To study the infection pathways of naturally inoculated *B. cinerea* during all the phenological stages of three wine grape cultivars.

2) To study the infection pathways of artificially inoculated *B. cinerea* during the phenological stages of three wine grape cultivars.

3) To compare the pathogenicity and virulence on grape and nectarine of isolates obtained from different host plants.

LITERATURE CITED

Agrios, G.N. 1997. Fruit and general diseases caused by ascomycetes and imperfect (asexual) fungi. Pages 339-342 in: Plant Pathology 4th Edition. Vol II: Plant Diseases caused by fungi. Academic Press, London.

Alexander, D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weymann, K., Freidrich, L., Maddox, D., Ahl-Goy, P., Luntz, T., Ward, E. & Ryals, J. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenicity-related protein 1a. *Proclamation of the National Academy of Science of the U.S.A.* 90: 7327-7331.

Avissar, I. & Pesis, E. 1991. The control of postharvest decay in the table grapes using acetaldehyde vapours. *Annals of Applied Biology* 118: 229-237.

Backhouse, D. & Willetts, H.J. 1987. Development and structure of infection cushions of *Botrytis cinerea. Transactions of the British Mycological Society* 89: 89-95

Bar Nun, N., Tal Lev, A., Harel, E. & Mayer, A.M. 1988. Repression of laccase formation in *Botrytis cinerea* and its possible relation to phytopathogenicity. *Phytochemistry* 27: 2505-2509.

Bais, A.J., Murphy, P.J. & Dry, I.B. 2000. The molecular regulation of stilbene phytoalexin biosynthesis in *Vitis vinefera* during grape berry development. *Australian Journal of Plant Pathology* 27: 425-433.

Bavaresco, L., Petegolli, D., Cantu, E., Fregoni, M, Chiusa, G. & Trevisan, M. 1997. Elisitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. Vitis 36: 77-83

Benlioğlu, S. & Yilmaz, D. 1992. Influence of plant growth regulators on mycelial growth gennination of canidia and pathogenicity of *Botrytis cinerea*. Pages 119-122 in: Proceedings of the 10th international Botrytis symposium. K. Verhoeff, N.E. Malathrakis and B. Williamson, eds. Pudoc Scientific Publishers, Wageningen.

Blakeman, J.P. 1975. Germination of *Botrytis cinerea* conidia in vitro in relation to nutrient conditions on leaf surfaces. *Transactions of the British Mycological Society* 65: 39-247.

Borecka, H. & Millikan, D.F. 1973. Stimulatory effect of pollen and pistillate parts of some horticultureral species upon the germination of *Botrytis cinerea* spores. *Phytopathology* 63: 1431-1432

Breuil, A.C., Jeandet, P, Adrian, M., Chopin, F., Pirio, N., Meunier, P. & Bessis, R. Characterization of a pterostilbene dehydrodimer produced by *Botrytis cinerea. Phytopathology* 89: 298-302.

Bristwo, P.R., McNicol, R.J. & Williamson, B. 1986. Infection of strawberry flowers by *Botrytis cinerea* and its relevance to gray mould development. *Annals of applied Biology 109: 545-554*

Brooke, P.J. 1991. *Botrytis* stem-end rot and other storage diseases of kiwifruit - a review. *Acta Horticulturae* 297: 545-550

Broome, J.C., English, J.T., Marois, J.J., Latorre, B.A. & Aviles, J.C. 1995. Development of an infection model for *Botrytis* bunch rot of grapes based on wetness duration and temperature. *Phytopathology* 85: 97-102.

Bulger, M.A., Ellis, M.A. & Madden, L.V. 1986. Effect of wetness duration and temperature on infection of strawberry flowers and fruit by *Botrytis cinerea. Phytopathology* 76: 1114 (Abstract)

Bulger, M.A., Ellis, M.A. & Madden, L.V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. *Phytopathology* 77: 1225-1230

Chardonnet, C., L'Hyvernay, A. & Doneche, B. 1997. Effect of calcium treatment prior to *Botrytis cinerea* infection on the changes in pectic composition of grape berry. *Physiological and Molecular Plant Pathology* 50: 213-218.

Chou, M.C. & Preece, T.F. 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. *Annals of applied Biology* 62: 11-22.

Clark, C.A. & Lorbeer, J.W. 1976. Comparative histopathology of *Botrytis squamosa* and *Botrytis cinerea* on onion leaves. *Phytopathology* 66: 1279-1289.

Coertze, S. & Holz, G. 1999. Surface colonization, penetration and lesion formation on grapes inoculated fresh or after cold-storage with single airborne conidia of *Botrytis cinerea. Plant Disease* 83: 917-924.

Coertze, S., Holz, G. & Sadie, A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea. Plant Disease* 85: 668-677.

Cole, L., Dewey, F.M. & Hawes, C.R. 1996. Infection mechanisms of *Botrytis* species: pre-penetration and pre-infection processes of dry and wet conidia. *Mycological Research* 100: 277-286.

Coley-Smith, J.R. 1980. Sclerotia and other structures in survival. Pages 85-114 in: The biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Creasy, L.L. & Coffee, M. 1988. Phytoalexin production potential of grape berries. *Journal of the American Society for Horticultural Science* 113: 230-234.

Dashwood, E.P. & Fox, R.A. 1988. Infection of flowers and fruits of red raspberry by *Botrytis cinerea. Plant Pahtology* 37: 423-430.

De Kock, S.L. & Holz, G. 1992. Blossom-end rot of pears: Systematic infection of flowers and immature fruit by *Botrytis cinerea. Journal of Phytopathology* 135: 317-327.

De Kock, P.J. & Holz, G. 1991. Colonisation of table grapes by *Botrytis cinerea* in the Western Cape Province. *Phytophylactica* 23: 73-80.

Derckel, J.P., Baillieul, F., Manteau, S., Audran, J.P., Haye, B., Lambert, B. & Legendre, L. 1999. Differential induction of grapevine defenses by two strains of *Botrytis cinerea. Phytopathology* 89: 197-203.

Di Lenna, P., Marciano, P. & Magro, P. 1981. Comparative investigation on morphological and physiological features of three isolates of *Botrytis cinerea. Phytopathologiche Zeitschrift* 100: 203-211.

Doss, R.P., Potter, S.W., Chastagner, G.A. & Christian, J.K. 1993. Adhesion of nongerminated *Botrytis cinerea* conidia to several substrata. *Applied and Environmental Microbiology* 59: 1786-1791.

Doss, R.P., Potter, S.W., Soeldner, A.H., Christian, J.K. & Fukunaga, L.E. 1995. Adhesion of germlings of *Botrytis cinerea*. *Applied and Evironmental Microbiology* 61: 260-265.

Duncan, R.A., Stapleton, J.J. & Leavitt, G.M. 1995. Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as influenced by leaf removal. *Plant Pathology* 44: 956-965.

Edlich, W., Lorenz, G., Lyr, H., Neg. & Pommer, E.H. 1989. New aspects on the infection mechanism of *Botrytis cinerea* Pers. *Netherlands Journal of Plant Pathology* 95: 53-62.

Elad, Y. & Kirshner, B. 1992. Calcium reduces *Botrytis cinerea* damages to plants of *Ruscus hypoglossum*. *Phytoparasitica* 20: 285-291.

Elad, Y. & Yunis, H. 1993. Effect of microclimate and nutrients on development of cucumber gray mold (*Botrytis cinerea*). *Phytoparasitica* 21: 257-268.

Ellerbrock, L.A. & Lorbeer, J.W. 1977. Sources of primary inoculum of *Botrytis squamosa*. *Phytopathology* 67: 363-372.

English, J.T., Bledsoe, A.M. & Marois, J.J. 1990. Influence of leaf removal from the fruit cluster zone on the components of evaporative potential within grapevine canopies. *Agriculture, Ecosystems and Environmnet* 31: 49-61.

English J.T., Kaps, M.L., Moore, J.F., Hill, J. and Nakova, M. 1993. Leaf removal for control of *Botrytis* bunch rot of wine grapes in the midwestern United States. *Plant Disease* 77: 1224-1227.

English J.T., Thomas, C.S., Marois, J.J. & Gubler, W.D. 1989. Microclimates of grapevine canopies associated with leaf removal and control of *Botrytis* bunch rot. *Phytopathology* 79: 395-401.

Fermaud, M. & Gaunt, R. 1995. *Thrips obscuratus* as a potential vector of *Botrytis cinerea* in kiwifruit. *Micological Research* 99: 267-273.

Fermaud, M. & Le Menn, R. 1989. Association of *Botrytis cinerea* with grape berry moth larvae. *Phytopathology* 79: 651-656.

Fermaud, M. & Le Menn, R. 1992. Transmission of *Botrytis cinerea* to grapes by grape berry moth larvae. *Phytopathology* 82: 1393-1398.

Fourie, J.F. & Holz, G. 1994. Infection of plum and nectarine flowers by *Botrytis cinerea*. *Plant Pathology* 43: 309-315.

Fourie, J.F. & Holz, G. 1995. Initial infection process by *Botrytis cinerea* on nectarine and plum fruit and the development of decay. *Phytopathology* 85: 82-87.

Garrett, S.D. 1960. Inoculum potential, Page 675 in: Plant Pathology. J.G. Horsfall and A.E. Dimond, eds. Academic Press, New York and London.

Gessler, A. & Jermini, M. 1985. Role of flower infections of grapy by *Botrytis cinerea* and consequences for the spraying schedule. *Quaderni Della Scuola Di Specializzazione In Viticoltrua Ed Enologia, Università du Torino* 9: 255-266.

Giraud, T., Fortini, D., Levis, C., Lamarque, C., Leroux, P., LoBuglio, K. & Brygoo, Y. 1999. Two sibling species of the *Botrytis cinerea* complex, transposa and vacuma, are found in sympatry on numerous host plants. *Phytopathology* 89: 967-973.

Good, H.M. & Athureczky, P.G.M 1967. Effects of drying of the viability of germinated spores of *Botrytis cinerea, Cercospora musae,* and *Monilinia fructicola. Phytopathology* 57: 719-722.

Gronover, C.S., Kasulke, D., Tudzynski, P. & Tudzynski, B. 2001. The role of G protein alpha subunits in the infection process of the gray mold fungus *Botrytis cinerea. Molecular Plant- Microbe Interaction* 14: 1293-1302.

Gubler, W.D., Marois, J.J., Blesoe, A.M. & Bettiga, L.J. 1987. Control of *Botrytis* bunch rot of grape with canopy management. *Plant Disease* 781: 599-601.

Hain, R., Reif, H.J., Krause, E., Langebartels, R., Kindl, H., Vorman, B., Wiese, W., Schmelzer, E., Schreier, P.H., Stöcker, R.H. & Stenzel, K. 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* 361: 153-156.

Hill, G. 1985. Suberisation of cell walls: A Defence reaction of grape stem tissue against invading mycelium of *Botrytis cinerea. Quaderni Della Scuola Di Specializzazione In Viticoltura Ed Enologia, Università di Torino* 9: 229-230.

Hill, G. Stellwaag-Kittler., Huth, G. & Schlösser, E. 1981. Resistance in different developmental stages to *Botrytis cinerea. Phytopatologische Zeitschrift* 102: 328-338.

Hobbs, E.L. & Walters, W.E. 1964. Influence of nitrogen and potassium on susceptibility of chrysanthemum morifolium to *Botrytis cinerea. Phytopathology* 54: 674-676.

Holz, G., Coertze, S. & Basson, E.J. 1997. Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. Annual Meeting of the American Phytopathological Society, Rochester, USA. *Phytopathology* 87 (supplement): S43.

Hoos, G. & Blaich, R. 1988. Metabolism of *stilbene phytoaliexins* in grapevines: Oxidation of resveratrol in single-cell cultures. *Vitis* 27: 1-12.

Hunter, J.E. & Rohrbach, K.G. 1969. *Botrytis cinerea* development on macadamia racemes in relation to meteorological conditions. *Phytopahtology* 59: 1033 (Abstract).

Hunter, J.E., Rohrbach, K.G. & Kunimoto, R.K. 1972. Epidemiology of *Botrytis* blight of macadamia racemes. *Phytopathology* 62: 316-319.

Hutcheson, S.W. 1998. Current concepts of active defense in plants. *Annual Review of Phytopathology*. 36: 59-90.

Hyre, R.A. 1972. Effect of temperature and light on colonisation and sporulation of the *Botrytis* pathogen on gernaium. *Plant Disease Reporter* 56: 126-130.

Islam, S.Z., Honda, Y. & Senhaji, M. 1998. Phototropism of Canidial Genn Tube of *Botrytis cinerea* and its implication in plant infection process. *Plant Disease* 82: 850-856.

Jarvis, W. R. 1962a. The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. *Transactions of the British Mycological Society* 45:549-559.

Jarvis, W.R. 1962b. Splash dispersal of spores of *Botrytis cinerea* Pers. *Nature (London)* 193: 599.

Jarvis, W.R. 1980. Epidemiology. Pages 219-250 in: The biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, New York.

Jeandet, P. & Bessis, R. 1989. Une réflexion sur les mécanismes morphologiques et biochimiques de l'interaction vigne-*Botrytis*. *Bulletin de l'O.I.V.* 62: 673-657.

Jeandet, P., Sbaghi, M. & Bessis, R. 1992. The use of phytoalexin induction and of *in vitro* methods as a tool for screening grapevines for resistance to *Botrytis cinerea*. Pages 109-112 in: Proceedings of the 10th international Botrytis symposium. K. Verhoeff, N.E. Malathrakis and B. Williamson, eds. Pudoc Scientific Publishers, Wageningen.

Kamoen, O., Jamart, G., Van Vaerenbergh, J. & Gouwy, P. 1985. *Botrytis cinerea* infections on petals and green leaves. *Mededelingen van de Faculteit Landbouwwetenscahppen Universiteit Gent.* 50: 1053-1059.

Kapat, A., Zimand, G. & Clad, Y. 1998. Biosynthesis of pathogenicity hydrolic enzymes by *Botrytis cinerea* during infection of green leaves and *in vitro*. *Mycological Research* 102: 1017-1028.

Kerssies, A. 1993. Influence of environmental conditions on dispersal of *Botrytis cinerea* conidia and on post-harvest infection of gerbera flowers grown under glass. *Plant Pathology* 42: 754-762.

Kerssies, A., Bosker-van Zessen, A.I., Wagemakers, C.A.M. & van Kan, J.A.L. 1997. Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. *Plant Disease* 81: 781-786.

Kombrink, E. & Somssich, I. 1995. Defense responses of plants to pathogens. *Advances in Botanical Research* 21: 1-34.

Kosuge, T. & Hewitt, W.B. 1964. Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea*. *Phytopathology* 54: 167-172.

Kolattukudy, P.E. & Crawford, M.S. 1987. The role of polymer degrading enzymes in fungal pathogenesis. Pages 75-95 in: Molecular Determination of Plant Diseases. Nishimura *et al*, eds. Japan Sci. Soc.Press, Tokyo/Springer-verslag, Berlin.

Langcake, P. 1981. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. *PhysiologicalPlant Pathology* 18: 213-226.

Langcake, P. & McCarthy, W.V. 1979. The relationship of resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. *Vitis* 18: 244-253.

Lavy-Meir, G., Barkai-Golan, R. and Kopeliovitch, E. 1988. Initiation at the flowering stage of postharvest *Botrytis* stem-end rot in normal and non-ripening tomato fruits. *Annals of applied Biology* 112: 393-396.

Liswidowati, Melchior, F., Hohmann, F., Schwer, B. & Kindl, H. 1991. Induction of stilbene synthase by *Botrytis cinerea* in cultured grapevine cells. *Planta* 183: 307-314.

Louis, C., Girard, M., Kuhl, G. & Lopez-Ferber, M. 1996. Persistance of *Botrytis cinerea* in its vector *Drosophila melanogaster. Phytopathology* 86: 934-939.

Lourens, P. 1986. Parasitism by *Botrytis cinerea. Deciduous fruit grower* 36: 133-137.

Mansfield, J.W. & Hutson, R.A. 1980. Microscopical studies of fungal development and host responses in broad bean and tulip leaves inoculated with five species of *Botrytis*. *Physiological Plant Pathology* 17: 131-144.

Marais, P.G. 1985. Infection of table grapes by *Botrytis cinerea. Deciduous fruit grower* 35: 166-170.

Marois, J.J., Nelson, J.K., Morrison, J.C., Lile, L.S. & Bledsoe, A.M. 1986. The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. *American Journal of Enology and Viti*culture 37: 293-296.

McClellan, W.D. & Hewitt, B. 1973. Early *Botrytis* rot of grapes: Time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* 63: 1151-1157.

McKeen, W.E. 1974. Mode of Penetration of epidermal cell walls of *Victa Faba* by *Botrytis cinerea. Phytopathology* 64: 461-467.

Molot, B. 1987. La Modélisation de la pourriture grise de la vigne par systéme d' *E.P.I Progrés Agricole et Viticole* 104: 355-358.

Nair, N.G. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. *Australian Journal of Agricultural Research* 36: 435-442.

Nair, N.G. & Allen, R.N. 1993. Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. *Mycological Research* 97:1012-1014

Nair, N.G., Emmett, R.W. & Parker, F.E. 1988. Some factors predisposing grape berries to infection by *Botrytis cinerea. New Zealand Journal of Experimental Agriculture* 16: 257-263.

Nair, N.G., Guilbaud-Oulton, S., Barchia, I. & Emmett, R. 1995. Siginificance of carry over inoculum, flower infection and latency on the incidence of *Botrytis cinerea* in berries of grapevines at harvest in New South Wales. *Australian Journal of Experimental Agriculture* 35: 1177-1180.

Nair, N.G. & Parker, F.E. 1985. Midseason bunch rot of grapes: an unusual disease phenomenon in the Hunter Valley, Australia. *Plant Pathology* 34: 302-305.

Nair, N.G. & Martin, A.B. 1987. Ultrastructure and development of sclerotia of *Botrytis cinerea* Pers. *in vitro*. *Journal of Phytopathology* 119: 52-63.

Nair, N.G. & Nadtotchei, A. 1987. Sclerotia of *Botrytis* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. *Journal of Phytopathology* 119: 42-51.

Nelson, K.E. 1951a. Factors influencing the infection of table grapes by *Botrytis cinerea* (Pers.). *Phytopathology* 41: 319-326.

Nelson, K.E. 1951b. Effect of humidity on infection of table grapes by *Botrytis cinerea*. *Phytopathology* 41: 859-864.

Nelson, K.E. 1956. The effect of *Botrytis* infection on the tissue of Tokay grapes. *Phytopathology* 46: 223-229.

Northover, J. 1987. Infection sites and fungicidal prevention of *Botrytis cinerea* bunch rot of grapes in Ontaria. *Canadian Journal of Plan Pathology* 9:129-136.

Padgett, M. & Morrison, J.C. 1990. Changes in grape berry exudates during fruit development and their effect on mycelial growth of *Botrytis cinerea*. *Journalof the American Society of Horticultural Science* 115: 269-273.

Percival, D.C., Sullivan, J.A. & Fisher, K.H. 1993. Effects of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot (*Botrytis cinerea* Pers.: Fr.) with 3 *Vitis vinifera* L. cultivars. *Vitis* 32: 87-97.

Pezet, R. 1998. Purification and characterization of a 32-kDa laccase-like stilbene oxidase produced by *Botrytis cinerea* Pers.:Fr. *FEMS Microbiology Letters* 167: 203-208.

Pezet, R. & Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans les grappes de *Vitis vinifera* (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18: 317-322.

Pezet, R. & Pont, V. 1992. Differing biochemical and histological studies of two grape cultivars in the view of their respective susceptibility and resistance to *Botrytis cinerea*. Pages 93-98 is: Proceedings of the 10th international Botrytis symposium. K.Verhoeff, N.E. Malathrakis and B. Williamson, eds. Pudoc Scientific Publishers, Wageningen.

Powelson, R.L. 1960. Initiation of strawberry fruit rot caused by *Botrytis cinerea*. *Phytopathology* 50: 491-494.

Pucheu-Planté, B. & Mercier, M. 1983. Étude ultrastructurale de l'interrelation hôte-parasite entre le raisin et le champignon *Botrytis cinerea:* exemple de la pourriture noble en Sauternais. *Canadian Journal of Botany* 61: 1785-1797.

Punja, Z.K. & Raharjo, S.H.T. 1996. Response of transgenic cucumber and carrot plants expressing different chitinase enzymes to inoculation with fungal pathogens. *Plant Disease* 80: 999-1005.

Quidde, T., Buttner, P. & Tudzynski, P. 1999. Evidence for three different specific saponin-detoxifying activities in *Botrytis cinerea* and cloning and functional analysis of a gene coding for a putative avenacinase. *European Journal of Plant Pathology* 105: 273-283.

Rijkenberg, F.H.J., De Leeuw, G.T.N. & Verhoeff, K. 1980. Light and electron microscopy studies on the infection of tomato fruits by *Botrytis cinerea. Canadian Journal of Botany* 58: 1394-1404.

Sackston, W. E. 1960. *Botrytis cinerea* and *Sclerotinia Sclerotiorum* in seed of Safflower and Sunflower. Plant Disease Reporter 44: 664 (Abstract).

Salinas, J. & Verhoeff, K. 1995. Microscopical studies of the infection of gerbera flowers by *Botrytis cinerea. European Journal of Plant Pathology* 101: 377-386.

Salinas, J., Warnaar, F. & Verhoeff, K. 1986. Production of cutin hydrolyzing enzymes by *Botrytis cinerea* in vitro. *Journal of Phytopathology* 116: 299-307.

Salinas, J., Glandorf, D.C.M., Picavet, F.D. & Verhoeff, K. 1989. Effects of temperature, relative humidity and age of conidia on the incidence of spotting on gerbera flowers caused by *Botrytis cinerea. Netherlands Journal of Plant Pathology* 95: 51-64.

Sasaki, I. & Nagayama, H. 1994. β-Glucosidase of *Botrytis cinerea*: Its involvement in the pathogenicity of this fungus. *Bioscience, Biotechnology and Biochemistry* 58: 616-620.

Sasaki, I. & Nagayama, H. 1996. β-Glucosidase of *Botrytis cinerea*: Its involvement in the pathogenicity of this fungus. *Bioscience, Biotechnology and Biochemistry* 60: 54-56.

Savage, S.D. & Sall, M.A. 1983. *Botrytis* bunch rot of grapes: The influence of selected cultural practices on infection under California conditions. *Plant Disease* 67: 771-774.

Savage, S.D. & Sall, M.A. 1984. *Botrytis* bunch rot of grapes: Influence of trellis type and canopy microclimate. *Phytopathology* 74: 65-70.

Sbaghi, M., Jeandet, P., Faire, B., Bessis, R. & Fournioux, J.C. 1995. Development of methods using phytoalexin (resveratrol) assessment as a selection criterian to screen grapevine *in vitro* cultures for resistance to grey mould (*Botrytis cinerea).Euphytica* 86: 41-47.

Sbaghi, M., Jeandet, P., Bessis, R. & Leroux, P. 1996. Degradation of stilbene-type phytoaliexin in relation to the pathogenicity of *Botrytis cinerea* to grapevines. *Plant Pathology* 45: 139-144.

Sharman, S. & Heale, J.B. 1977. Penetration of carrot roots by the grey mould fungus *Botrytis cinerea* Pers. ex pers. *Physiological Plant Pathology* 10: 63-71.

Sharrock, K.R. & Hallett, I.C. 1991. Physiology of *Botrytis* infection in kiwifruit. *Acta Horticulturae* 297: 551-545.

Shaul, O., Elad, Y. & Zieslin, N. 1996. Suppression of *Botrytis* blight in cut rose flowers with gibberellic acid. Effects of exogenous application of abscisic acid and paclobutrazol. *Postharvest Biology and Technology* 7: 145-150.

Shoemaker, P.B. & Lorbeer, J.W. 1977. The role of dew and temperature in the epidemiology of *Botrytis* leaf blight of onion. *Phytopathology* 67: 1267-1272.

Staples, R.C. & Mayer, A.M. 1995. Putative virulence of *Botrytis cinerea* acting as a wound pathogen. *FEMS Microbiology Letters* 134: 1-7.

Ten Have, A., Mulder, W., Visser, J. & Van Kan, J.A.L. 1998. The endopolygalacturonase gene *Bcqp*1 is required for full virulence of *Botrytis cinerea*. *Molecular Plant-Microbe Interactions* 11: 1009-1016.

Thompson, J.R. & Latorre, B.A. 1999. Characterization of *Botrytis cinerea* from table grapes in Chile using RAPD-PCR. *Plant Disease* 83: 1090-1094.

Thomas, C.S. and Marois, J.J. 1986. Effect of wind and relative humidity on sporulation and external mycelium formation of *Botrytis* on grape. *Phytopathology* 76: 1114 (Abstract).

Thomas, C.S., Marois, J.J. & English, J.T. 1988. The effects of wind speed, temperature and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. *Phytopathology* 78: 260-265.

Tronsmo, A. & Raa, J. 1977. Life cycle of the dry eye rot pathogen *Botrytis cinerea* Pers. on apple. *Phytopatologische Zeitschrift* 89: 203-207.

Tronsmo, A., Tronsmo, A.M. & Raa, J. 1977. Cytology and biochemistry of pathogenic growth of *Botrytis cinerea* Pers. in apple fruit. *Phytopatologische Zeitschrift* 89: 208-215.

Vail, M.E. & Marois, J.J. 1991. Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. *Phytopathology* 81: 188-191.

Van den Bergh, L. & Lentz, C.P. 1968. The effect of relative humidity and temperature on survival and growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*. *Canadian Journal of Botany* 46: 1477-1481.

Van der Vlugt-Bergmans, C.J.B., Wagemakers, C.A.M. & Van Kan, J.A.L. 1977. Cloning and expression of the cutinose A gene of *Botrytis cinerea*. *Plant Microbe Interactions* 10: 21-29.

Vercesi, A., Locci, R. & Prosser, J.I. 1997. Growth kinetics of *Botrytis cinerea* on organic acids and sugars in relation to colonisation of grape berries. *Mycological Research* 101: 139-142.

Verhoeff, K. 1970. Spotting of tomato fruits caused by *Botrytis cinerea*. *Netherlands Journal of Plant Pathology* 76: 219-226.

Verhoeff, K. 1980. The infection process and host-pathogen interactions. Pages 153-180 in: The Biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press London.

Verhoeff, K & Liem, J.I. 1978. Presence of endo-polygalacturonase in conidia of *Botrytis cinerea* before and during germination. *Phytopahtologische Zeitschrift* 91: 110-115.

Volpin, H. & Elad, Y. 1991. Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight. *Phytopathology* 81: 1390-1394.

Wasfy, E.H., Farag, S.A., Tarabieh, M.A. & Abd-Elmoety, S.H. 1978. Studies on enzymes of different strains of *Botrytis cinerea*. *Phytopathologische Zeitschrift* 92: 168-179.

Williamson, B., Duncan, G.H., Harrison, J.G., Harding, L.A., Elad, Y. & Zimand, G. 1995. Effect of humidity on infection of rose petals by dry-ipoculated canidia of *Botrytis cinerea*. *Mycological Research* 99: 1303-1310.

Williamson, B., McNicol, R.J. & Dolan, A. 1987. The effect of inoculating flowers and developing fruits with *Botrytis cinerea* on post-harvest grey mould of red raspberry. *Annals of applied Biology* 111: 285-294.

Wisniewski, M., Droby, S., Chalutz, E. and Eilam, Y. 1995. Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum in vitro* and on the biocontrol activity of *Candida oleophila*. *Plant Pathology* 44: 1016-1204.

Wubben, J.P., ten Have, A., van Kan, J.A.L. & Visser, J. 2000. Regulation of endopolygalacturonase gene expression in *Botrytis cinerea* by galacturonic acid, ambient ph and carbon catabolite repression. *Current Genetics* 37: 152-157.

Yunis, H., Elad, Y. & Pressman, E. 1991. Calcium enrichment of fertilizer to reduce grye mould of greenhouse-grown eggplant pepper and cucumber. *Phytoparasitica* 19: 246 (Abstract).

Yunis, H., Elad, Y. & Mahrer, Y. 1990. Effects of air temperature, relative humidity and canopy wetness on grey mould of cucumbers in unheated greenhouses. *Phytoparasitica* 18: 203-215.

Zalewska-Sobczak, J., Borecka, H & Urbanek, H. 1981. Comparison of pectinase, xylanase and acid protease activities of virulent and less virulent isolates of *Botrytis cinerea*. *Phytopathologische Zeitschrift* 101: 222-227.

Zheng, L., Campbell, M., Murphy, J., Lam, S. & Xu, J. R. 2000. The BMP1 gene is essential for pathogenicity in the gray mould fungus *Botrytis cinerea*. *Molecular Plant-Microbe Interactions* 13: 724-732.

2. OCCURRENCE OF *BOTRYTIS CINEREA* AND SUBSEQUENT DISEASE EXPRESSION AT DIFFERENT POSITIONS IN BUNCHES OF THREE WINE GRAPE CULTIVARS

ABSTRACT

The occurrence of *Botrytis cinerea* and subsequent disease expression at different positions in bunches of wine grapes (cultivars Chenin Blanc, Shiraz and Chardonnay) was determined from 1999 to 2001. Different techniques were used to detect viable inoculum at different positions (rachises, laterals, pedicels, and the pedicel-end, cheek and style-end of berries) in bunches. Isolations were made on Kerssies' *B. cinerea* selective medium, or bunches were used untreated, or treated with paraquat. Paraquat was used to terminate host resistance and to promote the development of the pathogen from the tissues. The material was used untreated to detect the pathogen on the surface, or were surface-sterilised to detect mycelia (latent infection) in the tissue. *B. cinerea* occurred in a consistent pattern in bunches of the three cultivars. In the isolation studies, *B. cinerea* consistently developed from the tissue of the rachis, laterals, pedicel, pedicel-end and berry cheek. In the humidity chamber studies, it also developed from all positions, except from the berry cheek. The rachis, lateral and pedicel parts contained much higher levels of *B. cinerea* than any position on the berry. Furthermore, the pathogen consistenly occurred at relatively high levels on the rachises throughout the season. Collectively, the data showed that in the Western Cape province, *B. cinerea* occured more regularly in wine grape bunches during the early part of the season, than later in the season.

INTRODUCTION

Botrytis cinerea Pers.:Fr. causes grey mould on grapevine (*Vitis vinifera* L) (Nair & Hill, 1992). Although the fungus can attack bunches, leaves, buds and canes (Nair & Hill, 1992; Gütschow, 2001), the most prominent symptom is found on the berries (Nair & Nadtotchei, 1987). Grape berries are considered resistant to infection when immature (Du Plessis, 1937; Hill *et al.*, 1981; Nair & Hill, 1992). McClellan & Hewitt (1973) and Nair & Parker (1985) found that *B. cinerea* invades the stigma and stylar end during the bloom period and becomes

latent in necrotic stigma and style tissue at the stylar end of the berry. Then, at véraison or later the fungus establishes a pathogenic relationship and rot the berries. Pezet and Pont (1986) found no evidence for the stylar end infection pathway. They showed that latent infection was pedicel-associated. Holz *et al* (1997, 1998) also suggested the pedicel to be the predominant pathway for berry infection.

Ripe grapes are considered susceptible to decay by clusters of conidia (Broome *et al.*, 1995; Hill *et al.*, 1981; Nair & Allen, 1993; Nelson, 1951). However, workers in South Africa (G. Holz, *unpublished data)* and in California (Duncan *et al.*, 1995) showed that the amount of *B. cinerea* on the berry surface stays low throughout the season and that the pathogen is only present as single colony forming units. Solitary conidia of *B. cinerea* did not readily penetrate the berry skin (Coertze & Holz, 1999; Coertze *et al.*, 2001; Gütschow, 2001) and were unable to induce disease symptoms on ripe Dauphine grape berries (Coertze & Holz, 1999). In addition, it was demonstrated (Coertze *et al.*, 2001) that neither surface colonisation, nor wetness regime had any positive effect on berry skin penetration by single conidia. These findings imply that the berry skin provides an effective barrier to penetration by single conidia, and confirmed the decisive role of wounding in both symptom expression and the epidemiology of *B. cinerea* on grape (Coertze *et al.*, 2001). Wounds can be caused by insects, frost, hail, windblown sand, sunburn or the rapid uptake of water leading to splitting of the berry (Jarvis, 1980; Savage & Sall, 1983).

Gütschow (2001) showed that grape cultivars may differ in their resistance reaction to *B. cinerea*, and more so in the pedicel tissue than in the berry cheek. There are furthermore strong indications that factors (compact bunch structure, wind damage, wounding, berry burst) which cause "weak spots" at the pedicel-end of the berry, may play a determining role in symptom expression. The aim of the study was to determine natural *B. cinerea* infection and subsequent disease expression at specific positions in bunches of three wine grape cultivars differing in cluster tightness.

MATERIALS AND METHODS

Vineyards and grape material. Bunches of the cultivars Chenin Blanc, Shiraz and Chardonnay were collected from 1999 to 2001 from vineyards in the Western Cape province. Shiraz has a loosely spaced bunch architecture whereas Chenin Blanc and Chardonnay have a more compact berry arrangement. Shiraz grapes were collected from Great Constantia,

which is situated near Cape Town. No fungicide sprays for the control of *B. cinerea* were applied in the vineyard during the 1999 – 2001 period. Chardonnay grapes were collected from the farm Buitenverwachting, which is adjacent to Great Constantia. The spray programme for the control of *B. cinerea* consisted of the following applications: 14 November 2000 – procymidone (Sumisclex 250 SC, Sanachem); 11 December 2000 – fludioxonil/cyprodinil (Switch 375/250 WG, Novartis); 11 January 2001 – fludioxonil/cyprodinil (Switch 375/250 WG, Novartis). Chenin Blanc grapes were collected from Dellrust, which is situated between Stellenbosch and Strand. The spray programme for the control of *B. cinerea* consisted of the following applications: November 1999 – pyrimethanil (Scala 400 EC, Aventis); December 1999 – iprodione (Rovral Flo 250 EC, Aventis); January 2000 – procymidone (Sumisclex 250 SC, Sanachem); November 2000 – procymidone (Sumisclex 250 SC, Sanachem); December 2000 - iprodione (Rovral Flo 250 EC, Aventis); January 2001 – procymidone (Sumisclex 250 SC, Sanachem). Shoots with sound, unblemished bunches, each from a different arbitrarily chosen vine, were obtained at pea size, bunch closure (approximately 6°Brix), véraison (approximately 11°Brix), and the harvest stage (approximately 16°Brix) for the different investigations. Thirty bunches were used for each cultivar at each growth stage.

Detection of *B. cinerea*. Two techniques were used to detect the pathogen in bunch tissue. For the first technique, 20 bunches of each cultivar were surface sterilised (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol). This treatment eliminated *B. cinerea* from the bunch surface (Sarig *et al.*, 1997). The treated bunches were divided in two groups. One group was immersed in paraquat solution (30 ml/l water) (WPK Paraquat, 200 g/l [bipyridyl], WPK Agricultural, Cape Town, South Africa) for 30 seconds, rinsed in sterile deionised water and air-dried. The other group was left untreated. The bunches of both groups were placed in transluscent plastic (Addisware) containers (33×22×11 cm) (five per container) lined with a wet paper towel (Kimberley–Clark; 324cm×240cm) to establish high relative humidity (≥93% RH). The bunches 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. Positions observed on structural bunch parts were rachises, laterals and pedicels. Positions on berries were the base, cheek and stylar end. The presence of *B. cinerea* at each position was recorded, and the incidence calculated after 14 days for each treatment.

For the second technique, isolations were made from the various positions in the bunches. Bunches of each cultivar were divided in two groups of five bunches each. One group was surface sterilised as described previously, the other group was left untreated. From each bunch, five berries and pedicels, and five lateral and rachis segments (approximately 10-20 mm each) were removed. One epidermal tissue segment (5 x 7 mm) was cut from the cheek of each berry, and the different segments (five segments per position on the berry) were placed in Petri dishes on Kerssies' *B. cinerea* selective medium (Kerssies, 1990). The plates were incubated at 22°C under diurnal light. Disease expression was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues. Disease expression at each position was recorded, and incidences for each position calculated after 14 days.

The treatments provided conditions that discriminate between conidial germination and the development of germlings on the surface, and the development of latent mycelia in the grape tissue during the incubation period. Paraquat (Baur *et al.*, 1969; Cerkauskas & Sinclair, 1980; Grindrat & Pezet, 1994; Pscheidt & Pearson, 1989) treatment terminated host resistance in the cells of the cuticular membrane without damaging host tissue, and thus facilitated the development of both surface conidia and latent mycelia in the tissue. Previous microscopic observations of berry skins on Kerssies medium (Coertze & Holz, 1999; Coertze *et al.*, 2001) showed that no superficial mycelial growth developed on the skin segments during the first 5 days of incubation. Hyphal growth usually occurred from cells underlying the cuticle into the medium after 5 days, which indicated direct penetration by surface conidia, or the development of latent mycelia from skin tissue during the incubation period. Surface sterilisation eliminated *B. cinerea* from the berry surface and facilitate the detection of mycelia in the skin tissue (Coertze & Holz, 1999; Coertze *et al.*, 2001; Gutschow, 2001). Therefore, according to these differential criteria, the development of *B. cinerea* from segments obtained from unsterile bunches resulted from the development of both surface conidia and latent mycelia. On segments obtained from surface-sterilised berries the pathogen developed from latent mycelia in the skin tissue only.

Statistical procedure. A complete randomised design experiment was performed and replicated over 2 seasons. For the first experiment, the experimental design was a 2x5x2x6 factorial with factors: seasons (1999/2000; 2000/2001); stages (flower, pea size, bunch closure, veraison, harvest); treatment (paraquat, non-paraquat) and pathways (cheek, laterals,

pedicel, rachis, style-end, pedicel-end). This experiment was conducted with three cultivars (Chardonnay, Chenin Blanc, Shiraz). An experimental unit was 10 bunches. For the isolation experiment the experiment design was a 5x2x4 factorial with factors: stages (pea size, bunch closure, harvest); treatments (sterile, non-sterile); and pathways (cheek, laterals, pedicel, rachis, style, pedicel-end). This experiment was conducted with three cultivars (Chardonnay, Chenin Blanc, Shiraz). An experimental unit was between 5-50 isolations. The data were transformed to a logit as well as percentage incidence and subjected to a factorial analysis of variance for each cultivar separately, using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Levene's test for homogeneity was performed to test if the cultivars were of comparable magnitude in the humidity chamber experiment. Student's t-test was performed at a 5% significance level to compare treatment means (Snedecor & Cochran, 1980).

RESULTS

Detection of *B. cinerea*. *Bunches in moist chambers.* Levene's test for homogeneity indicated that for cultivars the seasonal variability in data were of comparable magnitude ($P = 0.9516$) and hence a combined analysis was validated. The analysis of variance for the effects of cultivar, treatment, pathway and phenological stage is given in Table 1. There was a significant difference between cultivars ($P = 0.0019$), treatments ($P < 0.0001$), pathways ($P < 0.0001$) and phenological stages ($P < 0.0001$). The significant interactions were cultivar and position ($P = 0.0018$), treatment and position ($P = 0.0003$), phenological stage and treatment ($P = 0.0002$) and phenological stage and position ($P < 0.0001$). Several general responses can be derived from the significant effect of the interactions. The paraquat test showed that the mean incidence of *B. cinerea* was significant higher in the structural bunch parts than on berries (Table 2). This tendency was also found in the untreated bunches, but levels did not differ significantly for the pedicel and pedicel-end of the berry. Furthermore, for both treatments, the mean incidence of *B. cinerea* was consistently low for both the cheek and stylar end of the berry. The mean incidence of *B. cinerea* was significantly higher in tissues exposed to paraquat than in the untreated tissues from bloom to bunch closure (Table 3). Data of the phenological stage x position interaction revealed that the pathogen occupied predominantly the rachis during this period (Table 4). The mean incidence of *B. cinerea* was significant higher in bunches of Chardonnay and Chenin Blanc than in Shiraz (Table 5). For

the rachis, the mean incidence of *B. cinerea* was consistenly high and did not differ significantly between the cultivars (Table 6).

Isolation studies. The analysis of variance for the effects of season, treatment, pathway and phenological stage is given in Table 7. There was a significant difference between seasons (P = 0.0015) and pathways (P = 0.0022). No significant interactions were recorded. The sterilisation test showed that the pathogen occurred predominantly on the rachis and the laterals in bunches (Table 8). For both sterilisation regimes, the mean incidence of *B. cinerea* was significant higher on the rachis than on the pedicel and the berry positions. Furthermore, for both sterilisation regimes the mean incidence of *B. cinerea* was consistlently low at the berry positions. The data of the cultivar x position interaction revealed that for the rachis, the mean incidence of *B. cinerea* was relatively high for the three cultivars (Table 9). On Chenin blanc (Table 10), the pathogen occurred at a consistent high level at this position at all growth stages. On Chardonnay (Table 11) and Shiraz (Table 12) the mean incidence of *B. cinerea* fluctuated between growth stages.

DISCUSSION

Different methods were used in this study to facilitate the natural development of *B. cinerea* from both surface and latent mycelia. In the isolation studies, *B. cinerea* consistently developed from the tissue of the rachis, laterals, pedicel pedicel-end and berry cheek. In the humidity chamber studies, it also developed from all the above-mentioned sites as well as from the style-end, but never from the berry cheek. The levels of decay were furthermore consistently higher on the paraquat-treated bunches than on the non-treated bunches. The data thus showed that although grape bunches in Western Cape vineyards do not normally develop grey mould during pea size and bunch closure, they may carry high amounts of *B. cinerea*. However, the frequency at which the pathogen occurred in bunches was relatively low. In the paraquat treatment, the highest level of *B. cinerea* was during flower (7.11%) and under sterile conditions, the rachis reached the highest levels with 16% infected. Host resistance was thus the limiting factor in the development of decay and not the amount of pathogen on or in the tissue.

The rachis, lateral and pedicel parts contained much higher levels of *B. cinerea* than any position on the berry. This finding confirmed that of Gütschow (2001) on table grapes. Observations of the pathogen's development showed that it developed mainly from the

receptacle part of the pedicel and then spread towards the pedicel-end of the berry. These findings support the hypothesis that berry rot is not caused by infection of the pistil and subsequent latency in the style-end, but rather infection in the structural parts (rachis, lateral, pedicel) and subsequent latency in the pedicel or pedicel-end of the berry. The latter finding is in agreement with observations made by Pezet and Pont (1986); Holz *et al.* (1997, 1998) and Gütschow (2001) and does not support the stylar end pathway for berry infection described by others worker (McClellan & Hewitt, 1973; Nair & Parker, 1985). Jarvis (1980) showed that when berries compress against each other it causes the pedicel-berry connections to brake from each other and form small cracks. My observations, together with that of Jarvis (1980), emphasise the profound importance that infection in the structural bunch parts and bunch architecture has on grey mould development in the vineyard. The observation that the inner part of the less compact Shiraz bunch has lower levels of *B. cinerea* than that of the more compact Chardonnay and Chenin Blanc bunches, supports the findings by Nair and Parker (1985) that more compact bunches are less well aerated and has more infections.

The occupation pattern of *B. cinerea* at different positions in bunches of the wine grape cultivars Chardonnay, Chenin blanc and Shiraz, confirmed the general pattern of inoculum ecology and subsequent disease expression on table grapes described by Holz and co-workers (Coertze & Holz, 1999; Coertze & Holz, 2002; Coertze *et al.* 2001; Gütschow, 2001). However, in the wine grape bunches, *B. cinerea* occupied predominantly the rachis. Furthermore, the pathogen consistenly occurred at relatively high levels on the rachises throughout the season. These findings indicate that grape cultivars may differ in resistance of their bunch parts to natural *B. cinerea* infection. 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; Coertze & Holz, 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; Vercesi *et al.*; 1997]) 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.

Working with natural infection, Holz *et al.* (2000) showed that in the Western Cape province, *B. cinerea* occurs more regularly in table grape bunches during the early part of the season, than later in the season. This suggests that in table grape vineyards, inoculum of the pathogen is more abundant from bloom to bunch closure than from véraison to harvest. The *B. cinerea* incidence data found in the wine grape cultivars Chardonnay, Chenin blanc and Shiraz indicate that the pathogen follows a similar pattern in wine grape vineyards. This phenomenon explains why grey mould develops mostly from the inner bunch (Nair, 1985; Nair & Hill, 1992; Nair & Parker, 1985) and why disease management strategies in both table and wine grape vineyards should concentrate on the pre-bunch closure stage and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season.

The motivation for studying the behaviour of a pathogen is that we can control its development in a vineyard. My studies, together with those of others, stress the importance of bunch architecture as well as infections in the structural bunch parts. It also stresses the low levels of inoculum in vineyards and the strong natural defense system of the grape plant. From this it is clear that control methods, as well as any further studies, must focus on the structural bunch parts and more so during the earlier phenological stages of the grapevine. Giving the host plant a healthy, balanced and microbial rich soil to live in will enhance the natural cycling of nutrients in the soil, which in turn will enhance the nutritional status of the plant. This will reduce stress and put the plant in a position to produce all its cell structures and natural defense systems properly. Lastly, managing the bunch architecture so that it is less compact, may inhibit natural wounding caused by stress factors.

LITERATURE

Agulhon,R. 1971. Le triarimol dans la lutte contre l'oidium de la vigne *Uncinula necator*. *Phytiatrie-Phytopharmacie Revue Francaise de Medecine et de Pharmacie des Vegetaux* 20: 117-124.

Broome, J.C., English, J.T., Marois, J.J., Latorre, B.A. & Aviles, J.C. 1995. Development of an infection model for Botrytis bunch rot of grapes based on wetness duration and temperature. *Phytopathology* 85: 97-102.

Baur, J.R., Bovey, R.W., Baur, P.S. & El-Seify, Z. 1969. Effects of paraquat on the ultrastructure of mesquite mesophyll cells. *Weed Research* 9: 81-85.

Cerkauskas, R.F. & Sinclair, J.B. 1980. Use of paraquat to aid the detection of fungi in soybean tissues. *Phytopathology* 70: 1039-1038.

Coertze, S. & Holz, G. 1999. Surface colonization, penetration and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea*. *Plant Disease* 83: 917-924.

Coertze, S., and Holz, G. 2002. Epidemiology of *Botrytis cinerea* on grape: wound infection by dry, airborne conidia. *South African Journal of Enology and Viticulture* 23. In press.

Coertze, S., Holz, G and Sadie, A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*. *Plant Disease* 85: 668-677.

Du Plessis, S.J. 1937. Botrytis rot of grapes and its control during 1933-1934. *Farming South Africa* 9: 395-397, 439-442.

Duncan, R.A., Stapleton, J.J. & Leavitt, G.M. 1995. Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as influenced by leaf removal. *Plant Pathology* 44: 956-965.

Gessler, A. & Jermini, M. 1985. Role of flower infection of grape by *Botrytis cinerea* and consequences for the spraying schedule. Quaderni Della Scuola Di Specializzazione *In Vitiolture Ed Enologia, Università di Torino* 9: 245-250.

Grindrat, D. & Pezet, R. 1994. Le Paraquat, un Outil pour la Révélation Rapide d'Infections Fongiques Latentes et de Champignons Endophytes. *Journal of Phytopathology* 141: 86-89.

Gubler, W.D., Marois, J.J., Blesoe, A.M. & Bettiga, L.J. 1987. Control of Botrytis bunch rot of grape with canopy management. *Plant Disease* 781: 599-601.

Gütschow, M. 2001. Resistance to *Botrytis cinerea* in parts of leaves and bunches of grapevines. M.Sc. dissertation, University of Stellenbosch, Stellenbosch. 87pp.

Gütschow, M., & Holz, G. 2000. Resistance to *Botrytis cinerea* in morphological parts of a selected table and wine grape cultivar. (Abstr.). XIIth Int. *Botrytis* Symp. Reims, France, P46.

Hill, G. 1985. Suberization of cell walls: a defense reaction of grapes stem tissue against invading mycelium of *Botrytis cinerea*. Quarderni Della Scuola Di Specializzazione *In Viticoltura Ed Enologia, Università di Torino* 9: 229-230.

Hill, G.K., Stellwaag-Kittler, F., Huth, G. & Schlösser, E. 1981. Resistance of grapes in different development stages to *Botrytis cinerea*. *Phytopatologische Zeitschrift* 102: 329-338.

Holz, G., Coertze, S. & Basson, E.J. 1997. Latent infection of *Botrytis cinerea* in grape pedicel leads to post harvest decay. Annual Meeting of the American Phytopathological Society, Rochester, USA. *Phytopathology* 87 (supplement): S88.

Holz, G., Coertze, S. & Basson, E.J. 1998. Infection pathways and latency of *Botrytis cinerea* in grape bunches. Abstract. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 3: 6.22.

Holz, G., Gütschow, M., Frederiks, C., du Preez, I. F.; Coertze, S. 2000. Infection pathways of *Botrytis cinerea* on grape bunches. (Abstr.). XIIth Int. *Botrytis* Symp. Reims, France, L23.

Hoos, G., & Blaich, R. 1988. Metabolism of stilbene phytoalexins in grapevines: oxidation of resveratrol in single-cell cultures. *Vitis* 27:1-12.

Jarvis, W.R. 1980. Epidemiology. Page 219 in: The Biology of *Botrytis cinerea*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore-trap. *Netherlands Journal of Plant Pathology* 96: 247-250.

Kosuge, T., & Hewitt, W. B. 1964. Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea*. *Phytopathology* 54:167-17.

Langcake, P. 1981. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. *Physiological Plant Pathology* 18:213-226.

Marois, J.J., Nelson, J.K., Morrison, J.C., Lile, L.S. & Bledsoe, A.M. 1986. The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. *American Journal of Enology and Viticulture* 37: 293-296.

McClellan, W.D. & Hewitt, B. 1973. Early Botrytis rot of grapes: Times of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* 63: 1151-1157.

Nair, N. G. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. *Australian Journal of Agricultural Research* 36:435-442.

Nair, N.G. & Allen, R.N. 1993. Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. *Mycological Research* 97: 1012-1014.

Nair, N.G., Emmet, R.W. & Parker, F.E. 1988. Some factors predisposing grape berries to infection by *Botrytis cinerea*. *New Zealand Journal of Experimental Agriculture* 16: 257-263.

Nair, N.G. & Hill, G.K. 1992. Bunch rot of grapes caused by *Botrytis cinerea*. Pages 147-169 in: Plant Disease of International Importance. Vol III: Disease of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

Nair, N.G. & Nadtotchei, A. 1987. Sclerotia of *Botrytis cinerea* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. *Journal of Phytopathology* 119: 42-51.

Nair, N.G. & Parker, F.E. 1985. Midseason bunch rot of grapes: An unusual disease phenomenon in the Hunter valley, Australia. *Plant Pathology* 34: 302-305.

Nelson, K.E. 1951. Effect of humidity on infection of table grapes by *Botrytis cinerea*. *Phytopathology* 41: 859-864.

Northover, J. 1987. Infection sites and fungicidal prevention of *Botrytis cinerea* bunch rot of grapes in Ontario. *Canadian Journal of Plant Pathology* 9: 129-136.

Padgett, M., & Morrison, J. C. 1990. Changes in the grape berry exudates during fruit development and their effect on the mycelial growth of *Botrytis cinerea*. *Journal of the American Society of Horticultural Science.* 115:269-273.

Percival, D.C., Sullivan, J.A. & Fisher, K.H. 1993. Effects of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot. (*Botrytis cinerea* Pers.: Fr.) with 3 *Vitis vinifera* L. cultivars. *Vitis* 32: 87- 97.

Pezet, R., & Pont, V. 1984. *Botrytis cinerea*: Activité antifongique dans les jeunes grappes de *Vitis vinifera*, Varieté Gamay. *Phytopathologische Zeitschrift* 111:73-81.

Pezet, R. & Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans le grappes de Vitis vinifera (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18: 317-322.

Pscheidt, J.W. & Pearson, R.C. 1989. Time of infection and control of Phomopsis fruit rot of grapes. *Plant Disease* 73: 829-833.

Sarig, P., Zutkhi, Y., Monjauze, A., Lisker, N. & Ben-Arie, R. 1997. Phytoalexin elicitation in grape berries and their susceptibility to *Rhizopus stolonifer*. *Physiological and Molecular Plant Pathology* 50: 337-347.

SAS, 1999. SAS/STAT User's Guide, Version 8, 1st printing, Volume 2. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513.

Savage, S.D. & Sall, M.A. 1983. Botrytis bunch rot of grapes: the influence of selected cultural practices on infection under California conditions. *Plant Disease* 67: 771-774.

Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. 7th Ed. Iowa State University Press, Ames.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52: 591-611.

Vail, M.E. & Marois, J.J. 1991. Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. *Phytopathology* 81: 188-191.

Vercesi, A., Locci, R. & Prosser, J. I. 1997. Growth kinetics of *Botrytis cinerea* on organic acids and sugars in relation to colonization of grape berries. *Mycological Research* 101:139-142.

Table 1. Analysis of variance for effects of cultivar, treatment and position on *Botrytis cinerea* incidence (logit transformed) in grape bunches incubated in moist chambers

Source of variation	Df	Mean square	P > f
Cultivar (C)	2	25.866	0.0019
Error	3	0.267	.
Treatment (T)	1	52.209	0.0001
C * T	2	0.438	0.5778
Position (P)	5	227.988	0.0001
C * P	10	2.985	0.0018
T * P	5	5.064	0.0003
C * T * P	10	0.208	0.9847
Error	33	0.786	.
Growth stage (S)	4	13.457	0.0001
C * S	8	0.952	0.1295
S * T	4	3.555	0.0002
C * S * T	8	1.168	0.0550
S * P	20	1.710	0.0001
C * S * P	40	0.226	0.9997
S * T * P	20	0.515	0.6307
C * S * T * P	40	0.437	0.8720
Error	144	0.595	.
Corrected total	359	4.374	.

Table 2. Mean incidence[v] of *Botrytis cinerea* (logit transformed) recorded[w] at different positions[x] in grape bunches that were treated or not treated with paraquat and kept in moist chambers

Position	Paraquat	Control
Bunch		
Laterals	-4.37c[y] (1.77[z])	-5.10de (0.11)
Pedicel	-4.80d (1.73)	-6.35f (0.34)
Rachis	-1.81a (16.67)	-2.70b (4.33)
Berry		
Pedicel end	-5.39e (0.79)	-6.59f (0.24)
Cheek	-7.54g (0.00)	-7.55g (0.00)
Style end	-7.25g (0.08)	-7.47g (0.01)

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches at pea size, bunch closure and harvest stages.

[w] Bunches were kept in translucent plastic containers lined with a wet paper towel to establish high relative humidity (≥93% RH). The bunches 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.

[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.

[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$

[z] Values in parenthesis are the means of the logit transformed percentages.

Table 3. Mean incidence[v] of *Botrytis cinerea* (logit transformed) recorded[w] in grape bunches that were treated and not treated with paraquat and kept in moist chambers

Treatment	Flower	Pea Size	Bunch Closure	Véraison	Harvest
Paraquat	-4.45a[x] (7.11[y])	-4.85ab (5.68)	-5.12bc (3.67)	-6.04de (0.09)	-5.53cd (0.98)
Control	-5.59cd (3.37)	-5.98de (0.35)	-6.09de (0.07)	-6.39e (0.003)	-5.75d (0.40)

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches at flower, pea size, bunch closure, véraison and harvest stages.

[w] Bunches were kept in translucent plastic containers lined with a wet paper towel to establish high relative humidity (≥93% RH). The bunches 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.

[x] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$.

[y] Values in parenthesis are the means of the logit transformed percentages.

Table 4. Mean incidence[v] (logit transformed) of *Botrytis cinerea* recorded[w] at different positions[x] in grape bunches kept in moist chambers

Position	Flower	Pea Size	Bunch closure	Veraison	Harvest
Bunch					
Laterals	-4.28e[y] (1.88[z])	-4.42ef (0.21)	-4.78efgh (0.69)	-5.20ghij (0.00)	-5.00fghi (0.26)
Pedicel	-4.61efg (1.91)	-5.44ijk (1.28)	-5.52ijk (0.93)	-6.93lm (0.13)	-5.37hijk (0.94)
Rachis	-1.17a (26.67)	-1.97b (14.17)	-2.34bc (9.17)	-3.04d (0.00)	-2.74cd (2.50)
Berry					
Pedicel end	-5.62jk (0.77)	-5.60jk (0.79)	-5.90k (0.45)	-6.98lmn (0.16)	-5.83k (0.43)
Cheek	-7.68o (0.00)	-7.52no (0.00)	-7.54no (0.00)	-7.56no (0.00)	-7.44mno (0.00)
Style end	-6.76l (0.21)	-7.52no (0.00)	-7.54no (0.00)	-7.56no (0.00)	-7.44mno (0.00)

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches.

[w] Bunches were kept in translucent plastic containers lined with a wet paper towel to establish high relative humidity (≥93% RH). The bunches 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.

[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.

[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$.

[z] Values in parenthesis are the means of the logit transformed percentages.

Table 5. Mean incidence[w] of *Botrytis cinerea* (logit transformed) recorded[x] in grape bunches kept in moist chambers

Cultivar	Means (%)
Chardonnay	-5.29a[y] (2.66[z])
Chenin Blanc	-5.32a (2.57)
Shiraz	-6.11b (1.30)

[w] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches at flower, pea size, bunch closure, véraison and harvest stages.

[x] Bunches were kept in translucent plastic containers lined with a wet paper towel to establish high relative humidity (≥93% RH). The bunches 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.

[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$

[z] Values in parenthesis are the means of the logit transformed percentages.

Table 6. Mean incidence[v] of *Botrytis cinerea* (logit transformed) recorded[w] at different positions[x] in grape bunches kept in moist chambers

Position	Chardonnay	Chenin Blanc	Shiraz
Bunch			
Laterals	-4.42b[y] (1.33[z])	-4.76bc (1.24)	-5.03cd (0.25)
Pedicel	-5.13cde (1.00)	-4.88bc (1.77)	-6.72f (0.35)
Rachis	-2.03a (13.00)	-2.28a (11.50)	-2.45a (7.00)
Berry			
Pedicel end	-5.65ef (0.59)	-5.35de (0.81)	-6.97f (0.15)
Cheek	-7.42gh (0.00)	-7.44gh (0.00)	-7.78h (0.00)
Style end	-7.31gh (0.03)	-7.05fg (0.09)	-7.73h (0.003)

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches at pea size, bunch closure and harvest stages.

[w] Bunches were kept in translucent plastic containers lined with a wet paper towel to establish high relative humidity (≥93% RH). The bunches 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.

[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.

[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$.

[z] Values in parenthesis are the means of the logit transformed percentages.

Table 7. Analysis of variance of data for the effect of season, growth stage, sterilisation regime and position on the percentage (logit transformed) bunch parts isolated from different positions in grape bunches that yielded *Botrytis cinerea*

Source of variation	Degrees of freedom	Chardonnay		Chenin Blanc		Shiraz	
		Mean square	Significance level	Mean square	Significance level	Mean square	Significance level
Season	1	2.617	0.2169	64.149	0.0044	21.066	0.0015
Treatment (T)	1	0.523	0.5358	16.920	0.0282	0.940	0.0958
Position (P)	4	17.704	0.0222	17.397	0.0223	13.000	0.0022
T*P	4	0.772	0.6334	1.238	0.4688	0.068	0.7918
Error	3	1.077	.	1.063	.	0.163	
Growth Stage (G)	4	0.318	0.5356	0.866	0.4388	0.799	0.1299
G*T	4	2.651	0.0022	0.561	0.6385	0.687	0.1791
G*P	15	0.790	0.0875	1.099	0.3240	0.321	0.6304
G*T*P	15	0.279	0.7410	0.216	0.9950	0.272	0.7419
Error	16	0.391	.	0.871	.	.	.

Table 8. Means[v] (logit transformed) of the effect of the interaction sterilisation regime x position on the percentage bunch parts isolated[w] from grape bunches that yielded *Botrytis cinerea* on Kerssies' medium

Position	Untreated	Surface sterilised
Bunch		
Rachis	0.22 a[y] (56.00[z])	-1.47abc (16.00)
Lateral	-0.57ab (38.00)	-2.22bcd (10.00)
Pedicel	-2.80cde (8.00)	-3.94de (1.80)
Berry		
Pedicel end	-4.20de (1.00)	-4.34e (0.50)
Cheek	-3.97de (1.60)	-4.50e (0.20)

[v] Average values of data recorded over two seasons in material of Chardonnay, Chenin Blanc and Shiraz inoculated at pea size, bunch closure and harvest.

[w] Sections isolated from different positions in bunches were incubated for 14 days at 22°C. The bunches presence of *B. cinerea* was positively identified by the development and formation of sporulating colonies of the pathogen.

[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.

[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$

[z] Values in parenthesis are the means of the logit transformed percentages.

Table 9. Means[v] (logit transformed) of the effect of the interaction cultivar x position on the percentage bunch parts isolated[w] from grape bunches that yielded *Botrytis cinerea* on Kerssies' medium

Position[x]	Chardonnay	Chenin Blanc	Shiraz
Bunch			
Rachis	-1.53 a[y] (16.00[z])	-0.62a (36.00)	-1.73a (12.00)
Lateral	-2.57ab (4.00)	-1.40a (24.00)	-2.82b
Pedicel	-3.60bc (3.50)	-3.37b (4.90)	-4.26c (0.80)
Berry			
Pedicel end	-3.53bc (3.00)	-4.27b (0.75)	-4.62c (0.00)
Cheek	-4.51c (0.30)	-4.24b (0.90)	-4.53c (0.20)

[v] Average values of data recorded over two seasons in bunches at pea size, bunch closure and harvest stages.

[w] Sections isolated from different positions in bunches were incubated for 14 days at 22°C. The bunches presence of *B. cinerea* was positively identified by the development and formation of sporulating colonies of the pathogen.

[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.

[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$

[z] Values in parenthesis are the means of the logit transformed percentages.

Table 10. Means[v] (logit transformed) of the effect of the interaction growth stage x position on the percentage bunch parts isolated[w] from Chenin Blanc grape bunches that yielded *Botrytis cinerea* on Kerssies' medium

Position	Flower	Pea Size	Bunch Closure	Veraison	Harvest
Bunch					
Rachis	0.00a[y] (50.00[z])	0.00a (50.00)	-1.37abcde (20.00)	-1.37abcde (20.00)	-0.38abc (40.00)
Lateral	-0.80abcd (30.00)	0.23ab (55.00)	-1.52abcdef (25.00)	-2.45abcdefg (5.00)	-2.45abcdefg (5.00)
Pedicel	-3.66efg (3.50)	-3.30defg (6.50)	6.00 (-3.74) efg	-3.13cdefg (4.50)	-3.01bcdefg (4.00)
Berry					
Pedicel end	-4.62g (0.00)	-4.62g (0.00)	-4.62g (0.00)	-3.23cde (3.00)	na
Cheek	-4.11efg (1.50)	-3.92efg (1.50)	-4.20efg (1.00)	-4.34fg (0.50)	-4.62g (0.00)

[v] Average values of data recorded over two seasons in Chenin Blanc bunches.
[w] Sections isolated from different positions in bunches were incubated for 14 days at 22°C. The bunches presence of *B. cinerea* was positively identified by the development and formation of sporulating colonies of the pathogen.
[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.
[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$
[z] Values in parenthesis are the means of the logit transformed percentages.

Table 11. Means[v] (logit transformed) of the effect of the interaction growth stage x position on the percentage bunch parts isolated[w] from Chardonnay grape bunches that yielded *Botrytis cinerea* on Kerssies' medium

Position[x]	Flower	Pea Size	Bunch Closure	Veraison	Harvest
Bunch					
Rachis	-1.75ab[y] (10.00[z])	-0.72a (30.00)	-1.75ab (10.00)	-2.40bcd (0.00)	-1.03a (30.00)
Lateral	-1.85abc (10.00)	-2.45bcd (5.00)	-3.04bcdef (0.00)	-2.45bcd (5.00)	-3.04bcdef (0.00)
Pedicel	-3.83efg (2.00)	-4.05fg (2.00)	-3.12cdef (5.50)	-3.70defg (2.50)	-3.32def (5.50)
Berry					
Pedicel end	-4.62g (0.00)	-3.79efg (2.00)	-3.05bcdef (4.00)	-2.65bcde (6.00)	na
Cheek	-4.62g (0.00)	-4.62g (0.00)	-4.62g (0.00)	-4.62g (0.00)	-4.11g (1.50)

[v] Average values of data recorded over two seasons in Chardonnay bunches.
[w] Sections isolated from different positions in bunches were incubated for 14 days at 22°C. The bunches presence of *B. cinerea* was positively identified by the development and formation of sporulating colonies of the pathogen.
[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.
[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$
[z] Values in parenthesis are the means of the logit transformed percentages.

Table 12. Means[v] (logit transformed) of the effect of the interaction growth stage x position on the percentage bunch parts isolated[w] from Shiraz grape bunches that yielded *Botrytis cinerea* on Kerssies' medium

Position	Flower	Pea Size	Bunch Closure	Veraison	Harvest
Bunch					
Rachis	-1.37 ab[y] (20.00[z])	-1.75ab (10.00)	-2.40bc (0.00)	-2.40bc (0.00)	-0.72a (30.00)
Lateral	-1.90cd (15.00)	-3.04e (0.00)	-3.04cd (0.00)	-3.04cd (0.00)	-1.90b (15.00)
Pedicel	-4.62e (0.00)	-4.34e (0.50)	-3.83de (2.00)	-4.34e (0.50)	-4.20e (1.00)
Berry					
Pedicel end	-3.04e (0.00)	-4.62e (0.00)	-4.62e (0.00)	-4.62e (0.00)	na
Cheek	-4.62e (0.00)	-4.62e (0.00)	-4.62e (0.00)	-4.62e (0.00)	-4.20e (1.00)

[v] Average values of data recorded over two seasons in Shiraz bunches.
[w] Sections isolated from different positions in bunches were incubated for 14 days at 22°C. The bunches presence of *B. cinerea* was positively identified by the development and formation of sporulating colonies of the pathogen.
[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.
[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$
[z] Values in parenthesis are the means of the logit transformed percentages.

3. OCCURRENCE OF *BOTRYTIS CINEREA* AND SUBSEQUENT DISEASE EXPRESSION AT DIFFERENT POSITIONS IN BUNCHES OF THREE WINE GRAPE CULTIVARS INOCULATED WITH DRY, AIRBORNE CONIDIA

ABSTRACT

Bunches of wine grapes (cultivars Chenin Blanc, Chardonnay and Shiraz) at pea size, bunch closure, and harvest were dusted with dry conidia of *Botrytis cinerea* in a settling tower and incubated for 24 h at high relative humidity (±93%). Following incubation, the bunches were divided in two groups. The one group was surface-sterilised in 70% ethanol for 5 s, the other group was left untreated. Bunches of the sterile group, and from the untreated group were used for isolation. Rachis segments, laterals, pedicels and berry skin segments (from the pedicel-end, cheek and style-end) were removed from each bunch. The sections were placed in Petri dishes on Kerssies' *B. cinerea* selective medium and on a water agar medium supplemented with paraquat, and incubated at 22°C under diurnal light. Occupation by the pathogen was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues. The pathogen resided more often on the structural bunch parts than on the berries. Overall, the isolation studies revealed that conidia occurred predominantly on the rachis. The incidence of *B. cinerea* was furthermore constantly high in the inner bunch after each inoculation, and in bunches of different maturities. The data therefore indicated that, when available, conidia penetrated loose and tight clustered bunches in a similar way.

INTRODUCTION

Botrytis cinerea Pers.:Fr., a pathogen of grape (*Vitis vinifera* L.), can attack most of the plant's organs (Gütschow, 2001). The most prominent symptom is found on the berries (Nair & Nadtotchei, 1987). Different infection pathways have been described for infection of grape berries by *B. cinerea*, namely natural openings (Pucheu-Planté & Mercier, 1983), style ends (McClellan & Hewitt, 1973; Nair & Parker, 1985), wounds (Nair et al., 1988), direct penetration of the cuticle (Nelson, 1956) or through the pedicel (Gütschow, 2001; Holz *et al.*,

1997, 1998; Pezet & Pont, 1986). Laboratory studies with dry, airborne conidia, and estimations of the amount of viable *B. cinerea* occurring at different positions in grape bunches give perspective to the relative importance of these pathways in the development of *B. cinerea* epiphytotics. Working with single, airborne conidia of the pathogen on Dauphine table grapes, Coertze and co-workers (Coertze & Holz, 1999; Coertze *et al.*, 2001) proved that skins of fresh ripe berries, and berries at other growth stages, provided an effective barrier to penetration by this mode of infection. In the event of wounding, a combination of fresh wounds, freshly dispersed conidia and free water on the berry surface is necessary for successful wound infection (Coertze & Holz, 2002). Working with natural infection, Holz (1999) showed that the pathogen seldom occurs on the surface or in the skin tissue the berry. The stylar end of berries was furthermore found to be virtually free of naturally occurring *B. cinerea*. On the majority of berries that yielded *B. cinerea*, the pathogen developed first at the berry base before it spread into the central portion of the cheek. The data of Holz (1999) also showed that the incidence of *B. cinerea* was higher in bunches during the early part of the season, than later in the season. These findings suggest that the importance of *B. cinerea* occurring superficially at the berry base, and probably at other positions in the bunch, is underestimated in the epidemiology of *B. cinerea*, and the development of epiphytotics in grapevine. Furthermore, in the Western Cape province, inoculum is dispersed more regularly in bunches from bloom to bunch closure than from véraison to harvest.

A recent study (Part 2) on the occurrence of *B. cinerea* at different positions in bunches of the wine grape cultivars Chardonnay, Chenin blanc and Shiraz, confirmed the general pattern of inoculum ecology and subsequent disease expression on table grapes described by Holz and co-workers (Coertze & Holz, 1999; Coertze *et al.*, 2001; Coertze & Holz, 2002; Gütschow, 2001; Holz *et al.*, 1997, 1998). However, it was found that in the wine grape bunches, *B. cinerea* predominantly occupied the rachis. Furthermore, the pathogen consistently occurred at relatively high levels on the rachises throughout the season. These findings indicate that grape cultivars may differ in resistance of their bunch parts to natural *B. cinerea* infection. *B. cinerea* conidia and germlings may 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; Coertze *et al.*, 2001; Coertze & Holz, 2002; 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; Vercesi *et al.*; 1997]) 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 aim of this investigation was to study the occurrence of *B. cinerea* and disease expression at different positions in bunches of three wine grape cultivars differing in bunch structure under conditions simulating natural infection by dry, airborne conidia.

MATERIALS AND METHODS

Grapes. The cultivars used were Chenin Blanc, Chardonnay and Shiraz. Shiraz is considered to have a fairly lose bunch architecture whereas Chenin Blanc and Chardonnay are considered to have a more dense berry arrangement. Bunches were obtained from vineyards at the farm Jakkalsfontein, in the Malmesbury district, situated approximately 100 km north east of Cape Town. The region has a moderate, dry Mediterranean climate, and has a history of low incidence of *B. cinerea*. No fungicide sprays for the control of *B. cinerea* were applied in the vineyard during the 1999 – 2001 period. Shoots with sound, unblemished bunches, each from a different arbitrarily chosen vine, were obtained at pea size, bunch closure (approximately 6 °Brix), and at harvest (approximately 20 °Brix). At pea size stage, shoots with clusters were obtained from the vineyard, 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 clusters and two to three leaves. To maintain tugidity, the shoots were then inserted into sterile aluminium foil-wrapped "oases" (florist's sponge), which was soaked with a 20% sucrose solution. Due to the detrimental effect of ethanol and the fact that the clusters do not dry properly (G. Holz, unpublished data), surface sterilisation was not done at pea size stage. At the other stages, bunches were obtained, surface-sterilised (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried to prevent natural infection by surface inocula (Sarig *et al.*, 1996; Coertze & Holz, 1999; Coertze *et al.*, 2001). To maintain turgidity, the peduncles of bunches were inserted into sterile aluminium foil-wrapped "oases" (florist's sponge) soaked with a 20% sucrose solution. The oases with shoots or bunches were placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm).

Inoculation and incubation. A virulent isolate of *B. cinerea* 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 from 14-day-old cultures were harvested with a suction-type collector and stored at 5°C until use. Storage time did not affect germination, the dry conidia could therefore be used in all experiments (Spotts & Holz, 1996). 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 or bunches that were positioned on two screens. 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 post inoculation (hpi) at 22°C (100 conidia per Petri dish, three replicates). Germination percentage varied between 92 and 99%. After inoculation, the screens were placed in 12 ethanol-disinfected perspex chambers lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). Each chamber contained three screens containing eight bunches. Each chamber was considered as a replicate. 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 grape bunches, namely dry conidia on dry bunch parts under high relative humidity. Studies (Coertze & Holz, 1999, Coertze *et al.*, 2001; Gütschow, 2001) with dry conidia of *B. cinerea* under similar conditions showed that germination, surface colonisation and skin penetration reached a maximum during this period.

Detection of *B. cinerea*. Following incubation, the bunches were divided in two groups of 10 bunches each. The one group was surface-sterilised in 70% ethanol for 5 s, the other group was left untreated. Bunches of the sterile group, and from the untreated group were used for isolation. From each bunch 10 rachis segments (25 mm), 20 laterals (20 mm), 40 pedicels and 120 berry skin segments (5 x 7 mm) (40 each from the pedicel-end, cheek and style-end) were removed. Five rachis, 10 laterals and 20 each of the pedicels and berry skin segments were placed in Petri dishes on Kerssies' *B. cinerea* selective medium (Kerssies, 1990), and the remaining segments were placed on a water agar medium supplemented with paraquat (Grindrat & Pezet, 1994). The plates were incubated at 22°C

under diurnal light and the segments were monitored daily for symptom expression and the development of *B. cinerea*. After 14 days the number of segments yielding sporulating *B. cinerea* colonies were recorded, and the incidence of *B. cinerea* at each position in the bunch calculated.

Statistical analysis. A complete randomised design experiment was performed and replicated over 2 seasons. The experiment design was a 2x2x3x4 factorial with factors: treatments (sterile, non-sterile); media (paraquat, kerssies); stages (pea size, bunch closure, harvest) and pathways (cheek, laterals, pedicel, rachis, style, pedicel-end). The experiment was conducted with three cultivars (Chardonnay, Chenin Blanc, Shiraz). An experimental unit was between 5-50 isolations. The data were transformed to a logit as well as percentage incidence and subjected to a factorial analysis of variance for each cultivar separately, using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-LSD (Least significant difference) was calculated at a 5% significance level to compare treatment means (Snedecor & Cochran, 1980).

RESULTS

Detection of *B. cinerea*. Analysis of variance for effects of season, cultivar, treatment, pathways, growth media, and phenological stages is given in Table 1. There was a significant difference between seasons (P = 0.0001); sterilisation treatment (P = 0.0001); positions in bunches (P = 0.0001); media (P = 0.0017) and grape phenological stages (P = 0.0001). No significant difference occurred between the cultivars (P = 0.9034). Significant interactions were found between sterilisation treatment and bunch position (P = 0.0053); sterilisation treatment and phenological stage (P = 0.0027) and phenological stage and bunch position (P = 0.0059). The mean incidence of *B. cinerea* was high at positions in the structural bunch parts, and low at the positions on berries (Table 2). The sterilisation test (Table 2) showed that, in the case of the structural parts, the mean incidence of *B. cinerea* was significant higher in the rachis than the lateral and pedicel. For berries, the mean incidence of *B. cinerea* was significant higher at the cheek than at the other two positions. For the cheek, the mean incidence of *B. cinerea* was also significant higher on untreated than on surface sterilised berries. The significant difference was not found for the pedicel end and the stylar end. The mean incidence of *B. cinerea* detected in untreated bunches increased

significantly with bunch development, and was very high at harvest (Table 3). The mean incidence of *B. cinerea* detected in the structural bunch parts remained higher than the mean incidence in the berry parts, during pea size and bunch closure (Table 4). It was only at harvest, that the mean incidence of *B. cinerea* in the cheek part was similar to that of the lateral and pedicel parts. The mean incidence of *B.* cinerea detected in the rachis, still remained higher than the other parts, during harvest (Table 4).

DISCUSSION

In this study, natural *B. cinerea* infection of grapes of different phenological stages were simulated in the laboratory by dispersing dry, airborne conidia in grape bunches and by exposing them to conditions favouring infection and disease expression. The conidia penetrated loose and tight clustered bunches in a similar way and resided more often on the structural bunch parts than on the berries. Overall, the isolation studies revealed that conidia occurred predominantly on the rachis. Approximately 65% of the rachises yielded *B. cinerea.* The next prominent position occupied was the laterals and pedicels, of which approximately 48% and 36% respectively yielded *B. cinerea.* The pathogen occupied the berry cheek less often (18%), and infrequently (2%) the pedicel end of the berry. The stylar end of the berries, on the other hand, was free of the pathogen. These findings showed some similarities in the pattern of *B. cinerea* occurrence in bunches dusted with dry, airborne conidia, and in those carrying natural inoculum (Holz *et al.,* 2000). For example, in both artificially and naturally inoculated bunches, the incidence of *B. cinerea* was significantly higher on the structural bunch parts than on berries. Furthermore, in both inoculations, the stylar end of the berry was virtually free of the pathogen.

Working with natural infection, Holz *et al* (2000) showed that in the Western Cape province, *B. cinerea* occured more regularly in grape bunches during the early part of the season, than later in the season. This suggests that in this region, inoculum of the pathogen is more abundant from bloom to bunch closure than from véraison to harvest. This phenomenon is confirmed by the *B. cinerea* incidence data found in bunches dusted with dry, airborne conidia. In the latter instance, the incidence of *B. cinerea* was constantly high in the inner bunch after each inoculation, and in bunches of different maturities. This finding indicated that, when available, conidia penetrated loose and tight clustered bunches in a similar way. The *B. cinerea* occupation pattern explains why grey mould develops mostly

from the inner bunch (Nair, 1985; Nair & Hill, 1992; Nair & Parker, 1985) and why disease management strategies should concentrate on the pre-bunch closure stage and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season.

My findings on the reaction of grape berry cheeks to *B. cinerea* substantiated those of other workers (Bulit & Verdu, 1973; Cerkauskas & Sinclair, 1980; Chardonnet *et al.*, 1997; Grindrat & Pezet, 1994; Gütschow, 2001; Gütschow & Holz, 2000; Hewitt, 1974; Hill, 1985) on the resistance of berry cheeks to *B. cinerea* infection and to disease expression by airborne conidia. The finding that the amount of *B. cinerea* was consistently low on grape berry skins is also in accordance with the hypothesis that *B. cinerea* conidia are deposited as single cells on berry surfaces (Coertze & Holz, 1999, Coertze *et al.*, 2001; Gütschow, 2001; Gütschow & Holz, 2000; Holz, 1999; Holz *et al.*, 2000), and with the phenomenon that single conidia of the pathogen do not survive for extended periods on berry surfaces (Coertze & Holz, 2002; Coertze *et al.*, 2001; Gütschow, 2001). In most studies where grapes were artificially inoculated, mature berries were atomised with (De Kock & Holz, 1991; Nair, 1985; Nair *et al.*, 1988; Nelson, 1951), dipped in (Broome *et al.*, 1995), or injected with (Avissar & Pesis, 1991; Marois *et al.*, 1986; Thomas *et al.*, 1988) conidial suspensions, or suspension droplets were placed onto the berry cheek (Chardonnet *et al.*, 1997; Marois *et al.*, 1987; Marois *et al.*, 1986). These methods allowed for the deposition of clusters of conidia, and may differ from primary natural inoculation in the vineyard, where single conidia may be deposited simultaneously at several sites on the berry surface.

Pezet and Pont (1986) showed in their histological studies of laboratory-inoculated bunches that *B. cinerea* colonises the stamens during bloom and invades their base situated on the receptacle. From there it spreads to the pedicel, and later via the vascular tissue into the berries. Latent infection was therefore predominantly pedicel-associated. Careful observation of naturally infected bunches (Holz *et al.*, 2000) showed that in the case of berry rot, the pathogen first developed in the receptacle part of the pedicel and then spread into the pedicel-end of the berry. In the present study, which was conducted in tandem with the investigation on natural infection (Part 2), bunches were first inoculated at pea size when the filaments were already shed. In these vineyards climatic conditions were generally not conducive to *B. cinerea* infection from bloom to pea size stage. The region where the bunches were obtained, is furthermore known to have history of low incidence of *B. cinerea*. The fact that the incidence of *B. cinerea* was generally low in the berry-pedicel attachment

zone of bunches dusted with dry conidia, can be ascribed to the role that infected filaments play in the infection pathway of *B. cinerea* in the field, and the natural establishment of the pathogen in pedicel tissue. The findings on the behaviour of airborne conidia in artificially inoculated bunches, and in naturally infected bunches, give credit to the pedicel infection pathway originally described by Pezet and Pont (1986), and confirmed later by other workers (Holz *et al.*, 1997, 1998; Holz, 1999). It therefore emphasises the crucial role of flower infection in the epidemiology of *B. cinerea* on grapevine.

LITERATURE

Avissar, I. & Pesis, E. 1991. The control of post harvest decay in table grapes using acetaldehyde vapours. *Annals of Applied Biology* 118: 229-237.

Bessis, R. 1972. Etude en microscopie èlectronique à balayage des rapports entre l'hôte et la parasite dans le cas de la Pourriture grise. *Comptes Rendus Academie Sciences (Paris)* 274: 2991-2994.

Broome, J.C., English, J.T., Marois, J.J., Latorre, B.A. & Aviles, J.C. 1995. Development of an infection model for *Botrytis* bunch rot of grapes based on wetness duration and temperature. *Phytopathology* 85: 97-102.

Bulit, J. & Verdu, D. 1973. Annual variations in the aerial sporing of *Botrytis cinerea* in a vineyard. *Botrytis* meeting, 28/06-1/07, Teresin, Poland.

Cerkauskas, R.F. & Sinclair, J.B. 1980. Use of paraquat to aid the detection of fungi in soybean tissues. *Phytopathology* 70: 1039-1038.

Chardonnet, C., L'Hyvernay, A. & Doneche, B. 1997. Effects of calcium treatment prior to *Botrytis cinerea* infection on the changes in pectic composition of grape berry. *Physiological Molecular Plant Pathology* 50: 213-218.

Coertze, S. & Holz, G. 1999. Surface colonization, penetration and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea*. *Plant Disease* 83: 917-924.

Coertze, S., Holz, G and Sadie, A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*. *Plant Disease* 85: 668-677.

Coertze, S., and Holz, G. 2002. Epidemiology of *Botrytis cinerea* on grape: wound infection by dry, airborne conidia. *South African Journal of Enology and Viticulture* 23. In press.

Creasy, L.L. & Coffee, M. 1988. Phytoalexin production potential of grape berries. *Journal of American Society of Horticultural Science* 113: 230-234.

De Kock, P.J. & Holz, G. 1991. Colonization of table grapes by *Botrytis cinerea* in the Western Cape Province. *Phytophylactica* 23: 73-80.

Du Plessis, S.J. 1937. *Botrytis* rot of grapes and its control during 1933-1934. *Farming South Africa* 9: 395-397, 439-442.

Duncan, R.A., Stapleton, J.J. & Leavitt, G.M. 1995. Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as influenced by leaf removal. *Plant Pathology* 44: 956-965.

Grindrat, D. & Pezet, R. 1994. Le Paraquat, un Outil pour la Révélation Rapide d'Infections Fongiques Latentes et de Champignons Endophytes. *Journal of Phytopathology* 141: 86-89.

Gütschow, M. 2001. Resistance to *Botrytis cinerea* in parts of leaves and bunches of grapevines. M.Sc. dissertation, University of Stellenbosch, Stellenbosch. 87pp.

Gütschow, M., and Holz, G. 2000. Resistance to *Botrytis cinerea* in morphological parts of a selected table and wine grape cultivar. (Abstr.). XIIth International *Botrytis* Symposium Reims, France, P46.

Hewitt, W. B. 1974. Rots and bunch rots of grapes. *Californian Agricultural Experimental Station Bulletin* 868.

Hill, G. 1985. Suberization of cell walls: a defense reaction of grapes stem tissue against invading mycelium of *Botrytis cinerea. Quarderni Della Scuola Di Specializzazione In Viticoltura Ed Enologia, Università di Torino* 9: 229-230.

Hill, G.K., Stellwaag-Kittler, F., Huth, G. & Schlösser, E. 1981. Resistance of grapes in different development stages to *Botrytis cinerea. Phytopatologische Zeitschrift* 102: 329-338.

Holz, G. 1999. Behaviour and infection pathways of diverse fungal pathogens on fruit. (Abstr.). Page 257 in: Conference Handbook. *Biennial Australian Plant Pathology Society Conference, 12th.* Canberra, Australia.

Holz, G., Körte, V. & Coertze, S. 1995. Behaviour of *Botrytis cinerea* on grape, nectarine and plum fruit surfaces under different wetness regimes. Page 56 in: Scientific Programme and Abstracts. *Biennial Australian Plant Pathology Society Conference, 10th.* Lincoln University, New Zealand.

Holz, G., Coertze, S. & Basson, E.J. 1997. Latent infection of *Botrytis cinerea* in grape pedicel leads to post harvest decay. Annual Meeting of the American Phytopathological Society, Rochester, USA. *Phytopathology* 87 (supplement): S88.

Holz, G., Coertze, S. & Basson, E.J. 1998. Infection pathways and latency of *Botrytis cinerea* in grape bunches. Abstract. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 3: 6.22.

Holz, G., Gütschow, M., Frederiks, C., du Preez, I. F., and Coertze, S. 2000. Infection pathways of *Botrytis cinerea* on grape bunches. (Abstr.). XIIth International *Botrytis* Symposium Reims, France, L23.

Hoos, G. & Blaich, R. 1988. Metabolism of stilbene phytoalexins in grapevines: oxidation of resveratrol in single-cell cultures. *Vitis* 27: 1-12.

Jarvis, W.R. 1962b. Splash dispersal of spores of *Botrytis cinerea* Pers. *Nature (London)* 193: 599.

Jarvis, W.R. 1980. Epidemiology. Page 219 in: The Biology of *Botrytis cinerea.* J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore-trap. *Netherlands Journal of Plant Pathology* 96: 247-250.

Kosuge, T. & Hewitt, W.B. 1964. Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea. Phytopathology* 54: 167-172.

Langcake, P. 1981. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. *Physiological Plant Pathology* 18: 213-226.

Marois, J. J., Bledsoe, A. M., Gubler, W. D., and Luvisi, D. A. 1986. Control of *Botrytis cinerea* on grape berries during postharvest storage with reduced levels of sulfur dioxide. *Plant Disease* 70:1050-1052.

Marois, J.J., Bledsoe, A.M., Bostock, R.M. & Gubler, W.D. 1987. Effects of spray adjuvants on development of *Botrytis cinerea* on *Vitis vinifera* berries. *Phytopathology* 77: 1148-1152.

Marois, J. J., Nelson, J. K., Morrison, J. C., Lile, L. S., and Bledsoe, A. M. 1986. The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. *American Journal of Enology and Viticulture* 37:293-296.

McClellan, W.D. & Hewitt, B. 1973. Early *Botrytis* rot of grapes: Times of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* 63: 1151-1157.

Nair, N.G. 1985. Fungi associated with bunch rot of grapes in the Hunter valley. *Australian Journal of Agricultural Research* 36: 435-442.

Nair, N.G., Emmet, R.W. & Parker, F.E. 1988. Some factors predisposing grape berries to infection by *Botrytis cinerea. New Zealand Journal of Experimental Agriculture* 16: 257-263.

Nair, N.G., Guilbaud-Oulton, S., Barchia, I. & Emmet, R.W. 1995. Significance of carry over inoculum, flower infection and latency on the incidence of *Botrytis cinerea* in berries of grapevines at harvest in New South Wales. *Australian Journal of Experimental Agriculture* 35: 1177-1180.

Nair, N.G. & Hill, G.K. 1992. Bunch rot of grapes caused by *Botrytis cinerea.* Pages 147-169 in: Plant Disease of International Importance. Vol III: Disease of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

Nair, N.G. & Nadtotchei, A. 1987. Sclerotia of *Botrytis cinerea* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. *Journal of Phytopathology* 119: 42-51.

Nair, N.G. & Parker, F.E. 1985. Midseason bunch rot of grapes: An unusual disease phenomenon in the Hunter valley, Australia. *Plant Pathology* 34: 302-305.

Nelson, K.E. 1951. Effect of humidity on infection of table grapes by *Botrytis cinerea. Phytopathology* 41: 859-864.

Nelson, K.E. 1956. The effect of *Botrytis* infection on the tissue of Tokay grapes. *Phytopathology* 46: 223-229.

Padgett, M. & Morrison, J.C. 1990. Changes in the grape berry exudates during fruit development and their effects on the mycelial growth of *Botrytis cinerea. Journal of American Society of Horticultural Science* 115: 269-273.

Pezet, R. & Pont, V. 1984. *Botrytis cinerea:* Activité antifongique dans les jeunes grappes de *Vitis vinifera,* Varieté Gamay. *Phytopatologische Zeitschrift 111: 73-81.*

Pezet, R. & Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans le grappes de *Vitis vinifera* (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18: 317-322.

Pucheu-Panté, B. & Mercier, M. 1983. Étude ultrastructurale de l'interrelation hôte-parasite entre le raisin et le champignon *Botrytis cinerea*: exemple de la pourriture noble en Sauternais. *Canadian Journal of Botany* 61: 1785-1797.

Salinas, J., Glandorf, D.C.M., Picavet, F.D. & Verhoeff, K. 1989. Effects of temperature, relative humidity and age of conidia in the incidence of spotting on gerbera flowers caused by *Botrytis cinerea. Netherlands Journal of Plant Pathology* 95: 51-64.

Sarig, P., Zutkhi, Y., Monjauze, A., Lisker, N. & Ben-Arie, R. 1997. Phytoalexin elicitation in grape berries and their susceptibility to *Rhizopus stolonifer. Physiological and Molecular Plant Pathology* 50: 337-347.

Sarig, P., Zahavi, T., Zuthi, Y., Yannai, S., Lisker, N. & Ben-Arie, R. 1996. Ozone for control of post-harvest decay of table grapes caused by *Rhisopus stolonifer. Physiological and Molecular Plant Pathology* 48: 403-415.

SAS, 1999. SAS/STAT User's Guide, Version 8, 1st printing, Volume 2. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513.

Savage, S.D. & Sall, M.A. 1983. *Botrytis* bunch rot of grapes: the influence of selected cultural practices on infection under California conditions. *Plant Disease* 67: 771-774.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika,* 52: 591-611.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical methods. Seventh ed. Ames Iowa: State University Press.

Spotts, R.A., & Holz, G. 1996. Adhesion and removal of conidia of *Botrytis cinerea* and *Penicillium expansum* from the grape and plum fruit surfaces. *Plant Disease* 80: 688-691.

Thomas, C.S., Marois, J.J. & English, J.T. 1988. The effect of wind speed, temperature and relative humidity on the development of aerial mycelium and conidia of *Botrytis cinerea* on grapes. *Phytopathology* 78: 260-265.

Vercesi, A., Locci, R. & Prosser, J.I. 1997. Growth kinetics of *Botrytis cinerea* on organic acids and sugars in relation to colonization of grape berries. *Mycological Research* 101: 139-142.

Table 1. Analysis of variance for effects of season, cultivar, treatment and position on *Botrytis cinerea* incidence (logit transformed) in grape bunches inoculated with dry, airborne conidia of the pathogen

Source of Variation	Df	Mean Square	P>f
Season (S)	1	342.322	0.0001
Cultivar (C)	2	0.203	0.9034
Treatment (T)	1	176.322	0.0001
C*T	2	0.725	0.6981
Position (P)	5	50.019	0.0001
C*P	10	0.692	0.9567
T*P	5	8.921	0.0053
C*T*P	10	0.442	0.9912
Media (M)	1	27.456	0.0017
C*M	2	1.483	0.5526
T*M	1	5.663	0.1364
C*T*M	2	0.234	0.9102
P*M	3	1.666	0.5716
C*P*M	6	0.194	0.9980
T*P*M	3	4.714	0.1406
C*T*P*M	6	0.528	0.9706
Error	23	1.986	.
Stage (S)	2	33.567	0.0001
C*S	4	1.531	0.6505
S*T	2	16.562	0.0027
C*S*T	4	0.836	0.8505
S*P	8	7.808	0.0059
C*S*P	16	0.634	0.9977
S*T*P	8	1.641	0.7194
C*S*T*P	16	0.481	0.9996
S*M	2	1.581	0.5315
C*S*M	4	0.614	0.9089
S*T*M	2	1.772	0.4929
C*S*T*M	4	1.136	0.7644
S*P*M	4	2.284	0.4562
C*S*P*M	8	0.316	0.9977
S*T*P*M	4	0.282	0.9769
C*S*T*P*M	8	0.349	0.9968
Error	48	2.468	.
Corrected Total	227	5.653	.

Table 2. Mean incidence[v] (logit transformed) of *Botrytis cinerea* recorded[w] at different positions[x] in grape bunches inoculated with dry, airborne conidia of the pathogen that were surface sterilised or left untreated

Position	Surface sterilised	Untreated
Bunch		
Rachis	-0.53cd[y] (35.56[z])	2.11a (95.56)
Lateral	-1.89e (20.00)	1.13b (76.67)
Pedicel	-2.03e (20.00)	0.01c (52.56)
Berry		
Pedicel end	-4.11f (1.17)	-3.81f (2.50)
Cheek	-3.56f (5.72)	-1.55de (31.28)
Style end	-4.62f (0.00)	-4.62f (0.00)

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches inoculated at pea size, bunch closure and harvest stages.
[w] Parts, obtained from inoculated bunches, were isolated on Kerssies and paraquat media.
[x] Bunch = position on the structural parts of the bunch. Berry = position on the berries.
[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$.
[z] Values in parenthesis are the means of the logit transformed percentages.

Table 3. Mean incidence[v] (logit transformed) of *Botrytis cinerea* recorded[w] in grape bunches inoculated at different growth stages with dry, airborne conidia of the pathogen that were surface sterilised or left untreated

Stage	Non- sterile	Sterile
Pea size	-1.97b[x] (29.38[y])	-2.84cd (16.62)
Bunch Closure	-1.86b (27.95)	-3.40d (6.62)
Harvest	1.12a (73.00)	-2.19bc (15.47)

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches.
[w] Parts, obtained from inoculated bunches, were isolated on Kerssies and paraquat media.
[x] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$.
[y] Values in parenthesis are the means of the logit transformed percentages.

Table 4. Mean incidence[v] (logit transformed) of *Botrytis cinerea* recorded[w] at different positions[x] in grape bunches inoculated at different growth stages with dry, airborne conidia of the pathogen

Position	Pea size	Bunch Closure	Harvest
Bunch			
Rachis	1.04a[y] (73.33[z])	0.78ab (63.33)	0.56abc (60.00)
Lateral	0.70ab (66.67)	-1.14d (35.00)	-0.70cd (43.33)
Pedicel	-1.27d (34.17)	-1.31d (29.75)	-0.45bcd (44.92)
Berry			
Pedicel end	-4.09ef (2.00)	-3.83ef (1.67)	na
Cheek	-3.24e (10.33)	-3.58ef (5.33)	-0.85d (39.83)
Style end	-4.62f (0.00)	-4.62f (0.00)	na

[v] Average values of data recorded over two seasons in Chardonnay, Chenin Blanc and Shiraz bunches.
[w] Parts, obtained from inoculated bunches, were isolated on Kerssies and paraquat media.
[x] Bunch is the position on the structural parts of the bunch. Berry is the position on the berries.
[y] Means with the same letter are not significantly different according to the Student's *t*-test at $P = 0.05$.
[z] Values in parenthesis are the means of the logit transformed percentages.

4. PATHOGENICITY AND VIRULENCE ON GRAPE AND NECTARINE FRUIT OF *BOTRYTIS CINEREA* ISOLATES OBTAINED FROM DIFFERENT HOST PLANTS

ABSTRACT

Pathogenicity and virulence of *Botrytis cinerea* isolates obtained from different host plants were compared by simulating natural infection on grape and nectarine fruit. Cold-stored fruit, considered highly susceptible to *B. cinerea* were therefore inoculated with single, airborne conidia of the pathogen. Different tests were conducted to assess surface penetration and lesion formation. Isolations were made from fruit skins on Kerssies' *B. cinerea* selective medium. Nectarine fruit were treated with paraquat, and grape berries were frozen for 1 h at –12°C. Paraquat and freezing were used to terminate host resistance and to promote the development of the pathogen from the tissues. The isolation study showed that, irrespective of host origin, 10 out of the 14 isolates tested on both grape and nectarine fruit displayed similar penetration ability. A similar pattern was found where fruit were subjected to paraquat and freezing treatment. On untreated fruit, seven of these isolates were rated equal in virulence on both fruits. Collectively these findings showed that no host specialisation exists in the *B. cinerea* isolates used in this study.

INTRODUCTION

Botrytis cinerea Pers.:Fr. is a pathogen with a wide host range that attacks at least 235 plant species (McFarlane, 1968). The fungus causes grey mould on many economically important crops including tomato, cucumber, gerbera, rose, onions, strawberry and grape (Coley-Smith *et al.*, 1980). On grape, the fungus causes grey mould under a wide range of climatic conditions at the pre- and post harvest stage. Plants have various physical and chemical mechanisms to stop infection by the fungus. The defenses may be preformed (cell wall and cuticle) or induced after pathogen penetration (Kombrink & Somssich, 1995). The induced mechanisms emerge at different stages after infection and different distances from the site of infection. The order of these defenses is firstly the production of oxidative molecules, cell wall strengthening, phytoalexin biosynthesis and the accumulation of

pathogenesis related proteins (Kombrink & Somssich, 1995). The grape phytoalexin, resveratrol, can control *B. cinerea* growth (Hoos & Blaich, 1990). Other active defense mechanisms include suberisation (Hill, 1985) and lignification (Hoos & Blaich, 1988). Genetic variation for resistance to *B. cinerea* has been observed within species, but no gene-for-gene resistance has been identified (Elad & Evensen, 1995).

For a plant pathogen, pathogenicity is seen as having the characteristics to cause disease. Virulence is the observable effects of the pathogen on a plant; it is not synonymous with, but describes degrees of pathogenicity (Agrios, 1997). It has been claimed that two genes, BMP1 (Zheng *et al.*, 2000) and the endopolygalaturonase gene Bcpg1 (Ten Have *et al.*, 1998), are vital for the pathogenicity of *B. cinerea*. Sasaki and Nagayama (1994) showed that β-glucosidase might be important in the initial infection process. Some authors showed that *B. cinerea* isolates differ morphologically and physiologically (Lorbeer, 1980; Gorienko & Manturovskaya, 1971). *B. cinerea* isolates from different host plants may also vary in their secretion of enzymes like pectin methyl esterase (Wasfy *et al*, 1978), β-glucosidase (Sasaki & Nagayame, 1994), phenol oxidase (Wasfy *et al*, 1978), hexose oxidase (Edlich *et al.*, 1989), acid proteases (Zalewska-Sobczak *et al.*, 1981) and polygalacturonase (Di Lenna *et al.*, 1981). According to Jarvis (1980), there is no specialisation in *B. cinerea*. Jarvis (1980) stated that it is normal for *B. cinerea* to attack dicotyles, monocotyles and sometimes even species of the Pteridophyta. According to Jarvis (1980), McNeill found one isolate, which parasitised only the roots and the dead foliage of the lettuce plant. Contrasting to the traditional view that *B. cinerea* is a clonal population without specialisation, Giraud *et al.* (1999) suggested that *B. cinerea* is a complex of sibling species that occur sympatrically on many host plants.

Pathogenicity and virulence of isolates of *B. cinerea* on plants have been studied by various workers (Di Lenna *et al.*, 1980; Derckel *et al.*, 1999; Giraud *et al.*, 1999; Zalewska-Sobczak *et al.*, 1980; Coley-Smith *et al.*, 1980). In these investigations inoculation have been done using spore suspensions, and the spores were mostly deposited on the plant surface as a cluster. However, in the field, infection by solitary conidia, and not by conidial clusters, should play a prominent role in disease (Coertze *et al.*, 2001; G. Holz, *unpublished data*). Results may differ following inoculation by single conidia compared to masses of conidia, and this is important in the interpretation and application of research on *B. cinerea*. The penetration of clusters of conidia at a single site may alter the host response to infection

(Fourie & Holz, 1995; Holz *et al.*, 1995; Holz *et al.*, 1998). Giraud *et al.* (1999) also mentioned that laboratory experiments might not reflect the natural infection conditions. It is thus important to simulate natural infection when investigating pathogenicity and virulence factors. The aim of this study was to simulate natural infection by single airborne *B. cinerea* conidia, and to compare the pathogenicity and virulence on grape and nectarine fruit of isolates obtained from different host plants.

MATERIALS AND METHODS

Grape and nectarine fruit. Freshly harvested grapes (cultivar Dauphine at 17° Brix) were obtained from De Doorns in the Hex river valley. The grapes were packed with an SO_2 generator (0.3-0.55 g $Na_2S_2O_5$ affixed to a paper sheet [Holz *et al.*, 1997; Nair, 1985]) inside a polyethylene bag in corrugated boxes (Patent no. RSA 75/6116) and kept in cold storage for 6 weeks at -0.5°C. Grapes are considered susceptible to *B. cinerea* infection when ripe (Broome *et al.*, 1995; Hill *et al.*, 1981; Nair & Allen, 1993; Nelson, 1951), but highly susceptible when subjected to SO_2 treatment and prolonged after cold storage (Nelson, 1951; Nelson, 1956). Freshly harvested nectarines (cultivar Mayglo) were obtained from Stellenbosch, and fruits were selected for optimum ripeness (average firmness [7.2 kg] and total soluble solids [10.6%]. The firmness was determined with a dial type penetrometer. The plunger had a diameter of 11 mm and skin was removed on opposite sides of the fruit. Readings were taken on each side and always on a healthy part of the fruit. The skin colour was determined with a chart for nectarine, chart N 1A. Total soluble solids were measured with a hand–held refractometer. The nectarines were packed in cartons as for export and stored for one and a half weeks at –0.5°C before used. No fungicides were applied to the nectarines used in these trials two weeks before harvest.

Isolates and inoculum. *B. cinerea* was obtained from naturally infected symptomatic tissue of different plants obtained from different regions in the Western Cape province during 1999. The plant material was placed in individual polyethylene bags to prevent cross-contamination. The bags were sealed and kept at 22°C under diurnal light to stimulate sporulation. Single conidiophores of *B. cinerea* were selected under a dissecting microscope, placed on potato dextrose agar (PDA, amended with 40 mg/l streptomycin sulfate), and incubated at 22°C for 72 h. Pure sub-cultures were obtained from hyphal tips growing on streptomycin amended PDA. Isolates selected for further use were maintained on potato

dextrose agar (PDA) at 5°C in the dark. Resistance to iprodione (Rovral 250 SC, Aventis) of 15 isolates, selected for the pathogenicity and virulence studies, was determined according to the protocols of the Fungicide Resistance Action Committee (FRAC) (Löcher & Lorenz, 1991). The mycelium growth sensitivity of the isolates was determined on PDA amended with 3 µg iprodione/ml. Mycelial plugs (5 mm in diameter) were taken from the active growing colony margins (after 3 days of active growth) and put on each of three non-amended plates, as well as on three iprodione-amended plates. The plates were incubated for 36 h at 22°C and the radial mycelial growth determined. Since a discriminatory concentration of the fungicide was used, an isolate was designated resistant if it grew on the control and fungicide-amended plates and sensitive if it grew only on the control plates (Fourie & Holz, 1998). For the preparation of inoculum, each 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 diurnal light regime (12 h near-ultraviolet light). Conidia were harvested dry with a suction-type collector from 14-day-old cultures and stored dry at 5°C until use (1-10 weeks). The storage time did not affect the germination (Spotts & Holz, 1996); consequently, the dry conidia could be used in all the experiments.

Inoculations. After cold storage the fruits were kept for 24 h at 22°C to reach ambient temperature. The fruits were then surface sterilised (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried. This treatment completely eliminated *B. cinerea* from the fruit surface (Nelson, 1956) and prevented natural infection to occur in the experiments. At each inoculation, berries were cut from clusters with short stem segments attached and packed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm). In order to recognise the inoculated side of the fruits at a later stage, a 1-cm mark was made on the fruit near the pedicel with a soft-tipped felt pen. Previous studies (Coertze & Holz, 1999; Spotts & Holz, 1996) showed no phytotoxic effect of the ink on fruit. For inoculation, 3 mg of 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 20 min to settle onto the fruits that were positioned on the screens. At this dosage, approximately three conidia were evenly deposited as single cells on each mm^2 of fruit surface (Coertze & Holz, 1999). Petri dishes with water agar (WA) and PDA were placed on the floor of the settling towers at each inoculation and percentage germination of conidia was determined after 6 h incubation at 22°C (100 conidia per Petri dish, three replicates). Germination was between 91 and 99% at 12 h post inoculation for all

experiments. Following inoculation, the screens were placed in separate ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). Each chamber contained five screens with 63 berries, or 18 nectarines per screen. Each screen in a chamber was randomly assigned to one of the five isolates used. Each chamber was considered as a block and the screens were randomised within each chamber. The chambers were kept at 22°C for 24 h with a 12 h photoperiod daily. Studies with airborne conidia of *B. cinerea* on grape (Coertze *et al.*, 2001) and nectarine (G. Holz, *unpublished data*) under similar wetness regimes showed that germination and surface colonisation reached a maximum during this period. After 24 h, the screens were removed from the different chambers and the fruits were used for isolation and symptom expression studies. The experiment was repeated.

Pathogenicity and virulence tests. Three techniques were used to determine pathogenicity and virulence. For the first technique, screens with fruit from the three chambers were transferred to identical dry perspex chambers (≤56% RH) for 10 days, followed by a 4-day incubation period at high relative humidity to promote disease expression. The fruit were examined daily for symptom expression. For the second technique, isolations were made from the fruit skins. The fruits were surface sterilised as described previously. Three epidermal tissue segments (approximately 5 x 7 mm) were cut from each fruit, placed with the cuticle upward on Kerssies' *B. cinerea* selective medium (Holz *et al.*, 1998) and incubated at 22°C. For the third technique, the fruits were surface sterilised as described previously, frozen (grape berries), or treated with paraquat (nectarine). Screens with berries were kept for 1 h at –12°C in an industrial freezer. Nectarines were immersed in paraquat solution (30 ml/l water) (WPK Paraquat, 200 g/l [bipyridyl], WPK Agricultural) for 30 seconds, rinsed in sterile deionised water and air-dried. Following treatment, the screens with fruit were kept in dry ethanol-disinfected perspex chambers (≤56% RH) for 10 days at 22°C, followed by a 4-day incubation period at high RH to promote symptom expression and sporulation.

The treatments provided conditions that facilitated conidial germination and the development of germlings on the fruit surface, and the development of latent mycelia in the skin during the incubation period. Both the paraquat (Baur *et al.*, 1969; Cerkauskas & Sinclair, 1980; Grindrat & Pezet, 1994; Pseidt & Pearson, 1989) and the freezing (Coertze &

Holz, 1999; Coertze *et al.*, 2001; Holz *et al.*, 2000) treatments terminated host resistance in the cells of the cuticular membrane without damaging host tissue, and thus facilitated the development of both surface conidia and latent mycelia in the skin tissue. Previous microscopic observations of fruit skins on Kerssies' medium (Coertze & Holz, 1999; Coertze *et al.*, 2001) and paraquat medium (Gütschow, 2001; Holz *et al.*, 2000) showed that no superficial mycelial growth developed on the skin segments during the first 5 days of incubation. Hyphal growth usually occurred from cells underlying the cuticle into the medium after 5 days, which indicated direct penetration by surface conidia, or the development of latent mycelia from skin tissue during the incubation period. Surface sterilisation eliminated *B. cinerea* from the fruit surface and facilitated the detection of mycelia in the skin tissue (Coertze & Holz, 1999; Coertze *et al.*, 2001; Gütschow, 2001). Therefore, according to these differential criteria, the development of *B. cinerea* from surface-sterilised fruit, and on surface-sterilised nectarine exposed to paraquat, or frozen berries, was from latent mycelia in the skin tissue only. The fruits 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. In the case of isolation studies, the incidence of segments yielding the pathogen was calculated. With the other teqhniques the incidence berries yielding *B. cinerea* symptom expression were calculated, or the number of lesions formed on nectarine counted.

Statistical procedure. For the grape experiment, the treatment design was a 15x2 factorial with factors isolates and treatments (freeze, non-freeze). An Anova was not done on the data from the grape isolation study, due to a lack of repititions. For the nectarine experiment, the treatment design was a 15x3x2 factorial with factors isolates, chambers and treatments (paraquat, non-paraquat). Data of fruit observations were transformed to a logit as well as percentage incidence and were subjected to a factorial analysis of variance. Data from the isolation studies were subjected to a two-way analysis. Statistical computations were performed using SAS (SAS institute Inc., Cary, NC). The experiments were subjected to analyses of normality of residuals ($P > 0.05$ = normality) using the Shapiro and Wilk test for normality (Shapiro & Wilk, 1965). Treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor & Cochran, 1980).

RESULTS

The analysis of variance for the effect of moist chamber, isolate and treatment showed that on grape (Table 1), isolate ($P = 0.0038$) and treatment ($P < 0.0001$) had a highly significant effect on the incidence of *B. cinerea* infected berries. On nectarine (Table 2), repetitions ($P < 0.0001$), isolates ($P < 0.0001$), chambers ($P = 0.0211$) and treatments ($P < 0.0001$) had a highly significant effect on the number of lesions caused by *B. cinerea* on fruit. In the nectarine skin isolation test, isolates ($P < 0.0001$) had a highly significant effect on the incidence of skins yielding *B. cinerea* (Table 3). Table 4 gives a summary of the isolates, their host's of origin and their geographical place of origin. The isolates were all pathogenic on both grapes and nectarine, but differed in virulence between the two fruits, and on a fruit type (Table 5). Virulence was also not regulated by the host of origin.

DISCUSSION

Investigations on pathogenicity and virulence can be influenced by inoculation method and ontogenic resistance in plant tissue (Coertze & Holz, 1999; Coertze *et al.* 2001). In most studies where plants were artificially inoculated with *B. cinerea*, parts or tissues were atomised with (De Kock & Holz, 1991; Nair, 1985; Nair *et al.*, 1988; Nelson, 1951), dipped in (Broome *et al.*, 1995), or injected with (Avissar & Pesis, 1991; Marois *et al.*, 1986; Thomson *et* al., 1988) conidial suspensions, or suspension droplets were placed onto the plant surface (Chardonnet *et* al., 1997; Marois *et al.*, 1987; Marois *et al.*, 1986). These methods allowed for the deposition of clusters of conidia, and may differ from primary natural inoculation in the vineyard, where single conidia may be deposited simultaneously at several sites on the berry surface (Coertze & Holz, 1999; Coertze *et al.* 2001). Furthermore, on both grape and nectarine, inoculation of fruit is often followed by a latent period (Holz *et al.*, 1997; Holz *et al.*, 1998), and a pathogenic relationship is generally established once the fruit ripens (Holz *et al.*, 1997; Holz *et al.*, 1998). Cold-stored fruit, considered highly susceptible to *B. cinerea* (Coertze & Holz, 1999) were therefore inoculated with single, airborne conidia. Different tests were conducted to assess surface penetration and lesion formation. The isolation study showed that, irrespective of host origin, 10 out of the 14 isolates tested on both grape and nectarine fruit displayed a similar penetration ability. A similar pattern was

found where fruit were subjected to paraquat and freezing treatment. On untreated fruit, seven of these isolates were rated equal in virulence on both fruits. Collectively these findings showed that no host specialisation excits in the *B. cinerea* isolates used in this study. This finding differs from that of other workers (Di Lenna *et al.*, 1980; Derckel *et al.*, 1999; Giraud *et al.*, 1999; Zalewska-Sobczak *et al.*, 1980; Coley-Smith *et al.*, 1980) who used clusters of conidia in their inoculation studies.

Differences in virulence on a specific host was found amongst the different isolates. However, contrary to the findings of Kerssies *et al.* (1997), the different tests revealed no major differences in virulence between isolates. Kerssies *et al.* (1997) suggested that the differences in virulence amongst isolates might be caused by differences in germination, which might be ascribed to differences in the conidia's reserve status. Conidia that were used in my study were harvested dry from 14-day-old cultures and stored dry at 5°C until use. Storage time did not affect germination; the dry conidia could therefore be used in all experiments (Spotts & Holz, 1996). The conidia furthermore did not receive water (Kerssies *et al.,* 1997) and nutrients (Derckel *et al.*, 1999) which could influence the growth of the pathogen on the host surface. An isolate originating from a specific host was also not necessarily the most virulent isolate on that host. For example, isolates R2 and 385.2, which were two of the most virulent isolates on grapes, were obtained from roses and strawberries respectively whereas solates 13B and 12E, which were the most virulent on nectarines, were isolated from grape. This finding differs from those made with *B. cinerea* on other hosts (Derckel *et al.,* 1999; Pie & Brower, 1993).

LITERATURE

Agrios, G.N. 1997. Plant Pathology. 4th ed. Academic Press, London. 635pp.

Avissar, I., and Pesis, E. 1991. The control of postharvest decay in table grapes using acetaldehyde vapours. *Annals of Applied Biology* 118:229-237.

Baur, J.R., Bovey, R.W., Baur, P.S. &El-Seify, Z. 1969. Effects of paraquat on the ultra structure of mesquite mesophyll cells. *Weed Research* 9: 81-85.

Broome, J.C., English, J. T., Marois, J. J., Latorre, B. A. and Aviles, J.C. 1995. Development of an infection model for *Botrytis* bunch rot of grapes based on wetness duration and temperature. *Phytopathology* 85: 97-102.

Cerkauskas, R.F. & Sinclair, J.B., 1980. Use of paraquat to aid detection of fungi in soybean tissue. *Phytopathology* 70: 1036-1038.

Chardonnet, C., L'Hyvernay, A., and Doneche, B. 1997. Effect of calcium treatment prior to *Botrytis cinerea* infection on the changes in pectic composition of grape berry. *Physiological and Molecular Plant Pathology* 50:213-218.

Coertze, S. and Holz, G. 1999. Surface colonization, penetration and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea. Plant Disease* 83: 917-924.

Coertze, S., and Holz, G. 2002. Epidemiology of *Botrytis cinerea* on grape: wound infection by dry, airborne conidia. *South African Journal of Enology and Viticulture* 23. In press.

Coertze, S., Holz, G and Sadie, A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea. Plant Disease* 85: 668-677.

Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R., eds. 1980. The Biology of Botrytis. Academic Press, London.

De Kock, P. J., and Holz, G. 1991. Colonization of table grapes by *Botrytis cinerea* in the Western Cape province. *Phytophylactica* 23:73-80.

Derckel, J.P., Baillieul, F., Manteau, S., Audran, J.P., Haye, B., Lambert, B. & Legendre, L. 1999. Differential induction of grapevine defenses by two strains of *Botrytis cinerea. Phytopathology* 89: 197-203.

Di Lenna, P., Marciano, P. & Magro, P. 1981. Comparative investigation on morphological and physiological features of three isolates of *Botrytis cinerea. Phytopathologiche Zeitschrift* 100: 203-211.

Duncan, R.A., Stapleton, J.J. & Leavitt, G.M. 1995. Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as influenced by leaf removal. *Plant Pathology* 44: 956-965.

Edlich, W., Lorenz, G., Lyr, H., Nega, E. & Pommer, E.-H. 1989. New aspects on the infection mechanism of *Botrytis cinerea* Pers. *Nehterland Journal of Plant Pathology* (Supplement 1) 95: 53-62.

Elad, Y. & Evensen, K. 1995. Physiological aspects of resistance to *Botrytis cinerea. Phytopathology* 85: 637-643.

Fourie, P.H. & Holz, G. 1998. Frequency of dicarboximide resistant strains of *Botrytis cinerea* in South African table grape vineyards and influence of spray schedules on resistant sub-populations. *South African Journal of Enology and Viticulture* 19: 1-9.

Giraud, T., Fortini, D., Levis, C., Lamarque, C., Leroux, P., LoBuglio, K. & Brygoo, Y. 1999. Two sibling species of the *Botrytis cinerea* complex, transposa and vacuma, are found in sympatry on numerous host plants. *Phytopathology* 89: 967-973.

Gorienko, M.V. & Manturovskaya, N.V. 1971. Interstrain differentiation of *Botrytis cinerea*. *Review of Plant Pathology* 51: 571.

Grindrat, D. & Pezet, R. 1994. Le Paraquat, un Outil pour la Révélation Rapide d'Infections Fongiques Latentes et de Champignons Endophytes. *Journal of Phytopatholgy* 141: 86-89.

Gütschow, M. 2001. Resistance to *Botrytis cinerea* in parts of leaves and bunches of grapevine. M.Sc. Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Hill, G. 1985. Suberization of cell walls: a defense reaction of grapes stem tissue against invading mycelium of *Botrytis cinerea*. *Quarderni Della Scuola Di Specializzazione In Viticoltura Ed Enologia, Università di Torino* 9: 229-230.

Hill, G., Stellwaag-Kittler, F., Huth, G. and Schlösser, E. 1981. Resistance of grapes in different development stages to *Botrytis cinerea*. *Phytopathologiche. Zeitschrift.* 102: 328-338.

Holz, G., Coertze, S., and Basson, E. J. 1997. Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. (Abstr.). *Phytopathology* 87:S43.

Holz, G., Coertze, S., and Basson, E. J. 1998. Infection pathways and latency of *Botrytis cinerea* in grape bunches. (Abstr.). 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 3:6.22.

Holz, G., Gütschow, M., Frederiks, C., du Preez, I. F., and Coertze, S. 2000. Infection pathways of *Botrytis cinerea* on grape bunches. (Abstr.). XIIth Int. *Botrytis* Symp. Reims, France, L23.

Holz, G., Körte, V. and Coertze, S. 1995. Behaviour of *Botrytis cinerea* on grape, nectarine and plum fruit surfaces under different wetness regimes. Page 56 in: Scientific Programme and Abstracts. Bien. Aust. Plathol. Soc. Conf., 10th. Lincoln University, New Zealand.

Hoos, G. & Blaich, R. 1988. Metabolism of stilbene phytoalexins in grapevines: oxidation of resveratrol in single-cell cultures. *Vitis* 27: 1-12.

Hoos, G. & Blaich, R. 1990. Influence of resveratrol on the germination of conidia and mycelial growth of *Botrytis cinerea* and *Phomopsis viticola*. *Journal of Phytopathology* 129: 102-110.

Jarvis, W.R. 1980. Taxonomy. Page 1 in: The Biology of Botrytis. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore-trap. *Netherlands Journal of Plant Pathology* 96: 247-250.

Kerssies, A., Bosker-van Zessen, A.I., Wagemakers, C.A.M. & van Kan, J.A.L. 1997. Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. *Plant Disease* 81: 781-786.

Kombrink, E. & Somssich, I.E. 1995. Defence respons of plants to pathogens. *Adv. Bot. Res.* 21: 1-34.

Lorbeer, J.W., 1980. Variation in *Botrytis* and *Botryotinia*. Pages 19-30 in: The Biology of *Botrytis*. J.R. Coley-Smith, K Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Löcher, F.J. & Lorenz, G., 1991. Methods for monitoring the sensitivity of *Botrytis cinerea* to dicarboximide fungicides. pp. 341-345 in FRAC Methods for Monitoring Fungicide Resistance. *EPPO Bulletin* 21: 291-355.

McFarlane, H.H.. 1968. Plant host-pathogen index to volumes 1-40 (1922-1961) of Review of Applied Mycology. Commonwealth Mycological Institute, Kew, England.

Marois, J. J., Bledsoe, A. M., Bostock, R. M., and Gubler, W. D. 1987. Effects of spray adjuvants on development of *Botrytis cinerea* on *Vitis vinifera* berries. *Phytopathology* 77:1148-1152.

Marois, J. J., Bledsoe, A. M., Gubler, W. D., and Luvisi, D. A. 1986. Control of *Botrytis cinerea* on grape berries during postharvest storage with reduced levels of sulfur dioxide. *Plant Disease* 70:1050-1052.

Marois, J. J., Nelson, J. K., Morrison, J. C., Lile, L. S., and Bledsoe, A. M. 1986. The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. *American Journal of Enology and Viticulture* 37:293-296.

Nair, N. G. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. *Australian Journal of Agricultural Research* 36:435-442.

Nair, N. G., and Allen, R. N. 1993. Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. *Mycological Research.* 97: 1012-1014.

Nair, N. G., Emmett, R. W., and Parker, F. E. 1988. Some factors predisposing grape berries to infection by *Botrytis cinerea*. *New Zealand Journal of Experimental Agriculture* 16:257-263.

Nelson, K. E. 1951. Effect of humidity on infection of table grapes by *Botrytis cinerea*. *Phytopathology* 41: 859-864.

Nelson, K. E. 1956. The effect of Botrytis infection on the tissue of Tokay grapes. *Phytopathology* 46: 223-229.

Northover, J. 1987. Infection sites and fungicidal prevention of *Botrytis cinerea* bunch rot of grapes in Ontario. *Canadian Journal of Plant Pathology* 9:129-136.

Pezet, R., and Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans les grappes de *Vitis vinifera* (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18:317-322.

Pie, K. & Brower, Y.J.C. 1993. Susceptability of cut rose flower cultivars to infections by different isolates of *Botrytis cinerea*. *Journal of Phytopathology* 137: 233-244.

Pscheidt, J.W. & Pearson, R.C. 1989. Time of infection and control of Phomopsis fruit rot of grapes. *Plant Disease* 73: 829-833.

Salinas, J., Glandorf, D.C.M., Picavet, F.D. and Verhoeff, K. 1989. Effects of temperature, relative humidity and age of conidia in the incidence of spotting on gerbera flowers caused by *Botrytis cinerea*. *Netherlands Journal of Plant Pathology* 95: 51-64.

Sarig, P., Zutkhi, Y., Monjauze, A., Lisker, N. & Ben-Arie, R. 1997. Phytoalexin elicitation in grape berries and their susceptibility to *Rhizopus stolonifer*. *Physiological and Molecular Plant Pathology* 50: 337-347.

SAS, 1999. SAS/STAT User's Guide, Version 8, 1st printing, Volume 2. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513.

Sasaki, I & Nagayama, H. 1994. β-Glycosidase from *Botrytis cinerea*: Its relation to the pathogenicity of this fungus. *Bioscience, Biotechnology and Biochemistry* 58: 616-620.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika,* 52: 591-611.

Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. 7th Ed. Iowa State University Press, Ames.

Spotts, R.A., & Holz, G. 1996. Adhesion and removal of conidia of *Botrytis cinerea* and *Penicillium expansum* from grape and plum fruit surfaces. *Plant Disease* 80: 688-691.

Ten Have, A., Mulder, W., Visser, J. & Van Kan, J.A.L. 1998. The endopolygalacturonase gene Bcpg1 is required for full virulence of *Botrytis cinerea*. *Molecular Plant Microbe Interactions* 11: 1009-1016.

Thomas, C. S., Marois, J. J., and English, J. T. 1988. The effects of wind speed, temperature and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. *Phytopathology* 78:260-265.

Wasfy, E.H., Farag, S.A., Tarabieh, M.A. & Abd-Elmoety, S.H. 1978. Studies on enzymes of different strains of *Botrytis cinerea*. *Phytopathologische Zeitschrift* 92: 168-179.

Zalewska-Sobczak, J., Borecka, H & Urbanek, H. 1981. Comparison of pectinase, xylanase and acid protease activities of virulent and less virulent isolates of *Botrytis cinerea*. *Phytopathologische Zeitschrift* 101: 222-227.

Zheng, L, Campbell, M., Murphy, J., Lam, S. & Xu, J.-R. 2000. The BMP1 gene is essential for pathogenicity in the grey mould fungus *Botrytis cinerea*. *Molecular Plant Microbe Interactions* 13: 724-732.

Table 1. Analysis of variance for the effects of chambers, isolates and treatments on the *Botrytis cinerea* incidence (logit transformed) on grapes inoculated with 15 isolates of the pathogen originating from different hosts

Source of variation	Df	Mean Square	P > F
Chamber	2	43.084	0.8990
Isolate	14	1099.881	0.0038
Treatment	1	28444.444	0.0001
Isolate * Treatment	14	372.962	0.5396
Error	58	403.811	.
Corrected Total	89	815.409	.

Table 2. Analysis of variance for the effects of chambers, isolates and treatments on the *Botrytis cinerea* incidence (logit transformed) on nectarines inoculated with 15 isolates of the pathogen originating from different hosts

Source of variation	Df	Mean Square	P > F
Repetitions (R)	1	30.989	0.0001
Isolates (I)	15	11.494	0.0001
Chambers (C)	2	5.919	0.0211
I*C	28	1.849	0.2071
Treatment (T)	1	444.262	0.0001
I*T	15	2.105	0.1493
C*T	2	1.108	0.4723
I*C*T	28	1.111	0.7931
Error	83	1.464	.
Corrected Total	1030	1.369	.

Table 3. Analysis of variance for the effects of isolate on the *Botrytis cinerea* incidence (logit transformed) on nectarines segments isolated on Kerssies' *B. cinerea* selective medium

Source of variation	Df	Mean Square	P > F
Isolates	15	4.527	0.0001
Error	15	0.289	.
Corrected Total	30	2.408	.

Table 4. Host, origin and dicarboximide sensitivity of *Botrytis cinerea* isolates used in the pathogenicity and virulence tests on grape and nectarine fruit

Isolates	Host plant of origin	Geographical place of origin	Dicarboximide sensitivity
R 2	Roses	Stellenbosch	Sensitive
15	Grapes	Constantia	Sensitive
17.1	Nectarines	Malmesbury	Sensitive
18	Peaches	Malmesbury	Sensitive
19	Citrus	Stellenbosch	Sensitive
385.2	Strawberries	Paarl	Sensitive
11D	Grapes	Robertson	Sensitive
12E	Grapes	Rawsonville	Sensitive
13B	Grapes	Paarl	Resistant
5A	Grapes	Hex River Valley	Sensitive
7C	Grapes	Stellenbosch	Sensitive
HS	Shrub	Stellenbosch	Sensitive
LR	Grapes	Western Cape	Resistant
S	Grapes	Western Cape	Sensitive
ULR	Grapes	Western Cape	Resistant
STD	Grapes	Western Cape	Sensitive

Table 5. Infectivity[v] of different *Botrytis cinerea* isolates shown by different tests on fruit grape and nectarine inoculated with dry, airborne conidia of the pathogen[w]

	Percentage Nectarine Lesions		Percentage Nectarine Segments	Percentage Grape Berries		Percentage Grape Segments
Isolates	**Control**	**Paraquat**	**Mean**	**Untreated**	**Freezed**	**Mean[x]**
R 2	0.633 l-q[y]	1.427 e-k	-0.486 f	55.556 b-I	71.429 a-d	14.268
15	0.408 n-q	2.126 a-e	1.821 bcd	28.571 i-l	61.905 b-h	7.937
17,1	1.172 g-m	2.548 ab	0.937 cde	20.644 jkl	65.794 a-h	15.873
18	0.534 l-q	2.044 a-e	1.874 bcd	31.746 h-l	63.492 a-h	19.048
19	0.188 pq	1.200 g-m	-0.592 f	9.524 kl	57.143 b-I	7.937
385,2	0.221 opq	2.367 abc	2.059 abc	46.032 d-j	71.429 a-d	22.222
11D	0.932 i-o	2.252 abc	1.083 cde	34.921 g-l	84.127 abc	47.619
12E	0.983 h-n	2.742 a	1.566 bcd	68.254 a-f	68.254 a-f	19.048
13B	1.256 f-l	2.603 ab	1.700 bcd	25.397 i-l	95.238 a	31.746
5A[z]	.	.	.	36.508 f-l	79.365 abc	20.635
STD[z]	0.739 j-p	1.507 d-I	1.645 bcd	.	.	.
7C	0.572 l-q	1.967 b-f	2.267 ab	44.444 d-j	85.714 ab	20.635
HS	0.942 i-o	2.167 a-d	0.774 de	20.635 jkl	52.381 c-j	12.698
LR	0.505 m-q	1.471 d-j	0.000 ef	4.762 l	39.683 d-k	1.587
S	0.710 k-q	1.909 b-g	2.553 ab	38.095 e-k	69.841 a-e	25.397
ULR	1.232 f-m	1.707 c-h	3.132 a	20.635 jkl	53.968 b-i.	0

[v] Average values of data recorded on fruit of Dauphine grapes and Mayglo nectarines.

[w] Three techniques were used to determine pathogenicity and virulence. For the first technique, fruit were kept at high relative humidity to promote disease expression. For the second technique, isolations were made from the fruit skins on Kerssies' *B. cinerea* selective medium. For the third technique, fruits were frozen (grape berries) or treated with paraquat (nectarine).

[x] An anova was not done on this data, due to a lack of repetitions.

[y] Means with the same letter in each column are not significantly different according to the Student's *t*-test at $P = 0.05$.

[z] Isolate 5A was used only on grape and STD only on nectarine.