RESISTANCE TO *BOTRYTIS CINEREA* IN PARTS OF LEAVES AND BUNCHES OF GRAPEVINE

MINIQUE GÜTSCHOW

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture at the University of Stellenbosch

Supervisor: Prof. G. Holz
March 2001

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

RESISTANCE TO *BOTRYTIS CINEREA* IN PARTS OF LEAVES AND BUNCHES OF GRAPEVINE

Knowledge of the presence of *Botrytis cinerea* in morphological parts of bunches and leaves of grapevine would help to find a reliable, sensitive, and specific assay to verify the actual occurrence of latent infection, and to plan strategies for the effective control of *B. cinerea* bunch rot. The aim of this study was (i) to determine natural *B. cinerea* infection at specific sites in leaves and bunches of grapevine at different phenological stages, and (ii) to determine resistance in the morphological parts to disease expression.

Bunches and leaves of the wine grape cultivar Merlot and the table grape cultivar Dauphine, were collected at pea size, bunch closure and harvest from five vineyards in the Stellenbosch and De Doorns regions respectively. The material was divided into two groups and sealed in polythene bags. The bags were lined with wet paper towels to establish high relative humidity. Leaves and bunches incubated in one group of bags were first treated with paraquat in order to terminate active host responses. These treatments provided conditions that facilitated disease expression under two host resistance levels by different inocula during the period of moist incubation. Disease expression was positively identified by lesion development, and the formation of sporulating colonies of *B. cinerea* at a potential infection site. Sites in leaves were the blades and petioles. Sites in bunch parts were rachises, laterals and pedicels, and on berries sites were the pedicel-end, cheek and style-end. In Dauphine, the various sites were at all stages classified as resistant to moderately resistant. However, at pea size and bunch closure, in spite of their resistance, nearly all the sites carried high to very high inoculum levels. The only exception was the berry cheek, which carried intermediate inoculum levels at pea size, and low inoculum levels at bunch closure. In nearly all sites, inoculum levels were lower at harvest. The decrease was the most prominent in petioles, rachises, laterals, pedicels and the pedicel-end of the berry. All these sites carried intermediate to low inoculum levels at harvest. In Merlot, sites constantly exibited a resistant reaction, except for the pedicel and pedicel-end of the berry, which changed from resistant at the early developmental stages to susceptible at harvest. Inoculum levels decreased during

the season in the rachises and laterals, but were constantly high during the season in the pedicel and pedicel-end of the berry. According to this pattern of natural occurrence, *B. cinerea* fruit rot in these vineyards was not caused by colonisation of the pistil, and subsequent latency in the style end of grape berries. However, fruit rot was primarily caused by colonisation of the pedicel, and subsequent latency in the pedicel or pedicel-end of the berry. These findings furthermore support the hypothesis of increased host resistance during development, but also indicate that in the Western Cape province, inoculum in vineyards is abundant during the early part of the season, and less abundant later in the season. More information is therefore needed on the behaviour of the different types of *B. cinerea* inocula on the different morphological parts of grapevine to validate the pathway described for natural *B. cinerea* infection in vineyards. The penetration and disease expression at the different morphological parts of bunches of two grape cultivars (Dauphine and Merlot) under conditions simulating natural infection by airborne conidia was therefore investigated.

The two cultivars did not differ in resistance of the berry cheek, which was at all stages classified as resistant. However, in Dauphine, latent inoculum levels in berry cheeks declined from intermediate at pea size to low at the following stages, whereas in Merlot, levels were intermediate during pea size and at harvest. Some differences between cultivars were found in the resistance of the structural bunch parts, and of their latent inoculum levels. In Dauphine, the rachis reacted susceptible at pea size, and was classified moderately resistant later in the season. Laterals and pedicels were moderate resistant at pea size, and resistant at later stages. Inoculum levels in rachises, laterals and pedicels were high at pea size, but intermediate at bunch closure and at harvest. The finding that *B. cinerea* infected and naturally occurred more commonly in the tissues of immature than mature bunches, that the structural parts of the bunch carried more *B. cinerea* than the berry cheek, and that these infections may be more important in *B. cinerea* bunch rot than infection of the cheek or the style end, suggest that emphasis should be placed on the disease reaction of the pedicel and related parts of immature bunches rather than on the berry.

The resistance reaction of leaf blades, petioles, internodes and inflorescences on cuttings, compared to those on older shoots from the vineyard were therefore investigated. In the case of vinelets, leaf blades, petioles, internodes and inflorescences were all classified susceptible to highly susceptible. The different parts furthermore all carried very high latent inoculum levels. In vineyard shoots the petioles and inflorescences showed resistance, and

carried intermediate to latent inoculum levels. This finding suggests that leaf blades are not appropriate parts for studying the behaviour of inoculum of *B. cinerea* and host responses in grape bunches. In stead, petioles and inflorescences of vineyard shoots should be used for this purpose.

OPSOMMING

WEERSTAND TEEN *BOTRYTIS CINEREA* IN MORFOLOGIESE DELE VAN BLARE EN TROSSE VAN WINGERD

Kennis oor die teenwoordigheid van *Botrytis cinerea* in morfologiese dele van wingerd word benodig vir die ontwerp van 'n betroubare, sensitiewe en spesifieke toets vir die bevestiging van latente infeksies, en vir die implementering van strategieë vir die effektiewe beheer van *B. cinerea*-vrot. Die doel van hierdie studie was om (i) natuurlike *B. cinerea* infeksie by spesifieke areas in blare en trosse van wingerd te bepaal, en (ii) om weerstand teen siekte-uitdrukking in hierdie morfologiese dele vas te stel.

Trosse en blare van die wyndruif kultivar Merlot en die tafeldruif kultivar Dauphine, is by ertjiekorrel, tros-toemaak en oes in vyf wingerde in die Stellenbosch- en De Doorns-omgewing, onderskeidelik, versamel. Die materiaal is in twee groepe verdeel en in polietileen sakkies verseël. Die sakkies is met klam papierdoekies uitgevoer om sodoende hoë relatiewe humiditeit te verseker. Blare en trosse wat in die een groep geïnkubeer is, is eers met paraquat behandel om aktiewe gasheerreaksies te beëindig. Hierdie behandelings het toestande geskep wat gedurende die periode van vogtige inkubasie gunstig was vir siekte-ontwikkeling deur verskillende inokula by twee gasheer-weerstandsvlakke. Siekte-uitdrukking is positief geïdentifiseer deur letsel-ontwikkeling en die vorming van sporulerende kolonies van *B. cinerea* by 'n potensiële infeksie-area. Dele waarop in die blare gekonsentreer is, was die blaarskyf en -steel. In die trosse was die dele die rachis, lateraal en korrelsteel, en op korrels was dit die korrelsteel-end, wang en styl-end. In Dauphine is die verskillende dele tydens al die fenologiese stadia as weerstandbiedend tot matig weerstandbiedend geklassifiseer. Die verskillende dele her egter, ten spyte van hul weerstandbiedendheid, hoë tot baie hoë inokulumvlakke by ertjiekorrel- en tros-toemaak-stadium gedra. Die enigste uitsondering was die korrelwang, wat 'n middelmatige inokulumvlak by ertjiekorrel, en 'n lae inokulumvlak by tros-toemaak, gedra het. Die inokulumvlakke was in byna al die dele laer by oes. Die afname in inokulumvlakke was die prominentste in die blaarstele, rachi, laterale, korrelstele en die korrelsteel-end van die korrel. Al hierdie dele het 'n middelmatige tot lae inokulumvlak by oes gehad. In Merlot was die

dele konstant weerstandbiedend, behalwe vir die korrelsteel en die korrelsteel-end van die korrel, wat gewissel het van weerstandbiedend by die vroeë ontwikkelingstadia, tot vatbaar by oes. Inokulumvlakke in die rachis en lateraal het gedurende die seisoen afgeneem, maar was deur die seisoen konstant hoog in die korrelsteel en korrelsteel-end van die korrel. Volgens díe patroon van natuurlike voorkoms, word *B. cinerea*-vrot in hierdie wingerde nie deur kolonisasie van die stamper, en die daaropvolgende latensie in die styl-end van die korrels, veroorsaak nie. Vrot word egter primêr deur kolonisasie van die korrelsteel, en die daaropvolgende latensie in die korrelsteel of korrelsteel-end van die korrel, veroorsaak. Hierdie bevindinge ondersteun die hipotese van toenemende gasheerweerstand gedurende ontwikkeling, en dui ook daarop dat inokulumvlakke in wingerde in die Wes-Kaap provinsie volop is gedurende die eerste deel van die seisoen, en minder volop is later in die seisoen. Meer inligting word dus benodig aangaande die gedrag van die verskillende inokulum tipes van *B. cinerea* op die verskillende morfologiese dele van wingerd, ten einde die infeksieweg vir natuurlike *B. cinerea* infeksie in wingerde te bevestig. Die vestiging van latente infeksies in die verskillende morfologiese dele van trosse van twee kultivars (Dauphine en Merlot), onder toestande wat natuurlike infeksie deur luggedraagde konidia simuleer, is dus ondersoek.

Die twee kultivars se weerstand in die korrelwang het nie verskil nie en is by alle fenologiese stadia as weerstandbiedend geklassifiseer. Die latente inokulumvlakke in die korrelwang van Dauphine het egter van middelmatig by ertjiekorrel, tot laag in die daaropvolgende stadia afgeneem, terwyl die vlakke in Merlot middelmatig by ertjiekorrel en oes was. Verskille tussen die twee kultivars is gevind ten opsigte van die weerstand in die trosdele, asook hulle latente inokulumvlakke. Die rachis van Dauphine was by ertjiekorrel vatbaar, en matig weerstandbiedend later in die seisoen. Die lateraal en korrelsteel was matig weerstandbiedend by ertjiekorrel en weerstandbiedend by latere stadia. Inokulumvlakke in rachi, laterale en korrelstele was hoog by ertjiekorrel, maar middelmatig by tros-toemaak en oes. Die bevindinge dat *B. cinerea* natuurlik meer algemeen in die weefsel van onvolwasse trosse voorgekom en laasgenoemde meer algemeen geïnfekteer het, dat *B. cinerea* se voorkoms hoër was in die morfologiese dele van die tros as in die korrelwang, en dat hierdie infeksies van groter belang in *B. cinerea*-vrot mag wees as infeksie van die wang of styl-end, dui daarop dat klem gelê moet word op die siektereaksie van die strukturele dele van onvolwasse trosse, eerder as van die korrel.

Die weerstand van blaarskywe, blaarstele, internodes en blomtrossies van steggies, in vergelyking met díe op ouer lote in wingerde, is dus ondersoek. Blaarskywe, blaarstele, internodes en blomtrossies van steggies is almal as vatbaar tot hoogs vatbaar geklassifiseer. Die verskillende dele het verder ook almal baie hoë latente inokulumvlakke gedra. By die ouer lote van wingerde het die blaarstele en blomtrossies weerstandbiedend vertoon, en middelmatige latente inokulumvlakke gedra. Hierdie bevindinge dui daarop dat blaarskywe nie die ideale morfologiese deel is vir gedragstudies van *B. cinerea* in druiwetrosse nie. Blaarstele en blomtrossies van ouer lote moet eerder vir die doel gebruik word.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the following:

Prof. Gustav Holz, my supervisor, for his guidance, advice, experience, knowledge, assistance with the preparation of the manuscript and especially his faith and enthusiasm;

Prof. Pedro Crous, Paul Fourie and Fred Walters for valuable comments on the manuscript;

Laboratory and research assistants of the Department of Plant Pathology, for practical and administrative support;

To all the producers who made their vineyards available for this study;

The Deciduous Fruit Producers Trust, The National Research Foundation, THRIP and The University of Stellenbosch for financial assistance;

Phyllis Burger and Willem Loubscher of the ARC Infruitec-Nietvoorbij for their assistance, input and friendship;

Mardie Booyse of the ARC for assisting me with the statistical analyses and data interpretation;

My husband, parents, family, friends and colleagues for their wonderful support, advice, prayers and encouragement;

My Heavenly Father for giving me vision and grace to accomplish this task.

CONTENTS

1. THE BIOLOGY OF *BOTRYTIS CINEREA* ON GRAPEVINE, WITH REFERENCE TO INFECTION AND HOST RESISTANCE

INTRODUCTION

Botrytis cinerea Pers.:Fr., a pathogen of grapevine (*Vitis vinifera* L.) causes grey mould and can attack most of the plant's organs (Nair and Hill, 1992). Grey mould is associated with early-season latent infections (McClellan and Hewitt, 1973; Nair, 1985; Nair and Parker, 1985) and infections of mature grapes favoured by late-season rains or prolonged periods of high relative humidity (Harvey, 1955). Other factors include the production and dispersal of various inocula, infection, and pathogen survival. Each event is predisposed and determined by different sets of environmental and agricultural factors such as temperature, rainfall, humidity and crop protection practices, nutrition and crop phenology (Jarvis, 1980). It is still uncertain however, how these modes contribute to the development of *B. cinerea* (Bulit and Dubos, 1988; English *et al.,* 1989).

To effectively combat a grey mould epidemic, research has led to the development of prediction models (Bulit and Lafon, 1970; Strizyk, 1983; Molot, 1987; Nair and Allen, 1993; Broome *et al.*, 1995) for recommendations on the effective application of fungicides for the control of *B. cinerea* bunch rot on grapevine. These prediction models use in-field monitoring stations to warn when conditions as mentioned previously are favourable for the disease to occur. The above measures are satisfactory solutions for farmers, but grapevine breeders have a more serious problem when selecting *B. cinerea* resistant cultivars from seedlings not yet bearing grapes. This problem, mentioned by Nair and Hill (1992), is the challenge addressed in this project, and deals with the question of old-age resistance of leaves and other morphological parts compared with old-age susceptibility of berries. Knowledge of *B. cinerea* behaviour on the grapevine and its morphological parts at different morphological stages is extremely important in the solution of this challenge.

INFECTION

Inoculum dispersal and germination

Botrytis cinerea maintains itself in grapevines as sclerotia (Nair and Nadtotchei, 1987), conidia (Corbaz, 1972; Bulit and Verdu, 1973) and mycelia (Gessler and Jermini, 1985; Northover, 1987). Kosuge and Hewitt (1964) observed that nutrients taken up by free water on the surface of the berry appear to serve as a source of energy to germinating conidia. Germination of *B. cinerea* depends on the micro-environmental conditions of the phylloplane, especially free water and nutrient availability (Blakeman, 1975). Free water is required for germination and this is why it is important to avoid condensation. An intact cuticle prevents diffusion of cellular solutions and limits water and nutrient availability on the surface. The hydrophobic character of the cuticle reduces the probability of rain, irrigation water or condensation accumulation on the surface (Carre, 1984). Washings from mature and immature berries were equally effective in stimulating germination of conidia and development of germ tubes (Kosuge and Hewitt, 1964). Hill *et al.* (1981) however, found no significant difference between germination of conidia on mature and immature berries, while McClellan and Hewitt (1973) showed that germination was poor in immature berry extracts. The grape flower aqueous extracts of the pollen, stigma and style enhanced germination and germ tube growth of conidia (McClellan and Hewitt, 1973).

Penetration

Different infection pathways have been described for *B. cinerea* on grape berries, namely stylar ends (McClellan and Hewitt, 1973; Nair and Parker, 1985), pedicels (Pezet and Pont, 1986; Holz *et al.*, 1997, 1998), natural openings (Pucheu-Planté and Mercier, 1983), wounds (Nair *et al.*, 1988), or by direct penetration of the cuticle (Nelson, 1956).

It is generally assumed that *B. cinerea* primarily attacks berries through the skin and causes rot. Successful penetration, and therefore infection, mainly takes place through the cracks around the stoma or through wounds (Nair and Nadtotchei, 1987). Bessis (cited in Verhoeff, 1980) found no proof for direct penetration of the berry cuticle, and concluded that the pathogen penetrates through minute openings or cracks in the cuticle. This process is only successful when natural resistance mechanisms in and on the skin are lacking, and the

berries are susceptible to infection (Nair and Hill, 1992). Resistance is normally provided in the first instance by the cuticle and secondly by active host responses in the tissue (Nair and Hill, 1992). The cuticle seems to be the major resistance mechanism of berries above 12% sugar content. Unripe berries with a sugar content of less than this are still resistant with or without the cuticle (Hill, 1985a).

During infection, free radicals are produced and they may damage membranes and increase susceptibility to the pathogen. Membrane damage increases leakage of nutrients to the surface, where they support growth and penetration of the fungus, and into the apoplast, where post-penetration growth occurs (Elad and Evensen, 1995). *Botrytis cinerea* is predominantly a wound pathogen under field conditions (Elad and Evensen, 1995) and injuries of the clusters due to insect damage or expansion of berries in tight cluster may be important avenues for infection (Savage and Sall, 1983). In response to pathogen attack, ethylene is often produced and increases the susceptibility of the berry. It promotes disease development by accelerating the senescing process, which favours the pathogen (Elad and Evensen, 1995). Treatment with antioxidants reduces ethylene production and disease development. This suggests that ethylene promotes oxidative reactions in the membranes and that membrane oxidation enhances ethylene production and action (Elad, 1992). Gibberellic acid (GA_3) inhibits the senescence-related increase in permeability of the membranes and therefore inhibits grey mould development (Sabehat and Zieslin, 1994). Auxins and cytokinins also increase resistance to grey mold (Elad and Evensen, 1995). Abscisic acid is associated with dormancy and stress responses and it accelerates senescence and increases ethylene sensitivity and therefore grey mould will be favoured (Borochov and Woodson, 1989).

In order for *B. cinerea* to effectively invade, it needs to soften the cell walls by exudation of cellulolytic and pectolytic enzymes. *Botrytis cinerea* is believed to penetrate the cuticle by way of enzymes and mechanical forces. Cutinase, which hydrolyses the primary alcohol ester linkages of the cutin polymer, seems to be the important factor. In a study of *B. cinerea* cutinase inhibition, treatment of inoculated gerbera flowers with a monoclonal antibody against cutinase from *B. cinerea,* lesion formation was reduced by up to 80% (Salinas *et al.*, 1992).

Cell wall degrading enzymes (CWDE) have been demonstrated in *B. cinerea* infected tissues and they include pectin methyl esterase, endo-polygalacturonase (PG), exo-PG, celactosidase, *B*-mannosidase and *alfa*-galactosidase (Barkai-Golan *et al.*, 1988; Johnston and Williamson, 1992). Grey mould accelerates production of hydrolytic enzymes associated with ripening and this might be via ethylene synthesis. It is a vicious circle in which plant hydrolases induce the production of fungal hydrolases and these enzymes are stimulated by the presence of galactose and other substances released from the cell wall of the plant (Verhoeff, 1974). Fungal CWDE's most important role is to degrade the cell walls and release nutrients for the pathogen. The cell wall hydrolysis creates osmotic stress on the protoplast resulting in cell death (Basham and Bateman, 1975). Cell death can be caused by these enzymes, but mostly commonly by a toxin of *B. cinerea* with a molecular size of 10-30000 daltons (Stein, 1984). Susceptibility of cell walls can be lessened by increasing the amount of calcium in the tissues (Elad and Volpin, 1988; Volpin and Elad, 1991).

Latency

The frequency of latent infections indicates that defences beyond the cuticle are very important. Latency is an important aspect in disease because early asymptomatic infection results in rotting later in the season. These infections are important because they are difficult to quantify, difficult to control and they fulfill a largely unexplored part in the development of infection. Latent infections are therefore feared by researchers, producers and thus the whole vine industry (Holz *et al.*, 1998). Pathogenic relationships are established once the fruit ripens (Mclellan and Hewitt, 1973). Grape clusters remain symptomless between the flowering period and the beginning of ripening, whereafter *B. cinerea* resumes its development (Pezet and Pont, 1986).

Resuming growth

At véraison or later the fungus resumes growth and rots the grape. Three explanations for the resuming of growth, leading to a pathogenic relationship has been suggested by Verhoeff (1980). In the first instance, the fungitoxic compounds in unripe fruit, disappear during ripening, especially high concentrations of phenols present in the outer layers of young grape berries. Secondly, concentration of sugars increases with ripening and a higher nutritional value exists. Thirdly, Verhoeff (1980) stated that the enzyme capabilities of the

fungus is insufficient to invade the unripe tissue, but as the tissue matures the cell wall undergoes chemical changes and the pectic material in the middle lamella becomes highly soluble.

Possner and Kliewer (1985) divided grape berries into four concentric zones to follow the developmental changes in the concentrations of malate, tartrate, glucose, fructose, potassium and calcium within the skin and the fruit flesh. In green berries the malate gradient increased in concentration from skin to seeds. Tartrate had the highest gradient in the periphery and was low in the centre. Towards maturity, the tartrate gradient decreased but the malate did not. In ripe berries the acid gradient was found to decrease in an axial direction from the pedicel towards the stylar scar. Before ripening, glucose and fructose had the highest levels in the skin and centre of the berry. After veraison, glucose and fructose had the highest levels in the centre and in the tissue below the peripheral vascular bundles of the berry. Potassium and calcium were localised near the peripheral and vascular bundles. Potassium increased constantly, but the calcium increase was completed 30 days after anthesis. Vercesi *et al.* (1997) found that hyphal growth was inhibited at high concentrations of tartaric and malic acid, but that it increased with greater sugar concentration. This data provides us with an explanation for the colonisation pattern of *B. cinerea* on grape berries. Growth will be poor during onset of ripening, when organic acids are the main carbon source. However, when sugar becomes the main carbon source, the fungus will have an enhanced growth rate as it is favoured by this carbon source.

Savage and Sall (1982) were unable to detect the fungus in immature berries. Pezet and Pont (1986) studied the effect of floral infections and latency, and found no evidence for the infection pathway as postulated by McClellan and Hewitt (1973). They showed that latent infection was predominantly pedicel-associated. De Kock and Holz (1991) consistently isolated *Botrytis cinerea* from apparently healthy and surface disinfected flowers and berries at all stages of bunch development. This finding confirmed the occurrence of latent infections but there was no evidence that berry infections arose from latent infections of the stigma. De Kock and Holz (1991) were furthermore unable to produce evidence that a relation exists between early infections and subsequent disease development or post harvest decay of table grapes. Decay was largely due to infection during storage by inoculum present

in bunches at veraison or during later stages. Infections occurring after veraison mask those that occur earlier.

Studies by Holz *et al.* (1998) on the behaviour of *B. cinerea* on the berry surface showed that the pathogen does not necessarily follow the infection pathway as described in the literature. It seems as if two inoculum types are involved in berry infection, namely mycelia and conidia. The more important infection pathway is via the pedicel (fruit stem) and this infection pathway is symptomless. There are clear indications that resistance mechanisms operate in the pedicel and that latency is settled here. These mechanisms are highly effective and destroy a large proportion of the latent infections in the pedicel. However, these mechanisms do seem to subside as bunches develop and the pathogen can systemically grow along the vascular tissue out of the pedicel and into the berry. This type of inoculum therefore reaches the berry from the inside and is not affected by the resistance mechanisms that normally stop it when trying to penetrate the berry skin (Holz *et al.*, 1998).

Infection of flower parts before berry infection

Infection of the generative organs nearly always results in reduced yield and early infection can destroy flower bunches (Nair and Hill, 1992). The flower infections can also be symptomless and the infection only manifests itself at a later stage of the grapevines growth (Nair, 1985; Nair and Parker, 1985; McClellan and Hewit, 1973). Evidence for the importance of latent infections by *B. cinerea* and the relation of early berry infections to late season bunch rot is primarily circumstantial (De Kock and Holz, 1991). On wine grapes in California (McClellan and Hewitt, 1973) and in Australia (Nair, 1985; Nair and Parker, 1985) early rot or midseason bunch rot is ascribed to the ability of *B. cinerea* to infect immature berries via senescing flower parts, thus resulting in latent infections. The establishment of *B. cinerea* on moribund or injured tissues normally allows the pathogen to infect the healthy tissues (Nair and Hill, 1992). Nair *et al* (1988) found that infected floral parts provide a large saprophytically based mycelial inoculum. In grape flowers, calyptras and stamens dehisce at the start and end of bloom respectively, and often these tissues adhere to the developing berries after being shed and become potent inocula for aggressive infections, as well as leaving wound sites as potential infection sites close to the pedicel (Powelson, 1960).

McClellan and Hewitt (1973) showed that the stigma and style are very turgid in the prebloom and early bloom stages and remain like this for a short period after the calyptra has dehisced. The stigma and style become dried or decayed necrotic tissue, which remains attached to the berries of some cultivars through maturity and harvest. The fungus did not appear to colonise the decayed stigmatic portion after bloom and the lack of moisture in this tissue and the inhibiting effect of berry extracts on this phase may explain this phenomenon.

When *B. cinerea* invades the stylar tissue there is also an abscission layer to bridge from the style to the ovary. Pollen and stigma extracts probably stimulate the bridging of this zone (Chou and Preece, 1968). Chou and Preece (1968) also reported that the enhanced aggressiveness of the fungus in the presence of aqueous pollen. They also demonstrated that the path of infection is through the stigma and style and then into the stylar end of the ovary. The fungus remains latent in the stylar end of the grape, and maximum infection takes place during bloom. Inoculations made during bloom, increased later fruit infections. Fungicide application during bloom therefore usually reduce infections appearing months later. Nair (1985) and Nair and Parker (1985) pointed to bloom as the time of primary infection of grapevines in the Hunter Valley, Australia. These flower infections are followed by a period of latency in the style-end where the pathogen remains in a quiescent phase.

In strawberries, infection is via the receptacle end by way of the stamen and calcycles. Mycelia present in developing fruit as a result of blossom infection remain quiescent until a certain stage of maturity is reached, or when favourable conditions reinitiate growth. The receptacle is then invaded and the rotting phase initiated (Jarvis, 1962). Botrytis rot is therefore dependent on the maturity of the tissue invaded. The stamens in strawberries remain attached to the receptacle throughout the growing season. Strawberry stamens have no abscission zone and they become necrotic shortly after pollen is released. In the grape flowers, stamens dehisce during the shatter stage, just after bloom. Therefore the necrotic stamens, although infected with *B. cinerea,* were not major infection sites (Powelson, 1960), but their wound sites might have been. Ogawa and English (1960) found that necrotic floral tissue was essential for infection of green apricots. He stated that styles, which failed to dehisce, were avenues of infection.

Infection of vegetative tissues before flower infection

Vegetative organs are not normally classified as susceptible. Heavy infection during periods of prolonged wetness, may lead to the colonisation of leaf tissue, but when it dries off the necrotic spots cease growing (Nair and Hill, 1992). Young leaves are very susceptible, whereas mature ones are relatively resistant (Hill *et al.*, 1981). However, these infections can produce conidia later in the season during wet periods. Germination of *B. cinerea* on green leaf tissue is often poor and penetration of healthy tissue is rare (Kamoen *et al.*, 1985). Infection of healthy green tissue will only occur in the field through the direct contact with infected senescent leaves, or infected flower parts (Garrett, 1960). In autumn *B. cinerea* sometimes invades nodes of shoots through the grape stalks and occasionally colonises the grape shoots (Agulhon, 1971). Healthy grape stalks undergo little risk of direct infection but can occasionally be invaded by mycelia growing from flower debris or attached berries (Hill, 1985b). In many cases the problem of stalk rot is related to grape stalk necrosis (stiellaehme), which is a physiological disease mainly based on mineral imbalances of the bunches (Theiller and Mueller, 1986). Because this disease is correlated with the vigorous growth of the vine, cultural practices that restrict growth (green manuring or low nitrogen fertilisation) result in a reduced occurrence of stalk rot and thus of *B. cinerea* infection (Hill, 1985b). Most of the cultivars classified as susceptible to *B. cinerea* stalk rot also show a high incidence of stalk necrosis (Nair and Hill, 1992).

HOST RESISTANCE

Genetic variation for resistance to *B. cinerea* has been observed within species, but no gene-for-gene resistance has been identified (Elad and Evensen, 1995). Leaf resistance may be based on a different mechanism than bunch rot resistance (Nair and Hill, 1992). The young berry shows high resistance due to different contributing factors. These include a preformed system of cuticle structure and tannin like blockages to fungal enzymes and an active defence system which entails stilbene production (Langcake, 1981), suberisation (Hill, 1985b) and lignification (Hoos and Blaich, 1988). However, physiological defence weakens during maturation (Blaich *et al.*, 1984; Hill *et al.*, 1985a; Creasy and Coffee, 1988). Conidia will however penetrate the skin during all developmental stages (Nelson, 1951; Kosuge and Hewitt, 1964; Bessis, 1972; Hill *et al.*, 1981), but are killed off by the resistance mechanisms

in the skin because unripe berries are less susceptible to *B. cinerea* rot than ripe berries. Conidia can only successfully infect after the natural resistance in the berry skin subsides with maturity (Nair and Hill, 1992). Susceptibility of berries increases after véraison and with sugar content above 6-8% (Stein, 1984).

Grape bunch architecture

Under field conditions other factors may contribute to resistance such as a loose grape architecture in the cluster (Lang and Thorpe, 1988). Looser bunches do not provide a moist microclimate or retain flower debris (Northover, 1987). Canopy management not only leads to a less humid environment (English *et al.,* 1989) which leads to a decrease in disease, but also allows better fungicide penetration (Gubler *et al.*, 1987).

Cuticular resistance

Exposure, cultivar and level of contact within the cluster are all important factors in the cuticular membrane formation process and contribute greatly to determining the overall susceptibility of a grape cultivar to bunch rot (Percival *et al.,* 1993). Prudet *et al.* (1992) showed that skin thickness influenced resistance and that it decreased towards maturity, especially after veraison. Pectins also become more digestible and Chardonnet and Donèche (1995) noted that higher calcium levels in the skin tissue results in the chelation state of the pectic substances.

Proanthocyanidins

Hill *et al.* (1981) gave pectins a minor role in resistance and considered the proanthocyanidins in the berry skins to be the major resistance factor. These are proteins that determine the resistance of the cell wall and inhibit endo-polygalacturonase secreted by fungi. The inhibitors are tannin like substances, and their activity decreases towards maturity by oxidation and condensation. High concentrations of this enzyme inhibitor acts against the fungal polygalacturonase (PG), and possibly inactivates toxins of *B. cinerea* as well (Hill *et al.*, 1981). *B. cinerea* has a high potential for breaking down tannins. Stein (1984) therefore considered proanthocyanidins as a minor factor for resistance.

Suberisation

Suberisation was detected in histochemical studies as a bright blue or yellow substance that becomes visible 18-20 h after inoculation. In grape stalks, the fungus was almost completely isolated after 24 h. Inoculation of unwounded stems resulted in a very low percentage of successful infections, suggesting that the intact cuticle is a very effective defence mechanism. Removing of the cuticle by wounding led to infections and stimulation of the suberisation response within 12-16 h after wounding. When side stems were cut off and the cutting surfaces inoculated, only the outer layers of the parenchymatic cells beneath the cuticle were suberised. No suberisation occurred in the vascular bundles. The hyphae grew unhindered into the xylem. Under field conditions attacks on the stems arose from infected berries and grew through the vascular bundles (Hill, 1985b). Grape berries show a similar pattern of suberisation and in unripe berries, the fungus can be isolated but in berries with a sugar concentration of 14% and higher *B. cinerea* infects successfully.

Suberisation is an effective resistance mechanism because it protects the tissue from fungal enzymes and toxins and in part from mechanical injury. The process can be triggered by a heat liable substance of low molecular weight produced by certain fungi. This product is not stable enough for implementing, but other chemical substitutes may exist that can be used for application in order to repair small holes or cracks in the cuticle for protection against infection (Hill, 1985b). Preformed fungitoxic substances are unlikely to be involved in early stages of direct infection through the cuticle but could play an important role in latency after flower infection (Pezet and Pont, 1986; McClellan and Hewitt, 1973; Nair and Parker, 1985).

Stilbenes

Phytoalexins are a group of chemicals of low molecular weight that are inhibitory to micro-organisms and whose accumulation in plants are initiated by interaction of the plant with micro-organisms (Langcake and McCarthy, 1979). In grapevine leaves, different stilbenes were found as well as resveratrol polymers (Langcake and Pryce, 1976). Resveratrol is a stress metabolite and possibly correlated with disease resistance (Langcake and Pryce, 1976, 1977). Pterostilbene and resveratrol are constitutive components of the woody parts of many species. However these compounds are only produced in the leaves and

fruits after exposure to UV - radiation or after fungal infection, and so they could act as phytoalexins (Hart, 1981; Pool *et al.,* 1981).

Formation of stilbenes becomes visible by irradiation of green tissue by UV light resulting in a bright bluish fluorescence. Production is induced soon after the tissue is damaged and is enhanced by chemicals, for instance galactaric acid, copper sulphate and several sugars (Stein and Hoos, 1984). The response decreases in ripened berries (Hill, 1985a). Stilbenes are toxic to *B. cinerea*, but their water solubility in water is low and they react with plant cell walls (Hill, 1985b). The fungus is restricted, but stilbenes do not inactivate the toxins of *B. cinerea* and might have a fungistatic rather than a fungitoxic activity (Stein, 1984). Hill (1985b) also remarks that stilbenes may only be indicators of a wound healing process and do not improve the defence reactions.

Several authors (Pool *et al.*, 1981; Barlass *et al.*, 1987; Bavaresco *et al.,* 1997 ; Dercks and Creasy, 1989) have shown that both the speed and intensity with which stilbenic compounds are formed are indicators of the plant's resistance to fungal infection. The analysis of resveratrol levels in grapevine tissues is therefore used as a basis for the selection of resistant cultivars.

If phytoalexins are important factors in the resistance of a plant to phytopathogenic fungi, the ability of the pathogen to detoxify these compounds could be an important component of the mechanisms of pathogenicity (Van Etten *et al.*, 1989). *Botrytis cinerea* is known to metabolise and thus detoxify phytoalexins from a number of plants (Mansfeld and Hudson, 1980; Pezet *et al.*, 1991). Sbaghi *et al.* (1996) reported that stilbene-degrading activity was related to the presence of a polyphenol oxidase (laccase-like enzyme) in the culture filtrate. Stilbene oxidases isolated from crude protein extracts of *B. cinerea* culture filtrates were shown to have simultaneous stilbene oxidase and laccase activity (Pezet *et al.*, 1991).

Proanthocyanidins of grape berries are potent inhibitors of stilbene oxidase. These tannins could contribute to the resistance of grape by inhibiting stilbene oxidase and preventing detoxification of phytoalexins as suggested by Nyerges *et al.* (1975). Jeandet *et al.* (1991) showed that levels were high in immature clusters but reached a low level in the ripe fruit. Resveratrol was synthesised especially in the skin cells and was absent from, or

low in the fruit flesh. This work showed a negative correlation between resveratrol content of grape skin and the developmental stages of berries. Jeandet *et al.* (1995a) showed that resveratrol was synthesised by living fruit cells surrounding infection sites and where a necrotic area later appeared. This localised response can help to arrest *B. cinerea* spreading lesions. These lesions may remain limited as long as climatic conditions are unfavourable to the pathogen. Spread of *B. cinerea* arises from these infections sites leading to the development of rapidly spreading lesions on fruit when highly favourable conditions prevail in the vineyard. Resveratrol production concurrently increases with further development of *B. cinerea*. At the ripe stage resveratrol production has been shown to be low (Jeandet *et al.*, 1991). The fungitoxic activity, however, was described as doubtful by Hoos and Blaich (1988), due to the water solubility of these stilbenes. Mycelium of *B. cinerea* can metabolise stilbenes quickly *in vitro* and it may therefore not reach effective concentrations to inhibit infecting hyphae of *B. cinerea* from the initially restricted lesions. This could lead to rapid colonisation of ripe clusters by *B. cinerea.*

Barlass *et al.* (1986) assessed a screening procedure for estimating resistance to infection by *Plasmopara viticola.* However, resveratrol production appeared to be highly sensitive to environmental changes, limiting its usefulness as a reproducible screening system. In addition, the technique did not transfer well to *in vitro* grown leaves or to young seedlings. Sbaghi *et al.* (1995) also completed a study in which it appeared that resveratrol could be considered as a good marker for grey mould resistance and would be able to serve as a means of screening for classifcation of susceptible and resistant varieties. The screening procedure represents a crucial step in any selection method for disease resistance. Tissue culture technique might be useful for this purpose (Hammerslag, 1984; Daub, 1986) because large numbers of genotypes might be screened *in vitro* in a limited amount of space and time. Recent results (Fanizza *et al.*, 1995) showed that there was a low relationship between the cultivar response *in vitro* and its susceptibility to grey mould under field conditions when using culture filtrates and phytotoxic polysaccharides for *in vitro* selection of resistant plants.

Hoos and Blaich (1988) suggested that stilbenes exercise a composite action in the defence system of the grapevine exhibiting fungistatic activity, as well as being precursors of the phenolic compounds such as lignin. Bavaresco *et al.* (1997) reported for the first time constitutive *trans*- and *cis*-resveratrol contents in cluster stems of different *V. vinifera*

cultivars at maturity. There is evidence in literature for constitutive resveratrol in lignified organs of grape vine such as canes (Langcake and Pryce, 1976; Pool *et al.,* 1981; Boukharta *et al.,* 1996) and seeds (Pezet and Cuenat, 1996). Variation in cluster stem compounds such as leucoanthocyanidins (Cantarelli and Peri, 1964) and procyanidins (Ricardo-Da-Silva *et al.,* 1991) has also been reported.

Jeandet *et al.* (1995b) suggest that there is a negative relationship between stilbene phytoalexin formation and anthocyanidin content of berry skins. Jeandet *et al.* (1991) found that the ability to produce phytoalexin decreases at véraison. They observed that chalcone synthase (enzyme for anthocyanin biosynthesis) may compete with stilbene (resveratrol) synthase causing a decrease in the ability of grapes to synthesise resveratrol in response to UV-radiation. This is observed after the onset of fruit ripening and may be a consequence of raised anthocyanin accumulation in fruits.

Resveratrol is produced after mechanical injury and fungal infection (Stein, 1984). Under UV - light, stilbenes emit bright blue fluorescence as it accumulates in boundary zones around injury zones of green tissues. Unripe berries have a significant potential for stlbene production but it lessens with maturity (Nair and Hill, 1992).

Different treatments exhibiting no direct fungitoxic or fungistatic activity reduced incidence of *B. cinerea* (Stellwaag-Kittler, 1969). This may, however, be due to an interaction between internal tissue-bound and external factors. For instance, the removal of leaves decreased the incidence of *B. cinerea* (English *et al.,* 1989). This might be due to a better microclimate with quicker drying-off after rain. Furthermore UV radiation hardens berries (Stelwaag-Kitler, 1969) and may also lead to a higher phytoalexin production (Langcake and Pryce, 1977).

CONCLUSION

As fungicide use becomes more restricted and resistance in pathogen populations becomes more widespread, the identification and manipulation of host disease resistance mechanisms are becoming more important (Elad and Evensen, 1995). Many factors contribute to resistance, but infection of the vegetative organs such as leaves, stalks, shoots and especially the pedicel and the resistance mechanisms operating in them, has yet to be

discovered as extensively as that of the generative organs. Most research is done on berry infection and all applied research is based upon this. In other words, all basic research done on disease resistance factors, chemical control, biological control, effect of nutrition (fertiliser), temperature, moisture, humidity (epidemiological studies and disease forecasting), bunch compactness, pruning practices etc., uses the berry as medium and criterion. This is however unpractical if researchers want to screen for resistance. Conventional genetic improvement has proved to be of limited use as the vine has broad heterozygosity (Bessis, 1986). Resistance tests for breeding purposes need to be conducted at a much earlier stage.

This study will therefore correlate berry behaviour with that of the other morphological parts of the grapevine such as leaves, leaf petioles, pedicels, rachises and laterals. Evidence that the disease reaction of the berry correlates with that of another organs, will simplify resistance screenings without having to wait until bunches develop.

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.

Barkai-Golan, R., Lavy-Meir, G. & Kopeliovich, E. 1988. Pectolytic and cellulolytic activity of *Botrytis cinerea* Pers. related to infection of non-ripening tomato mutants. *Journal of Phytopathology* 123: 174-183.

Basham, H.G. & Bateman, D.F. 1975. Killing of plant cells by pectic enzymes: The lack of direct injurious interaction between pectic enzymes or their soluble reaction products and plant cells. *Phytopathology* 65: 141-153.

Barlass, M., Miller, R.M. & Antcliff, A.J. 1986. Development of methods for screening grapevines for resistance to infection by downy mildew.--I. Dual culture *in vitro*. *American Journal of Enology and Vticulture* 37: 61-66.

Barlass, M., Miller, R.M. & Douglas, T.J. 1987. Development of methods for screening grapevines for resistance to infection by downy mildew. II. Resveratrol production. *American Journal of Enology and Vticulture* 38: 65-68.

Bavaresco, L., Cantù, E., Fregoni, M. & Trevisan, M. 1997. Constitutive stilbene contents of grapevine cluster stems as potential source of resveratrol in wine. *Vitis* 36: 115-118.

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.

Bessis, R. 1986. Grapevine physiology: the contribution of culture *in vitro*. *Experentia* 42: 927-933.

Blaich, R., Stein, U. & Wind, R. 1984. Perforationen in der Cuticula von Weinbeeren als morphologischer faktor der Botrytis resistenz. *Vitis* 23: 242-256.

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.

Borochov, A. & Woodson, W. 1989. Physiology and biochemistry of petal abscission. *Horticultural Review* 11: 15-43.

Boukharta, M., Girardin, M. & Metche, M. 1996. Isolement et caractérisation du trans-resveratrol et de l'ε-viniférine à partir du sarment de vigne (Vitis vinifera). XVIIIe Journée Int. Groupe Polyphénols, Bordeaux, 15-18 July. *Polyphenols Communication*. 1: 43-44.

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. & Dubos, B. 1988. Botrytis bunch rot and blight. Pages 13-15 In: Compendium of grape diseases. R.C. Pearson and A.C. Goheen, eds.. The American Phytopathological Society, St Paul, Minnesota.

Bulit, J. & Lafon, R. 1970. Quelques aspects de la biologie du *Botrytis cinerea* Pers., agent de la pourriture grise des raisins. *Vigne Vin* 4: 159-174.

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.

Cantarelli, C. & Peri, C. 1964. The leucoanthocyanins in white grapes: their distribution, amount, and fate during fermentation. *American Journal of Enology and Viticulture* 15: 146-151.

Carre, D.D. 1984. Influence of atmospheric humidity and free water on germination and germ tube growth of *Botrytis cinerea* Pers. M.S. thesis. Oregon State University, Corvallis.

Chardonnet, C. & Doneche, B. 1995. Relation entre la teneur en calcium et la resistance a la digestion enzymatique du tissu pelliculaire au cours de la maturation du raisin. *Vitis* 34: 95-98.

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.

Corbaz, R. 1972. Etudes des spores fongiques captèes dans l'air. *Phytopatologische Zeitschrift* 74: 314-328.

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

Daub, M.E. 1986. Tissue culture and the selection of resistance to pathogens. *Annual Review of Phytopathology* 24:159-186.

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

Dercks, W. & Creasy, L.L. 1989. The significance of stilbene type phytoalexins in the *Plasmopara viticola*-grapevine interaction. *Physiological and Molecular Plant Pathology* 34: 189-202.

Elad, Y. 1992. The use of antioxidants (free radical scavengers) to control grey mold (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. *Plant Pathology* 41: 417-426.

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

Elad, Y & Volpin, H. 1988. The envolvement of ethylene and calcium in grey mold of pelargonium, ruscus and rose plants. *Phytoparasitica* 16: 119-131.

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.

Fanizza, G., Bisignano, V., Pollastro, S., Miazzi, M. & Faretra, F. 1995. Effects of polysaccharides from *Botryotinia fuckeliana (Botrytis cinerea)* on *in vitro* culture of table and wine grapes (*Vitis vinifera). Vitis* 34: 41-44.

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 grape by *Botrytis cinerea* and consequences for the spraying schedule. *Quaderni Della Scuola Di Specializzazione In Viticoltura Ed Enologia, Università di Torino* 9: 255-266.

Gubler, W.D., Marois, J.J., Blesoe, L.J. 1987. Control of *Botrytis* bunch rot of grape with canopy management. *Plant Disease* 71: 599-601.

Hammerschlag, F.A. 1984. Optical evidence for an effect of culture filtrates of *Xanthomonas campestris* pv. *pruni* on peach mesophyll cell membranes. *Plant Science Letters* 34: 295-304.

Hart, J.H. 1981. Role of phytostilbenes in decay and disease resistance. *Annual Review of Phytopathology* 19: 437-458.

Harvey, J.M. 1955. A method of forecasting decay in California storage grapes. *Phytopathology* 45: 229-232.

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

Hill, G.K. 1985a. Botrytis control-current knowledge and future prospects. *Proceedings of Sixth Australian Wine Industry Technical Conference, Australia.*

Hill, G.K. 1985b. 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.

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): 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.

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. 1962. Splash dispersal of spores of *Botrytis cinerea* Pers. *Nature (London)* 193: 599.

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

Jeandet, P., Bessis, R. & Gautheron, B. 1991. The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. *American Journal of Enology and Viticulture* 42: 41-46.

Jeandet, P., Bessis, R., Sbaghi, M. & Meunier, P. 1995a. Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. *Phytopathologische Zeitschcrift* 143: 135-139.

Jeandet, P., Sbaghi, M., Bessis, R. & Meunier, P. 1995b. The potential relationship of stilbene (resveratrol) synthesis to anthocyanin content in grape berry skins. *Vitis* 34: 91-94.

Johnston, D.J. & Williamson, B. 1992. Purification and characterization of four polygalacturonases from *Botrytis cinerea*. *Mycological Research* 96: 343-349.

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

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

Lang, A. & Thorpe, M. 1988. Why do berries split? In Proceedings of the Second International Cool Climate Viticultural and Oenological Symposium., Auckland, New Zealand p. 69.

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

Langcake, P. & Pryce, R.J. 1976. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiological Plant Pathology* 9: 77-86.

Langcake, P. & Pryce, R.J. 1977. The production of resveratrol and the viniferins by grapevines in response to ultraviolet radiation. *Phytochemistry* 16: 1193-1196.

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

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

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.

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. & Hill, G.K. 1992. Bunch rot of grapes caused by *Botrytis cinerea*. Pages 147-169 in: Plant Diseases of International Importance. Vol III: Diseases 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* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. *Journal of Phytopathology* 119:52-63.

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

Nelson, K.E. 1951. Factors influencing the 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.

Nyerges, P., Szabó, E. & Donkó, E. 1975. The role of anthocyan and phenol compounds in the resistance of grapes against *Botrytis cinerea*. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 10: 21-32.

Ogawa, J.M. & English, H. 1960. Blossom blight and green fruit rot of almond, apricot and plum caused by *Botrytis cinerea*. *Plant Disease Reporter* 44:265-268.

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

Pezet, R. & Cuenat, P. 1996. Resveratrol in wine: Extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *American Journal of Enology and Viticulture* 47: 287-290.

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.

Pezet, R., Pont, V. & Hoang-Van, K. 1991. Evidence for oxidative detoxication of resveratrol and pterostilbene by a laccase-like stilbene oxidase produced by *Botrytis cinerea*. *Physiological and Molecular Plant Pathology* 129: 19-30.

Pool, R., Creasy, L.L. & Frackleton, A.S. 1981. Resveratrol and the viniferins, their application to screening for disease resistance in grape breeding programs. *Vitis* 20: 136-145.

Possner, D.R.E. & Kliewer, W.M. 1985. The localisation of acids, sugars, potassium and calcium in developing berries. *Vitis* 24: 229-240.

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

Prudet, S., Dubos, B. & Le Menn, R. 1992. Some characteristics of resistance of grape berries to grey mold caused by *Botrytis cinerea*. Recent advances in Botrytis research. Proceedings of the 10th International *Botrytis* symposium, Heraklion, Greece. K. Verhoeff, Malathakis, N.E. & Williamson, B.eds. Pudoc Scientific publishers, Wageningen.

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.

Ricardo-Da-Silva, J.M., Bourzeix, M., Cheynier, V. & Moutounet, M. 1991. Procyanidin composition of Chardonnay, Mauzac and Grenache-blanc grapes. *Vitis* 30: 245-252.

Sabehat, A. & Zieslin, N. 1994. GA**3** effects on postharvest alterations in cell membranes of rose (*Rosa x hybrida*) petals. *Journal of Plant Physiology* 144: 513-517.

Salinas, I., Schots, A. & Verhoeff, K. 1992. Prevention of gerbera flowers of infection by *Botrytis cinerea* using monoclonal anttibodies against cutinase. Pages 75-84 in: Function of Cutinolytic Enzymes in the Infection of Gerbera flowers by *Botrytis cinerea.* Ph.D. Dissertation. Utrecht University, Utrecht, the Netherlands.

Savage, S.D. & Sall, M.A. 1982. The use of a radio-immunosorbent assay for *Botrytis cinerea. European Plant Protection Bulletin* 12: 49-53.

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

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

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

Stein, U. 1984. Untersuchungen über biochemische und morphologische Merkmale de *Botrytis*-resistenz bei Vitaceen. Ph.D. Dissertation, Universität Karlsruhe, Karlsruhe.

Stein, U. & Hoos, G. 1984. Induktions- und Nachweismethoden für Stilbene bei Vitaceen. *Vitis* 23: 179-194.

Stellwaag-Kittler, F. 1969. Moeglichkeiten der Botrytiskaempfung an Trauben unter Bercksichtigung der epidemiologischen Grunlagen. *Weinberg Keller* 16: 109-134.

Strizyk, S. 1983. Modele d'etat potentiel d'infection application au *Botrytis cinerea*. Version 1983. *Association de coordination technique agricole.* France.

Theiller, R. & Mueller, H. 1986. Beziehungen zwischen Klimafaktoren und dem Steilaehmebefall bei Riesling x Silvaner. *Vitis* 25: 8-20.

Van Etten, H., Matthews, D.E. & Matthews, P.S. 1989. Phytoalexin detoxification: importance for pathogenicity and practical implications. *Annual Review of Phytopathology* 27: 143-164.

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. 1974. Latent infection by fungi. *Annual Review of Phytopathology* 12: 99-110.

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.

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

2. NATURAL *BOTRYTIS CINEREA* INFECTION AND DISEASE EXPRESSION IN PARTS OF LEAVES AND BUNCHES OF GRAPEVINE

ABSTRACT

Natural *Botrytis cinerea* infection at specific sites in leaves and bunches of grapevine and resistance to disease expression in the morphological parts was determined. Bunches and leaves of the wine grape cultivar, Merlot, and the table grape cultivar, Dauphine, were collected at pea-size, bunch closure and harvest from five vineyards in the Stellenbosch and De Doorns regions, respectively. The material was divided into two groups and sealed in polythene bags. The bags were lined with wet paper towels to establish high relative humidity. Leaves and bunches incubated in one group of bags were first treated with paraquat in order to terminate active host responses. These treatments provided conditions which facilitated disease expression under two host resistance levels by different inocula during the period of moist incubation. Disease expression was positively identified by lesion development, and the formation of sporulating colonies of *B. cinerea* at a potential infection site. Sites in leaves were the blades and petioles. Sites in bunch parts were rachises, laterals and pedicels, and sites on berries were the pedicel-end, cheek and style-end. In Dauphine, the various sites were at all stages classified as resistant to moderately resistant. However, at pea size and bunch closure, in spite of their resistance, nearly all the sites carried high to very high inoculum levels. The only exception was the berry cheek, which carried intermediate inoculum levels at pea size, and low inoculum levels at bunch closure. In nearly all sites, inoculum levels were lower at harvest. The decrease was, however, the most prominent in petioles, rachises, laterals, pedicels and the pedicel-end of the berry. All these sites carried intermediate to low inoculum levels at harvest. In Merlot, sites consistently gave a resistant reaction, except for the pedicel and pedicel-end of the berry, which changed from resistant at the early developmental stages to susceptible at harvest. Inoculum levels decreased during the season in the rachises and laterals, but were constantly high during the season in the pedicel and pedicel-end of the berry. According to this pattern of natural occurrence, *B. cinerea* fruit rot in these vineyards was not caused by colonisation of the pistil, and subsequent latency in the style end of of grape berries. However, fruit rot was primarily

caused by colonisation of the pedicel, and subsequent latency in the pedicel or pedicel-end of the berry. These findings suggest that the role of infection in rachises, laterals and pedicels is underestimated in the epidemiology of *B. cinerea* on grapevine.

INTRODUCTION

Botrytis cinerea Pers.:Fr. attacks bunches, leaves, buds, and canes of grapevine (*Vitis vinifera* L) and causes grey mould (Nair and Hill, 1992). Berries, on which the most prominent symptom of the disease is found (Nair and Nadtotchei, 1987), are considered resistant to infection when immature, and susceptible when mature (Hill *et al.*, 1981; Nair and Hill, 1992; Nelson, 1956). In spite of this differential susceptibility, infection of flowers and berries may destroy immature fruit (McClellan and Hewitt, 1973; Nair and Parker, 1985). In addition, colonised senescent floral tissues and aborted berries can serve as conidial and mycelial inoculum (Gessler and Jermini, 1985; Hill, 1985; Northover, 1987; Nair and Nadtotchei, 1987) for late-season infections of sound berries. Grape stalks undergo little risk from direct infection by conidia of *B. cinerea* but can occasionally be invaded by mycelial material growing from flower debris or attached berries (Hill, 1985). In autumn *B. cinerea* sometimes invades nodes of shoots through the grape stalks and occasionally colonises the grape shoots (Agulhon *et al.*, 1971). Leaves are not normally considered susceptible, but infection during periods of prolonged wetness may lead to colonisation of leaf tissue (Nair and Hill, 1992). Lesions formed on young leaves, can later in the season produce conidia in wet periods, thereby contributing to the total inoculum load in a vineyard.

Infection of immature berries is often followed by a latent period, defined as the interval from infection to the display of macroscopic symptoms (McClellan and Hewitt, 1973). Evidence for the importance of these latent infections in subsequent disease development is primarily circumstantial. In California and Australia, McClellan and Hewitt (1973) and Nair and Parker (1985) found that berry infection takes place during bloom. They showed that *B. cinerea* invades the stigma and style and then becomes latent in necrotic stigma and style tissue at the style end of the berry. Grape clusters remain symptomless between the flowering period and the beginning of ripening, and a pathogenic relationship is generally established once the fruit ripens. At veraison or later the fungus resumes growth and rots the berry (Mclellan and Hewitt, 1973; Nair and Parker, 1985; Pezet and Pont, 1986). In Switzerland, Pezet and Pont (1986) found no evidence for the style end infection pathway

and showed that latent infection was predominantly pedicel-associated. Their histological studies of laboratory-inoculated bunches showed that *B. cinerea* colonises the stamens during bloom and invades their base situated on the receptacle. From there it spreads to the pedicel and via the vascular tissue into the berries. Savage and Sall (1982), however, were unable to detect the pathogen in immature berries. De Kock and Holz (1991) found no relation between early infection and subsequent disease development or postharvest decay on table grapes. Decay was largely due to infection during storage by inoculum present in bunches at véraison or during later stages. It was later shown (Holz *et al.*, 1997, 1998; Holz, 1999) that berry cheeks were virtually free from natural *B. cinerea* infection during all developmental stages, and confirmed that berry infection was predominantly pedicel-associated. These workers (Holz *et al.*, 1997, 1998; Holz, 1999) furthermore showed that natural latent *B. cinerea* infection may generally occur in the other morpohological parts of grape bunches, and is therefore not exclusive to the grape pistil. Their findings furthermore suggest that natural latent infection levels are high in pedicels, and that resistance mechanisms operative in the pedicel suppress natural symptom expression.

There has been substantial difficulty in reproducibly demonstrating the presence of latent *B. cinerea* infections in grapevine. This phenomenon can be ascribed to a poor understanding of infection pathways followed by the pathogen (Coertze and Holz, 1999; Coertze *et al.*, 2001; Holz *et al.*, 1997, 1998; Holz, 1999), and thus of infection levels in grape tissue. Knowledge of the presence of *B. cinerea* in morphological parts of bunches and leaves of grapevine would help to find a reliable, sensitive, and specific assay to verify the actual occurrence of latent infection, and to plan effective disease control strategies. The aims of this study were (i) to determine natural *B. cinerea* infection at specific sites in leaves and bunches of grapevine at different phenological stages, and (ii) to determine resistance in the morphological parts to disease expression.

MATERIALS AND METHODS

Vineyards. Five vineyards (table grape cultivar Dauphine) were selected in De Doorns, a region well-known for table grape production, and five (wine grape cultivar Merlot) in Stellenbosch, which is well-known for wine grape production. The table and wine grape vineyards are approximately 120 km apart and separated by a series of mountain ranges. Both regions are in the winter rainfall region and has a moderate mediterranean

climate, with De Doorns being marginally drier. Dauphine vineyard blocks ranged from 1-5 ha and the vines were trained to a slanting trellis at 3 x 1.5 m spacings. All vines were micro-irrigated. Canopy management and bunch preparation were done according to the guidelines of Van der Merwe *et al.*, (1991). Merlot vineyard blocks ranged from 1-5 ha and the vines were trained to a two wire trellis system or goblet vines. All vines were drip-irrigated. A recommended programme for the control of downy and powdery mildew, and *B. cinerea* (De Klerk, 1985) was followed in all the vineyards. Sprays against downy mildew started at 10-15 cm shoot length and were applied every 14 days until pea size. Fungicides used were folpet (Folpan 50 WP, Agrihold), fosetyl-Al/mancozeb (Mikal M 44/26 WP, MayBaker), mancozeb (Dithane M45 80 WP, FBC Holdings) and mancozeb/oxadixyl (Recoil 56/8 WP, Bayer). Applications against powdery mildew started at 2-5 cm shoot length and were applied every 14 days until 3 wk before harvest. Fungicides used were penconazole (Topaz 10 EC, Syngenta), pyrifenox (Dorado 48 EC, Maybaker) and triadimenol (Bayfidan 25 EC, Bayer). Sprays against *B. cinerea* were applied at flowering, bunch closure, véraison and 2 weeks before harvest. Fungicides used were iprodione (Rovral Flo 25 EC, Aventis) and pyrimethanil (Scala 40EC, Aventis).

Infection periods. Temperature and rainfall for the 1998-2000 growing seasons were recorded at weather stations at Hexriver Valley (De Doorns) and Berg River Valley (Stellenbosch). Infection periods during each growing season were determined on the basis of the infection criteria of Sall *et al.* (1981). A rainy period was considered conducive to the natural development of *B. cinerea* if more than 5 mm rain was recorded during 24 h (relative humidity ≥92%; average temperature 15-22°C), or if 1-5 mm rain fell on each of two consecutive days (relative humidity ≥92%; average temperature 15-22°C).

Decay incidences. Sound unblemished leaves (20 per vineyard, 100 per cultivar) and bunches (10 per vineyard, 50 per cultivar) were selected for two consequtive seasons at pea-size, bunch closure and two weeks prior to harvest. At each sampling, leaves from each vineyard were divided in two groups of 50 leaves each. Bunches from each vineyard was carefully divided in two, more or less equal parts by cutting the rachis. The parts from each vineyard were divided in two groups of 50 bunch parts each. The material of one group was left untreated, and the other goup immersed in paraquat solution (30 ml/l water) for 30 seconds, rinsed in sterile deionised water and air-dried. Each leaf or bunch part was sealed in a polythene bag. The bags were lined with wet paper towels to establish high relative

humidity necessary for disease expression. The material was kept at 22°C under a diurnal light regime (12-h photoperiod) and examined daily for symptom development. Disease expression was positively identified by lesion development, and the formation of sporulating colonies of *B. cinerea* at a potential infection site. In the case of leaves, sites were the blades and petioles. Sites in bunch parts were rachises, laterals and pedicels, and on berries the pedicel-end, cheek and style-end (Fig. 1). Disease expression at each site was recorded for each sample, and incidences for each site calculated after 14 days. These treatments provided conditions which facilitated disease expression under two host resistance levels by different inocula during the period of moist incubation. On untreated material, disease expression at a given site was the result of infection by surface inoculum during incubation under high humidity and the development of latent mycelia in host tissue. Decay incidences therefore gave an indication of infection at a specific site as influenced by inoculum levels (surface and latent inoculum) and by host resistance. Paraquat terminated host resistance in the cells of the cuticular membrane without damaging host tissue (Baur *et al.*, 1969; Cerkauskas and Sinclair, 1980; Pscheidt and Pearson, 1989; Grindrat and Pezet, 1994). On paraquat-treated material, decay incidences gave an indication of infection by the inocula when host resistance was terminated.

Disease resistance and inoculum levels. At each developmental stage, sites were categorised for disease resistance according to the mean decay incidences recorded in the untreated material of a cultivar. Sites showing decay of ≤5%, 6-20%, 21-40% and ≥41% were classified respectively as resistant, moderately resistant, susceptible and highly susceptible to infection. The sites were also categorised into different sub-classes according to decay development in the paraquat treatment to describe their inoculum level (surface inoculum and latent mycelia). Sites showing decay of ≤5%, 6-20%, 21-40% and ≥41% were classified respectively as carrying low, intermediate, high and very high inoculum levels.

Statistical analysis. A split plot experimental design was used in all experiments. 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 and Wilk, 1965). The data was examined further by using the analysis of variance (ANOVA) and the treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor and Cochran, 1980).

RESULTS

Infection periods. Daily temperature and rainfall for the 1998-2000 growing seasons are shown in Figs. 2-5. The number of infection periods recorded before each sampling are given in Table 1-2. In 1998, climatic conditions favoured the natural development of *B. cinerea* in vineyards in both the Hexriver and Bergriver regions during bloom to pea size, and from pea size to bunch closure. Thereafter, conditions were generally unfavourable for the development of the pathogen. In 1999, conducive periods were recorded during bloom to pea size only in the Bergriver region.

Decay incidences. Analysis of variance for effects of season, phenological stage, cultivar and treatment on decay development is given in Table 3. As the pathogen did not in any of the samplings develop from the style-end, data of this site were not included in the analysis. In both seasons, significantly more sites in the paraquat-treated tissue than in the untreated tissue (Table 4) developed decay. Decay levels for both treatments were futhermore significantly higher in the first than the second season. For the untreated material, decay levels were relatively low at each developmental stage (Table 5). It was however significantly higher at harvest than at bunch closure. For the paraquat treatment, infection levels were high at pea size, then significantly declined at the later stages.

Based on the significance levels of decay incidences, sites in the untreated material were grouped in two classes (Table 6) consisting of the pedicels and the pedicel-end of the berries as one group, and the leaf blades, petioles, cheeks, laterals and rachises as a second group. In 1998, sites in the first group carried significantly higher infection levels than the other group. Decay incidences in the first group ranged between 9 and 14%, and in the second group between 4 and 6%. Decay incidences between the sites were not significantly different in the next season. However, decay was at a meaningful higher level in pedicels and the pedicel-end of the berries than in the other sites. Nearly a similar pattern in decay was found in the paraquat treated material. In 1998, decay incidences in the first group ranged between 62 and 67%. In 1999, incidences were between 38 and 42%. Decay incidences in the berry cheeks were consistently low and ranged between 2 and 7%. The sites furthermore reacted in a consistent pattern during the two seasons. In the untreated material, decay incidences at the different sites were at a similar level for both seasons, except for pedicels, which developed significantly more decay in the first than in the second season. In the

paraquat treatment, decay levels at all sites, except for the berry cheeks, were significantly higher in 1998 than 1999.

Decay at the different sites followed a characteristic pattern at each of the three developmental stages (Table 7). At pea size, decay levels at sites in the untreated material were low and did not differ significantly, except for differences between the rachises and the laterals. Incidences were, however, meaningfully higher in the laterals and rachises than at the other sites. In the paraquat-treated material, on the other hand, decay was exceptionally high (78%) in the pedicel-end of the berry. Decay levels were also significantly higher in the rachises, laterals and pedicels (ranging from 54 to 58%) than in the rest of the sites. Decay levels in the two treatments followed a similar pattern at bunch closure, but changed drastically at harvest stage. At this stage, in both treatments, decay incidences in the pedicel and pedicel-end of the berry were at a significantly higher level than at the other sites. The most significant changes in disease reaction during the season was found in the pedicels and the pedicel-end of the berry, and the rachises and laterals. In the first group, decay in the untreated material was low at pea size and bunch closure, but increased significantly at harvest stage. Decay at these sites followed an opposite trend in the paraquat treated material. It was significantly higher at pea size than at the harvest stage. In the rachises and laterals, levels in the untreated material did not differ significantly between stages, although it was markedly higher at pea size stage. Decay levels were, however, significantly higher at pea size than at harvest when the material was exposed to paraquat. The only site which showed a consistent reaction during the season was the berry cheek, which yielded low decay levels in both treatments and showed no significant change in decay pattern during the season.

In the comparison between cultivars (Table 8), meaningful differences were again found in the reaction of the pedicels and the pedicel-end of the berry, and the rachises and laterals. In Dauphine, decay levels were high in these sites at pea size, then decreased to a significant low level at harvest. In Merlot, decay levels increased significantly from pea size to harvest. In rachises and laterals of both cultivars, decay followed a similar decreasing pattern during the season. Again, berry cheeks were the only site that showed a constant reaction and which, in the case of both cultivars, yielded the pathogen at a low level during the season.

Disease resistance and inoculum levels. Mean decay levels for both cultivars, based on the data recorded at the various sites in two seasons, are given in Tables 9-10. Descriptions of disease resistance and of inoculum levels are given in Tables 11-12. In Dauphine, the various sites were at all stages classified as resistant to moderately resistant. However, at pea size and bunch closure, in spite of their resistance, nearly all the sites carried high to very high inoculum levels. The only exception was the berry cheek, which carried intermediate inoculum levels at pea size, and low inoculum levels at bunch closure. In nearly all sites, inoculum levels were lower at harvest. The decrease was however the most prominent in petioles, rachises, laterals, pedicels and the pedicel-end of the berry. All these sites carried intermediate to low inoculum levels at harvest. In Merlot, sites constantly reacted resistant, except for the pedicel and pedicel-end of the berry, which changed from resistant at the early developmental stages to susceptible at harvest. Inoculum levels decreased during the season in the rachises and laterals, but were constantly high during the season in the pedicel and pedicel-end of the berry.

DISCUSSION

In this study leaves and bunches were kept under conditions which facilitated disease expression by both surface inoculum and latent mycelia of *B. cinerea* under the influence of host resistance, or when host resistance was terminated. The pathogen consistently developed from tissues of the leaf blade, petiole, rachis, lateral, pedicel, the pedicel-end of the berry, and the berry cheek, but never from the style-end. Decay levels were furthermore consistently higher on paraquat-treated than non-treated tissues. This finding indicated that the amount of *B. cinerea* at different sites on leaves and bunches may be higher than generally assumed, and that moist incubation of non-paraquat treated tissues mostly does not give a good indication of the amount of *B. cinerea* occurring naturally on surfaces or in the tissues of grapevine. Disease expression by untreated parts was therefore not governed by the amount of *B. cinerea* occurring on their surfaces or in their tissues, but by the ability of their tissues to resist disease expression. My findings on the behaviour of the pathogen in the tissues of Dauphine and Merlot grapes furthermore indicate that cultivars may differ in the resistance reaction of their structural bunch parts to natural *B. cinerea* inoculum in the vineyard. Passive defence (proanthocyanidins in skins [Hill *et al.*, 1981], substances in berry exudates [Kosuge and Hewitt, 1964; Mclellan and Hewitt, 1973; Padgett and Morrison, 1990; Pezet and Pont, 1984; Vercesi *et al.*, 1997]) and active defence

mechanisms (lignification-like reactions [Hoos & Blaich, 1988], phytoalexins [Langcake, 1981] and suberin [Hill, 1985]) play an important role in the resistance of grapevine to infection by *B. cinerea*.

Decay levels for a specific site in the morhological parts differed between vineyards, and between seasons. These differences can normally be ascribed to the influence of different sets of environmental and climatical conditions, and cultivation practices exerted on *B. cinerea* in each vineyard (Jarvis, 1980). In this investigation, weather conditions were more conducive for the development of *B. cinerea* in the first season of the study. However, notwithstanding these differences, decay in the morphological parts followed a similar, constant pattern in all vineyards during both seasons. Based on the combined data for the different treatments, decay levels were the highest in the pedicels and the pedicel-end of the berry. Overall, approximately 30% of these sites yielded *B. cinerea.* Levels were lower in leaf blades, rachises and laterals, of which approximately 20% yielded *B. cinerea.* The pathogen caused decay of petioles (10%) and berry cheeks (5%) less often. The style end of the berries, on the other hand, were virtually free (≤0.02%) of *B. cinerea* decay. Data on the style-end was therefore not included in the statistical analyses. Careful observation furthermore 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. According to this pattern of natural occurrence of the pathogen in grape bunches, *B. cinerea* fruit rot in these vineyards was not caused by colonisation of the pistil, and subsequent latency in the style end of of grape berries, as was observed elsewhere (McClellan and Hewitt, 1973; Nair and Parker, 1985). However, fruit rot was primarily caused by colonisation of the pedicel, and subsequent latency in the pedicel or pedicel-end of the berry, as was shown by other workers (Pezet & Pont, 1986; Holz *et al.*, 1997,1998).

Nair *et al.* (1988) studied factors predisposing grape berries to infection by the pathogen, and concluded that infection cannot take place through uninjured skin. My findings on the reaction of grape berry cheeks to natural *B. cinerea* infection substantiated this finding. It furthermore confirmed those of Coertze *et al.* (2001) on the resistance of berry cheeks to *B. cinerea* infection and to disease expression by airborne conidia. In most studies where grapes were artificially inoculated, berries were atomised with (De Kock and Holz, 1991; Nair, 1985; Nair *et al.,* 1988; Nelson, 1951), dipped in (Broome *et al.*, 1995), or injected with (Avissar and Pesis, 1991; Marois *et al.*, 1986; Thomas *et al.,* 1988) conidial

suspensions, or suspension droplets were placed onto the berry cheek (Chardonnet, 1997; Marois *et al.*, 1987). These methods allowed for the deposition of groups 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. Working with ripe Dauphine table grapes inoculated fresh or after cold storage, Coertze and Holz (1999) recently proved the infectivity of single airborne conidia of the pathogen on grapevine. In a subsequent study, Coertze *et al.* (2001) simulated natural infection by airborne conidia in their studies with *B. cinerea* on grape, and found that natural resistance mechanisms render Dauphine grape berries resistant to both penetration and to disease expression when challenged from berry set to the ripe stage by solitary conidia at several sites on the berry surface. By using a differential set of segment isolation and freezing techniques on sterile and non-sterile berries, they proved that latent infections in grape berry cheeks established by this inoculum format were few, and may not contribute to a gradual built-up of secondary inoculum. Preliminary studies (Holz, 1999) with solitary conidia confirmed this trend on table grape cultivars Barlinka and Waltham Cross, and wine grape cultivars Merlot, Chenin Blanc, and Shiraz. These findings, and those of the present study suggest that the role of latent infection in rachises, laterals and pedicels is underestimated in the epidemiology of *B. cinerea* on grapevine. Pezet and Pont (1986) showed in their histological studies of laboratory-inoculated bunches that *B. cinerea* colonises the stamens and invades their base situated on the receptacle. From there it spreads to the vascular tissue in the berry and to the pedicel. These findings imply that incipient infections can cause both mid- or late-season bunch rot following a period of fungal latency in the rachises, laterals or pedicels, and not in berry cheeks and style ends.

For a facultative saprophyte such as *B. cinerea*, inoculum in vineyards is almost always present and may not be as important a parameter as the presence or absence of a conducive environment (Broome *et al.*, 1995). However, little is known about the relationship between conidial density of *B. cinerea* on parts of grape bunches and disease development. Corbaz (1972) and Bulit and Verdu (1973) found a fluctuation in the concentration of *B. cinerea* conidia in the air during the growing season in French vineyards; the highest numbers occurred from véraison to vintage. On the other hand, data on washings made from grape berries in Californian (Duncan *et al.*, 1995) and South African vineyards (G. Holz, unpublished data) indicated that the amount of *B. cinerea* on berry surfaces was very low throughout the season, and *B. cinerea* occurred as single colony forming units.

Holz and Coertze (1996), later showed that berry cheeks were virtually free from natural *B. cinerea* infection during all developmental stages. Other reports (Holz *et al.*, 1997, 1998; Holz, 1999; Part 3) indicated high natural latent infection levels in rachises, laterals and pedicels in immature bunches, which decline to low levels at maturity. These findings suggest that resistance mechanisms operative in the structural bunch parts suppress natural symptom expression, but also point to high inoculum loads in vineyards during bloom to bunch closure. My findings support the hypothesis of increased host resistance during development, but also indicate that in the Western Cape province, inoculum in vineyards is during the early part of the season, and less abundant later in the season. Disease management strategies should therefore concentrate on the pre-bunch closure stage, and coverage of the internal bunch parts.

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.

Avissar, I. & 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 ultrastructure of mesquite mesophyll cells. *Weed Research* 9: 81-85.

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 June – 1 July, Teresin, Poland.

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

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 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. & Sadie, A. 2001. Germination and establishment of latent infections on grape berries by single conidia of *Botrytis cinerea*. *Plant Disease*. In press.

Corbaz, R. 1972. Etudes des spores fongiques captées dans l'air. *Phytopatologische Zeitschrift* 74: 318-328.

De Klerk, C.A. 1985. Chemical control programme for vine diseases and pests. Farming in South Africa. Viticulture and Oenological Series 200. Nietvoorbij Institue for Viticulture and Oenology. Private bag X5026, 7599 Stellenbosch, Republic of South Africa.

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

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 infections of grape by *Botrytis cinerea* and consequences for the spraying schedule. *Quaderni Della Scuola Di Specializzazione In Viticoltura 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.

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.

Hill, G. 1985. Suberization 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.

Holz, G. 1999. Behaviour and infection pathways of diverse fungal pathogens on fruit. Page 257 In: Conference Handbook. Bien. Aust. Plant Pathol. Soc. Conf., 12th. Canberra, Australia.

Holz, G. & Coertze, S. 1996. Growth of separate, individual conidia of *Botrytis cinerea* on fruit surfaces. Page 47 In: Scientific Programme and Abstracts. XIth Int. *Botrytis* Symp., Wageningen, Netherlands.

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): 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.

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*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

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., Bledsoe, A.M., Gubler, W.D. & 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.

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.

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. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. *Austrialian Journal of Agricultural Research* 36:435-442.

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. *Phytopathology* 119:42-51.

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 Diseases of International Importance. Vol III: Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

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.

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 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.

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

Sall, M.A., Teviotdale, B.I. & Savage, S.D. 1981. Bunch rots. Pages 51-56 In: Grape Pest Management. D.L. Flaherty, F.L. Jensen, A.N. Kasimatis, H. Kido and W.J. Moller (eds). Publication No. 4105, Division of Agricultural Sciences, University of California, Berkeley.

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

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

Savage, S.D. & Sall, M.A. 1982. The use of a radio-immunosorbent assay for *Botrytis cinerea. European Plant Protection Bulletin* 12: 49-53.

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.

Van der Merwe, G.G., Geldenhuys, P.D. & Botes, W.S. 1991. Guidelines for the preparation of table grape cultivars for export. Unifruco, Bellville.

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.

Table 1. Number of infection periods recorded before each sampling in Dauphine vineyards in the Hexriver Valley

Sampling stage	1998/1999	1999/2000
Pea	4	0
Bunch closure	3	1
Harvest	1	0

Table 2. Number of infection periods recorded before each sampling in Merlot vineyards in the Bergriver Valley

Sampling stage	1998/1999	1999/2000
Pea	5	3
Bunch closure	1	1
Harvest	0	0

Table 3. Analysis of variance for effects on percentage decay in *Botrytis cinerea* infected grapevine tissue

Source of variation	Df[a]	MS[b]	SL[c]
Season (S)	1	23914.671	0.0001
Phenological Stage (P)	2	7674.300	0.0055
S x P	2	751.043	0.5370
Error (S x P)	24	1176.757	
Cultivar (C)	1	4452.805	0.0273
S x C	1	142.519	0.6779
P x C	2	3802.433	0.0187
S x P x C	2	721.633	0.4218
Error (S x P x C)	24	806.219	
Treatment (T)	1	174932.005	0.0001
S x T	1	6800.119	0.0008
P x T	2	11716.176	0.0001
S x P x T	2	1222.062	0.1116
C x T	1	3432.386	0.0144
S x C x T	1	820.119	0.2205
P x C x T	2	424.329	0.4564
S x P x C x T	2	53.319	0.9048
Error (S x P x C x T)	48	532.117	
Morphological Parts (MP)	6	11223.783	0.0001
S x MP	6	1182.894	0.0001
P x MP	12	1064.061	0.0001
S x P x MP	12	306.449	0.0008
C x MP	6	1461.071	0.0001
S x C x MP	6	131.319	0.2902
P x C x MP	12	466.983	0.0001
S x P x C x MP	12	318.650	0.0005
T x MP	6	7850.627	0.0001
S x T x MP	6	858.525	0.0001
P x T x MP	12	1431.082	0.0001
S x P x T x MP	12	291.012	0.0014
C x T x MP	6	224.252	0.0520
S x C x T x MP	6	86.875	0.5608
P x C x T x MP	12	329.979	0.0003
S x P x C x T x MP	12	345.925	0.0002
Error	576	106.9829	

[a] Degrees of freedom
[b] Mean square
[c] Significance level

Table 4. Mean decay incidences[w,x] recorded during two seasons in grapevine tissues[y] naturally infected with *Botrytis cinerea*

Treatment [z]	1998	1999
Paraquat	42.05 a	25.69 b
Untreated	7.49 c	2.50 d

[w] Bunches and leaves were sealed in polythene bags lined with wet paper towels to establish high relative humidity necessary for disease expression. Disease expression was positively identified by lesion development, and the formation of sporulating colonies of *B. cinerea* at a potential infection site. Sites in leaves were the blades and petioles. Sites in bunch parts were rachises, laterals and pedicels, and on berries the pedicel attachment area, cheek and style end.

[x] Values of each column or row followed by the same letter are not statistically different according to the Student's t – test ($P = 0.0001$).

[y] Material obtained at pea size, bunch closure and before harvest from five table grape (cultivar Daupine), and five wine grape (cultivar Merlot) vineyards.

[z] Paraquat = material immersed in paraquat solution (30 ml/l water) for 30 seconds; untreated = material left untreated.

Table 5. Mean decay incidences[w,x] caused by natural *Botrytis cinerea* infection in grapevine tissues[y] at three phenological stages

Treatment [z]	Pea-size	Bunch closure	Harvest
Paraquat	44.33 a	36.00 b	21.27 c
Untreated	4.49 de	3.86 e	6.67 d

[w,y,z] See Table 4.

[x] Values of each column or row followed by the same letter are not statistically different according to the Student's t – test ($P = 0.0008$).

Table 6. Mean decay incidences[w,x] recorded during two seasons at various sites in naturally *Botrytis cinerea* infected grapevine tissues[y] left untreated, or treated with paraquat

	1998		1999	
Site	**Paraquat**	**Untreated**	**Paraquat**	**Untreated**
Leaf				
Blade	46.60 a A	4.67 e B	30.20 g C	0.67 m B
Petiole	20.67 b D	4.53 e E	13.47 i F	0.67 m E
Structural bunch parts				
Rachis	44.27 a R	5.87e S	22.47 h T	1.93 m S
Lateral	49.40 a H	6.93 e I	25.53 gh J	2.40 m I
Pedicel	62.93 d N	14.60 f O	38.40 l P	4.67 m Q
Berry parts				
Cheek	2.60 c G	6.33 e G	7.67 j G	2.53 m G
Pedicel end	67.86 d K	9.53 fe L	42.06kl M	4.93 m L

[w,y] See Table 4.

[x] Values in each column followed by the same small letter, and in rows followed by the same capital letter are not statistically different according to the Student's t – test ($P = 0.0001$).

Table 7. Mean decay incidences[w, x] recorded at three phenological stages at various sites in naturally *Botrytis cinerea* infected grapevine tissues [y] left untreated, or treated with paraquat

	Pea Size		Bunch closure		Harvest	
Site	**Paraquat**	**Untreated**	**Paraquat**	**Untreated**	**Paraquat**	**Control**
Leaf						
Blade	35.70 a A	2.80 fg B	46.30 h C	4.40 m B	33.20 n A	0.80 q B
Petiole	18.50 b D	3.10 fg E	21.30 j D	4.10 m E	11.40 o F	0.60 q G
Structural bunch parts						
Rachis	54.60 d V	7.00 fg W	29.80 l X	1.30 m W	15.70 o Y	3.40 q W
Lateral	56.40 d I	7.50f J	40.80 h K	3.00 m J	15.20 o L	3.50 q J
Pedicel	58.60 d R	5.30 fg S	58.10 i R	7.10 m S	35.30 n T	16.20 r U
Berry parts						
Cheek	7.90 c H	4.80 fg H	3.70 k H	4.30 m H	3.80 p H	4.20 q H
Pedicel end	78.60 e M	0.90 g N	52.00 hi O	2.80 m N	34.30 n P	18.00 r Q

[w,y] See Table 4.

[x] Values in each column followed by the same small letter, and in rows followed by the same capital letter are not statistically different according to the Student's t – test ($P = 0.0001$).

Table 8. Mean decay incidences[w, x] recorded at three phenological stages for both treatments[v] at various sites in naturally *Botrytis cinerea* infected grapevine tissues [y] of two grape cultivars

	Dauphine			Merlot		
Site	**Pea size**	**Bunch closure**	**Harvest**	**Pea size**	**Bunch closure**	**Harvest**
Leaf						
Blade	20.70 a A	30.40 e B	10.80 i C	17.80 k A	20.30 o A	23.20 s B
Petiole	12.40 b D	15.00 f D	2.40 j F	9.20 l E	10.40 p D	9.60 t D
Structural bunch parts						
Rachis	31.10 c R	14.40 f ST	9.30 ij S	30.50 m R	16.70 o T	9.80 t S
Lateral	31.60 c I	21.70 g J	7.10 ij K	32.30 m I	22.10 o J	11.60 t K
Pedicel	28.60 c O	27.90 e O	12.10 i P	35.30 m Q	37.30 r Q	39.40 u Q
Berry parts						
Cheek	7.20 b G	4.10 h GH	2.30 j H	5.50 l GH	3.90 p GH	5.70 t GH
Pedicel end	38.40 d L	24.40 g M	7.90 ij N	41.10 n L	30.40 q M	44.40 u L

[w,y] See Table 4.

[x] Values in each column followed by the same small letter, and in rows followed by the same capital letter are not statistically different according to the Student's t – test ($P = 0.0001$).

[v] Control = untreated; P = paraquat treated.

Table 9. Mean *Botrytis cinerea* decay incidences recorded in Dauphine tissue

	Leaf				Structural bunch Parts						Berry parts					
	Blade		Petiole		Rachis		Lateral		Pedicel		Pedicel end		Cheek		Style End	
Stage	C[v]	P[v]	C	P	C	P	C	P	C	P	C	P	C	P	C	P
Pea size	3.2	38.2	4.6	20.2	13.6	48.6	13.2	50.0	8.2	49.0	1.6	75.2	5.2	9.2	0	0
Bunch closure	7.6	53.2	6.2	23.8	0.8	28.0	1.2	42.2	5.4	50.4	3.4	45.4	5.0	3.2	0	0
Harvest	1.2	20.4	1.0	3.8	3.0	15.6	2.4	11.8	5.6	18.6	4.2	11.6	2.6	2.0	0	0

[v] C = untreated; P = paraquat treated.

Table 10. Mean *Botrytis cinerea* decay incidences recorded in Merlot tissue

	Leaf				Structural bunch parts						Berry parts					
	Blade		Petiole		Rachis		Lateral		Pedicel		Pedicel end		Cheek		Style End	
Stage	C[v]	P[v]	C	P	C	P	C	P	C	P	C	P	C	P	C	P
Pea size	2.4	33.2	1.6	16.8	0.4	60.6	1.8	62.8	2.4	68.2	0.2	82.0	4.4	6.6	0	0
Bunch closure	1.2	39.4	2.0	18.8	1.8	31.6	4.8	9.4	8.8	65.8	2.2	58.6	3.6	4.2	0	0
Harvest	0.4	46.0	0.2	19.0	3.8	15.8	4.6	18.6	26.8	52.0	31.8	57.6	5.8	5.6	0	0

[v] C = untreated; P = paraquat treated.

Table 11. Description of disease resistance [u] assigned to various sites in bunches and leaves of table grape cultivar Dauphine, and of natural *Botrytis cinerea* inoculum levels [t]

	Leaf		Structural bunch parts			Berry parts	
Stage	**Blade**	**Petiole**	**Rachis**	**Lateral**	**Pedicel**	**Pedicel end**	**Cheek**
Pea size	R +++	R +++	MR ++++	MR ++++	MR ++++	R ++++	MR ++
Bunch closure	MR ++++	MR +++	R +++	R ++++	MR ++++	R ++++	R +
Harvest	R +++	R +	R ++	R ++	MR ++	R ++	R +

[u] Disease resistance: R = resistant (<5% decay in untreated material); MR = moderately resistant (6-20% decay in untreated material); S = susceptible (21-40% decay in untreated material); HS = highly susceptible (> 41% decay in untreated material).

[t] Inoculum levels: + = low infection levels (<5% decay in paraquat treated material); ++ = intermediate infection levels (6-20% decay in paraquat treated material); +++ = high infection levels (21-40% decay in paraquat treated material); ++++ = very high infection levels (> 41% decay in paraquat treated material).

Table 12. Description of disease resistance [u] assigned to various sites in bunches and leaves of table grape cultivar Merlot, and of natural *Botrytis cinerea* inoculum levels [t]

	Leaf		Structural bunch parts			Berry parts	
Stage	**Blade**	**Petiole**	**Rachis**	**Lateral**	**Pedicel**	**Pedicel end**	**Cheek**
Pea size	R +++	R ++	R ++++	R ++++	R ++++	R ++++	R ++
Bunch closure	R +++	R ++	R ++	R ++	MR ++++	R ++++	R +
Harvest	R ++++	R ++	R ++	R ++	S ++++	S ++++	MR ++

[u] Disease resistance: R = resistant (<5% decay in untreated material); MR = moderately resistant (6-20% decay in untreated material); S = susceptible (21-40% decay in untreated material); HS = highly susceptible (> 41% decay in untreated material).

[t] Inoculum levels: + = low infection levels (<5% decay in paraquat treated material); ++ = intermediate infection levels (6-20% decay in paraquat treated material); +++ = high infection levels (21-40% decay in paraquat treated material); ++++ = very high infection levels (> 41% decay in paraquat treated material).

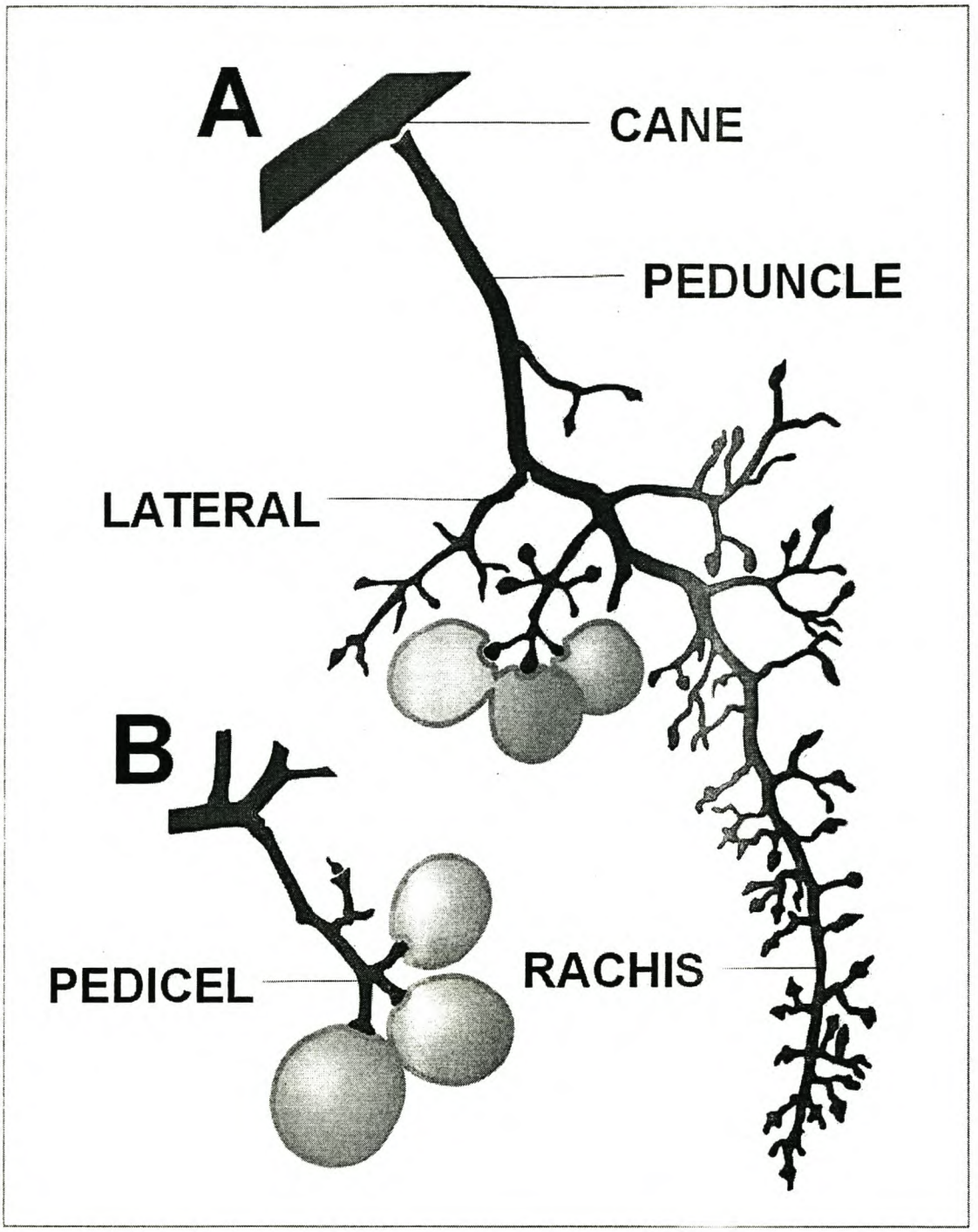

Fig. 1. Morphological parts of the grapevine
A = Structural bunch parts; B = Berry attachment parts

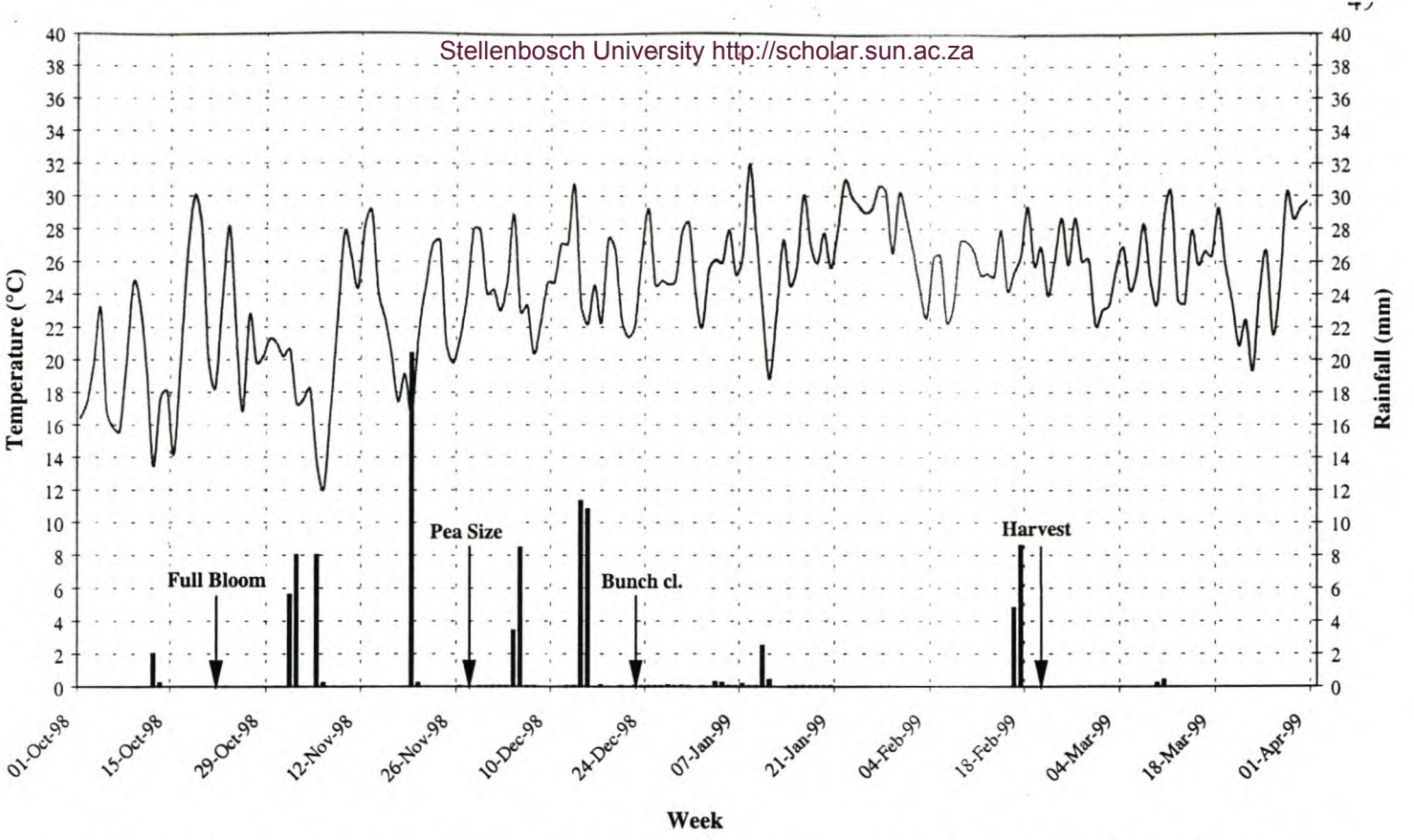

Figure 2. Precipitation and average daily temperature recorded during the 1998/1999 growth season in Dauphine vineyards in the Hexriver valley region. Precipitation (▌); average daily temperature (—); sampling stages = (↓)

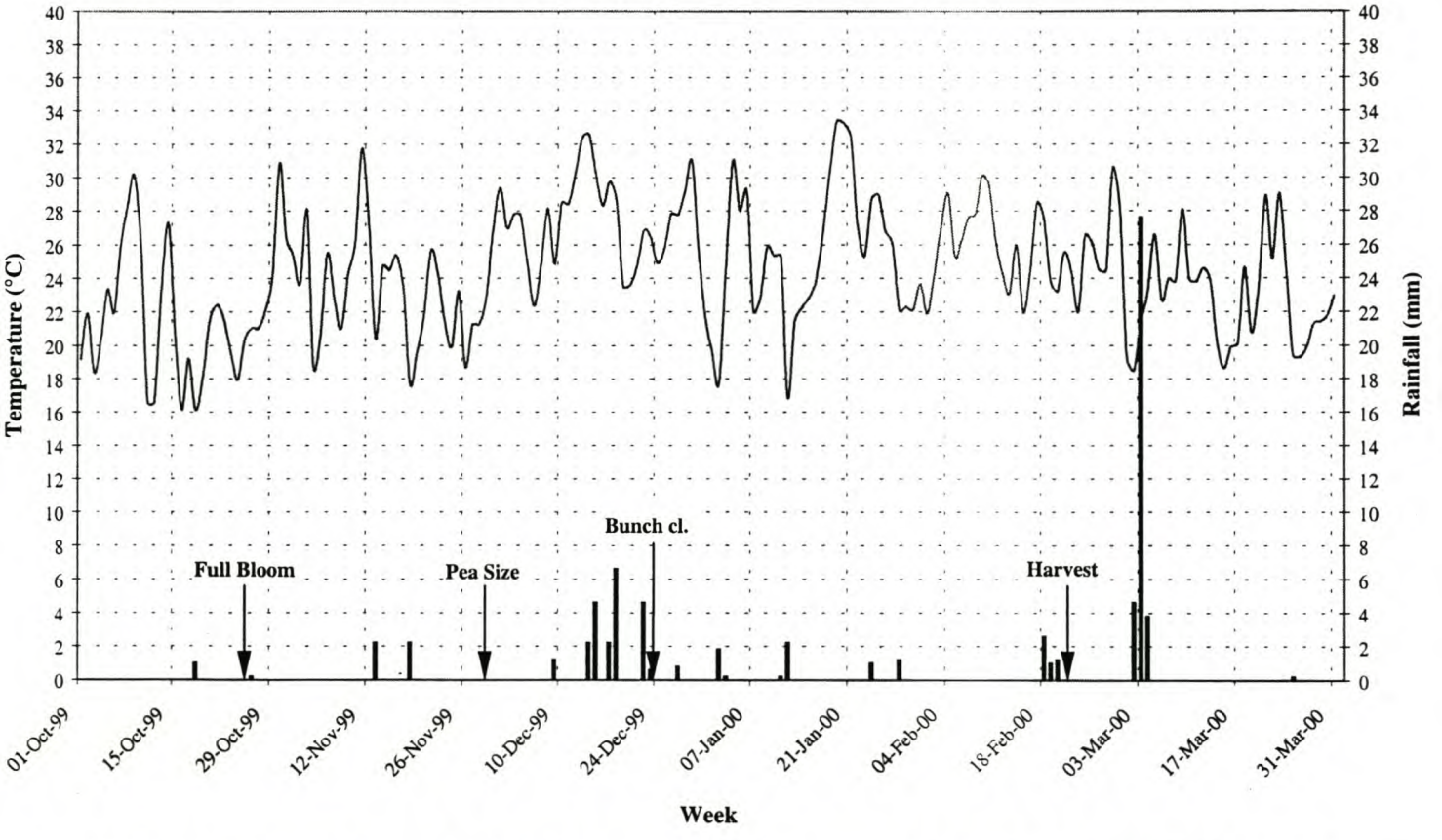

Figure 3. Precipitation and average daily temperature recorded during the 1999/2000 growth season in Dauphine vineyards in the Hexriver valley region. Precipitation (▌); average daily temperature (—); sampling stages = (↓)

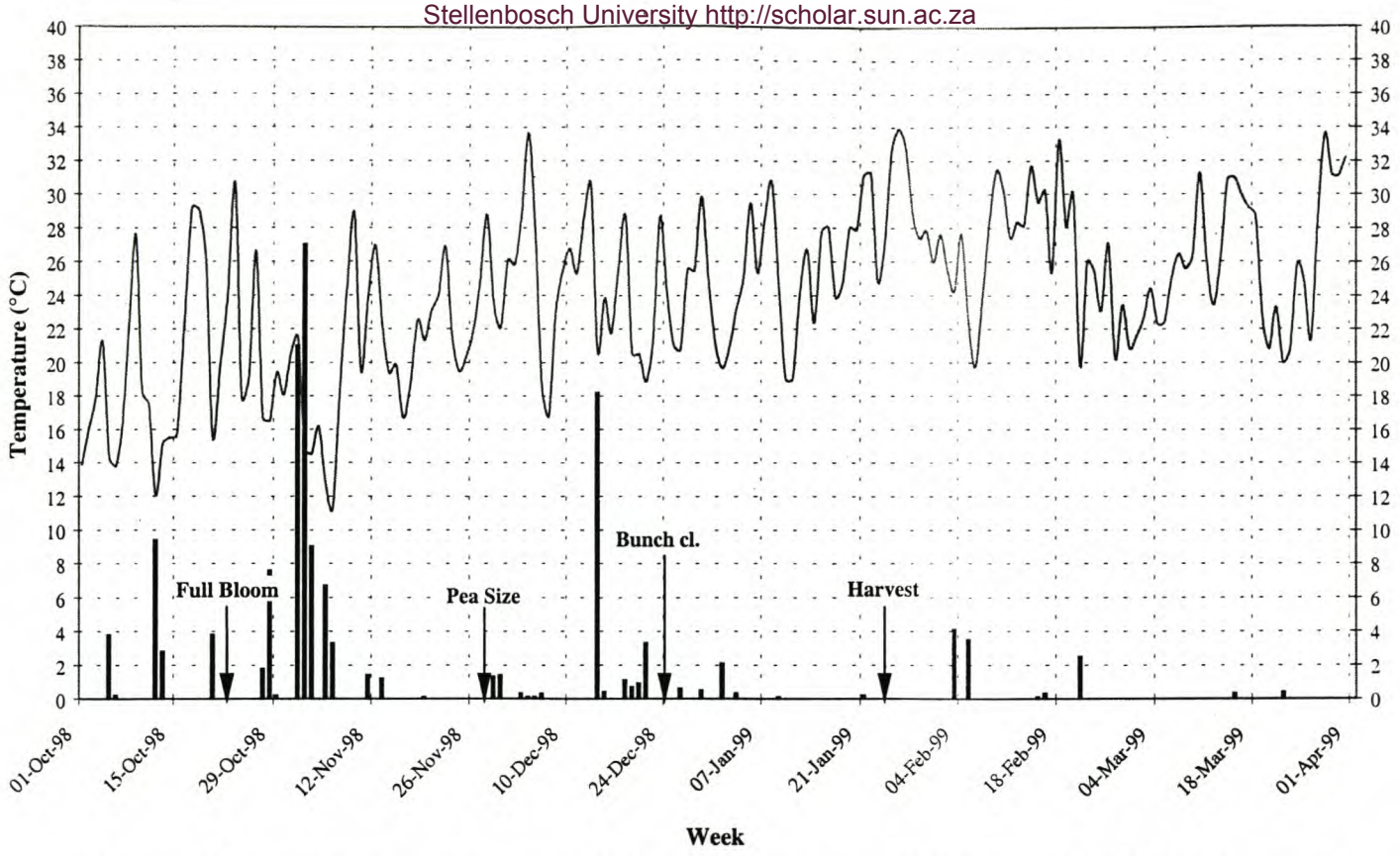

Figure 4. Precipitation and average daily temperature recorded during the 1998/1999 growth season in Merlot vineyards in the Bergriver valley region. Precipitation (▌); average daily temperature (—); sampling stages = (↓)

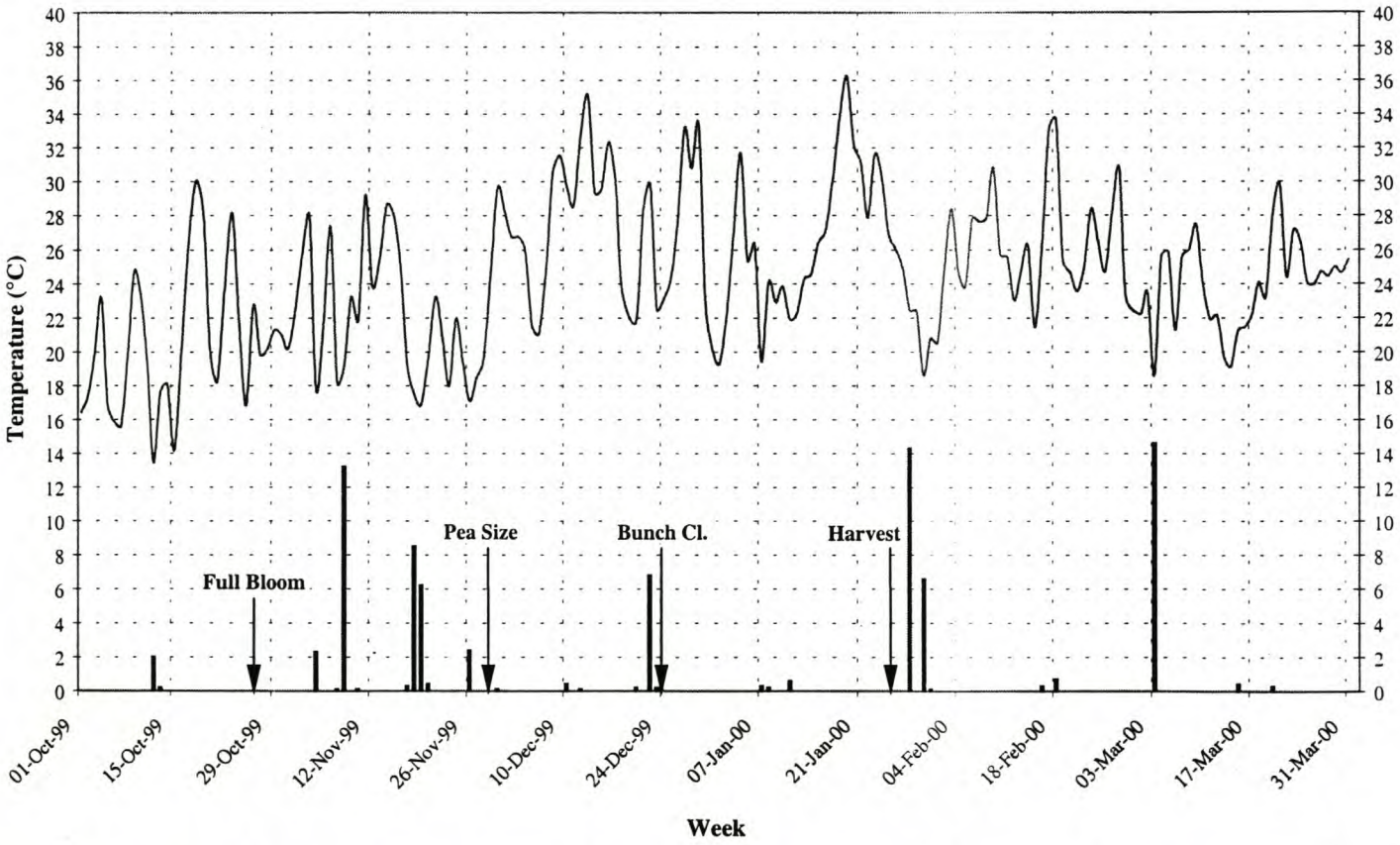

Figure 5. Precipitation and average daily temperature recorded during the 1999/2000 growth season in Merlot vineyards in the Bergriver valley region. Precipitation (▌); average daily temperature (—); sampling stages = (↓)

3. INFECTION AND DISEASE EXPRESSION IN PARTS OF GRAPE BUNCHES INOCULATED WITH AIRBORNE *BOTRYTIS CINEREA* CONIDIA

ABSTRACT

Grape bunches (table grape cultivar Dauphine, wine grape cultivar Merlot) 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, bunches were surface sterilised in 70% ethanol for 5 s to eliminate the pathogen on the bunch surface and to determine the development of latent infections established during moist incubation. From each bunch, 10 berries and pedicels, and 10 lateral and rachis segments (approximately 10-20 mm in length) 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 part per medium) were placed in Petri dishes on Kerssies' *B. cinerea* selective medium, or water agar medium supplemented with paraquat. Disease expression was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues. The two cultivars did not differ in resistance of the berry cheek, which was at all stages classified as resistant. However, in Dauphine, inoculum levels in berry cheeks declined from intermediate at pea size to low at the following stages, whereas in Merlot, levels were intermediate during pea size and at harvest. Some differences between cultivars were found in the resistance of the structural bunch parts, and of their inoculum levels. In Dauphine, the rachis reacted susceptible at pea size, and was classified moderately resistant later in the season. Laterals and pedicels were moderately resistant at pea size, and resistant at later stages. Inoculum levels in rachises, laterals and pedicels were high at pea size, but intermediate at bunch closure and at harvest. The finding that *B. cinerea* infected and naturally occurred more commonly in the tissues of immature than mature bunches, that the structural parts of the bunch carried more *B. cinerea* than the berry cheek, and that these infections may be more important in *B. cinerea* bunch rot than infection of the cheek or the style end, suggest that emphasis should be placed on the disease reaction of the pedicel and related parts of immature bunches rather than on the berry.

INTRODUCTION

Botrytis cinerea Pers.:Fr., a pathogen of grapevine (*Vitis vinifera* L.), is associated with early-season infection (McClellan and Hewitt, 1973; Nair, 1985; Nair and Parker, 1985) and infection of mature grapes favoured by late-season rains or prolonged periods of high relative humidity (Harvey, 1955; Jarvis, 1980). Different infection pathways have been described for conidial infection by *B. cinerea* on grape berries, namely style ends (MCclellan and Hewitt, 1973; Nair and Parker, 1985), pedicels (Holz *et al.*, 1997, 1998; Pezet and Pont, 1986), natural openings (Pucheu-Planté and Mercier, 1983), wounds (Nair *et al.*, 1988), or by direct penetration of the cuticle (Nelson, 1956). Passive defence (Hill *et al.*, 1981; Kosuge and Hewitt, 1964; McClellan and Hewitt, 1973; Padgett and Morrison, 1990; Pezet and Pont, 1984; Vercesi *et al.*, 1997) and active defence mechanisms (Creasy and Coffee, 1988; Hill, 1985; Hoos and Blaich, 1988; Langcake, 1981) to infection by *B. cinerea*, are strongly expressed in immature berries but tend to become weaker during berry ripening. Grapes are therefore resistant to disease expression from berry set to véraison when challenged by conidial clusters of the pathogen, and susceptible from véraison to harvest (Hill *et al.*, 1981; Nair and Hill, 1992; Nelson, 1951). Incipient flower infections cause late-season bunch rot, following a period of fungal latency in the style end of the berry (MCclellan and Hewitt, 1973; Nair and Parker, 1985), or in the receptacle part of the pedicel (Holz *et al.*, 1997, 1998; Pezet and Pont, 1986).

A recent study (Part 2) on the pattern of natural occurrence of *B. cinerea* in different sites in grape bunches indicated that the role of infection in rachises, laterals and pedicels is underestimated in the epidemiology of *B. cinerea* on grapevine. My observations (Part 2) on the behaviour of the pathogen in the the different morphological parts of Dauphine and Merlot grape bunches furthermore suggest that cultivars may differ in their resistance reaction to natural *B. cinerea* inoculum in the pedicel tissue, and not in the berry cheek. My findings support the hypothesis of increased host resistance in the structural parts of grape bunches during development, but also suggest that in the Western Cape province, inoculum in vineyards is abundant during the early part of the season, and less later in the season. These findings can have a major impact on quantitative studies involving host responses on grapevine. Disease prediction models, evaluation of fungicide efficacy, implementation of biological control and screening for host resistance were primarily based on the behaviour of

groups of conidia after inoculation with conidial suspensions on mature berries. The deposition of groups of conidia was used as a standard procedure in most studies where grapes are artificially inoculated. Grape bunches and berries are atomized with (De Kock and Holz, 1991; Nair, 1985; Nair *et al.*, 1995; Nelson, 1951) or dipped in (Broome *et al.*, 1995) conidial suspensions, or suspension droplets were placed onto the berry cheek (Chardonnet *et al.*, 1997; Marois *et al.*, 1987) or injected into berries (Avissar and Pesis, 1987; Nair and Parker, 1985; Thomas *et al.*, 1988). By using these methods, the importance of a primary infection event in the vineyard, namely natural infection of pedicels and latency in pedicel tissue, might have been overlooked. More information is therefore needed on the behaviour of the different types of *B. cinerea* inocula (single airborne conidia, groups of conidia; mycelia) on the different morphological parts of grapevine to validate the pathway described for natural *B. cinerea* infection in vineyards. The aims of this investigation was to study penetration and disease expression on the different morphological parts of bunches of two grape cultivars (Dauphine and Merlot) under conditions simulating natural infection by airborne conidia.

MATERIALS AND METHODS

Grapes. Sound unblemished bunches were obtained from two vineyards (table grape cultivar Dauphine, wine grape cultivar Merlot) in the Stellenbosch region, with a history of low *B. cinerea* incidences. Bunches were selected at pea size, bunch closure and two weeks prior to harvest. To prevent infection from surface inoculum, the bunches were surface sterilised at each sampling for 2 min in 0.35% sodium hypochlorite, rinsed in distilled water and air-dried. The bunches were suspended with their peduncles into sterile aluminum foil-wrapped "oases" (florist's sponge) soaked with a 20% sucrose solution to maintain turgidity, and placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm).

Inoculation. A virulent isolate of *B. cinerea* obtained from a naturally infected grape berry was maintained on potato dextrose agar (PDA) at 5°C. For the preparation of inoculum, the isolate was first grown on canned apricot halves. Conidiophores from the colonised fruit were transferred to PDA in Petri dishes and incubated at 22°C under a diurnal regime (12h near-ultraviolet light; 12h dark light). Conidia were harvested dry with a suction -type collector from 14-day-old cultures and stored dry at 5°C until use (1 to 16

weeks). Storage time did not affect germination; the dry conidia could therefore be used in all experiments (Spotts and 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 20 min to settle onto the bunches which were positioned on two screens. At this dosage, approximately three conidia were evenly deposited as single cells on each mm^2 of berry surface (Coertze and 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). Following inoculation, the screens were placed in 12 ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). Each chamber contained one screen carrying three oases with bunches. Each chamber was considered as a replicate. These conditions provided conditions commonly encountered in nature by the pathogen in grape bunches, namely dry conidia on dry berries under high relative humidity (humid berries). The chambers were incubated at 22°C with a 12 h photoperiod daily. After 24 h, the oases with bunches were removed from the chambers and placed in dry chambers (≤60% RH) for 48 h before the bunches were used for histological investigations and the determination of infection and disease expression.

Conidial dispersal and viability. At 24 h post inoculation, five berries and pedicels, and five rachis segments sections were randomly selected for microscopic studies. Thin hand-sectioned pieces (approximately 5 x 5 mm) of skin comprising the cuticle, epidermis, and a few cell layers, were cut with a razor blade. The sections were stained for 5 min in a differential stain containing fluorescein diacetate ([FDA] Sigma Chemical Co., St Louis, MO), aniline blue ([AB] BDH laboratory chemicals division, Poole, England) and blankophor ([BP] Bayer, Germany), mounted on a glass slide in 0.1 M KH_2PO_4 buffer (pH 5.0) and covered with a cover slip. FDA (2 mg per ml acetone) and AB (0.1% in KH_2PO_4 buffer, pH 5.0) were prepared as stock solutions and stored at –20°C and 5°C, respectively. Before a histology session, BP (0.5%) was added to the AB solution and a fresh stain was prepared by mixing 25 µl of the FDA stock solution with 1 ml of the AB/BP stock solution in a 1.5 ml polypropylene Eppendorf tube, which was then kept on ice. Conidial germination and viability of fungal structures were examined with a Zeiss Axioskop microscope equipped

with an epifluorescence condenser, a high-pressure mercury lamp, Neofluar objectives and Zeiss filters 02, 06 and 18. These sets include excitation filters G 365, BP 436/8 and BP 395-425, respectively. With this set-up, protoplasts of viable fungal structures fluoresced brilliant yellow-green with filters 02, 06 and 18. Protoplasts of dead cells were blue-black (filters 06 and 18), whereas cells without protoplasts fluoresced white (filter 02) or yellow (filter 18) (O'Brien and McCully, 1981).

Infection and disease expression. Following incubation, bunches were surface sterilised in 70% ethanol for 5 s to elminate the pathogen on the berry surface and promote the development of latent infections established during moist incubation (Coertze and Holz, 1999). From each bunch, 10 berries and pedicels, and 10 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 part per medium) were placed in Petri dishes on Kerssies' *B. cinerea* selective medium (Kerssies, 1990), or water agar medium supplemented with paraquat (Grindrat and Pezet, 1994). 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 site was recorded for each morphological part, and incidences for each part calculated after 14 days. These treatments provided conditions which facilitated the development of disease expression by latent infections established during moist incubation. On Kerssies' medium, disease expression was the result of latent infection as influenced by host resistance. Previous studies (Coertze and Holz, 1999) showed that no superficial mycelial growth developed on the berry skin segments during the early phases of incubation on Kerssies' medium. Hyphal outgrowth usually occurred from cells underlying the cuticle into the medium after 5 days. Uninfected skin segments retained their turgidity and remained green for 6 days, whereafter colour changes indicative of natural cell death, appeared. Fungal structures that penetrated the skin during the period of moist incubation, therefore grew further under the influence of active defence. Incidences therefore described infection levels of the morphological part as regulated by host resistance. Paraquat terminated host resistance in the cells of the cuticular membrane without damaging host tissue (Baur *et al.*, 1969; Cerkauskas and Sinclair, 1980; Pscheidt and Pearson, 1989; Grindrat and Pezet, 1994). On paraquat medium, disease expression was the result of latent infection developing after

surface sterilisation and the termination of host resistance. Incidences therefore described infection levels of a morphological part when host resistance was negated.

Disease resistance and inoculum levels. At each developmental stage, parts of a cultivar were catagorised for disease resistance according to the mean decay incidences recorded on Kerssies' medium. Sites showing decay of ≤5%, 6-20%, 21-40% and ≥41% were classified respectively as resistant, moderately resistant, susceptible and highly susceptible to infection. The sites were also catagorised into different sub-classes according to decay development on paraquat medium to describe their inoculum level. Sites showing decay of ≤5%, 6-20%, 21-40% and ≥41% were classified respectively as carrying low, intermediate, high and very high inoculum levels.

Statistical analysis. A split plot experimental design was used in all experiments. 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 and Wilk, 1965). The data was examined further by using the analysis of variance (ANOVA) and the treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor and Cochran, 1980).

RESULTS

Conidial dispersal and viability. Conidia used at each inoculation were highly viable and germinated freely on PDA and WA. Germination on both PDA and WA usually varied between 85 - 92%. Fluorescence microscopy showed that conidia were consistently dispersed at each inoculation on berry cheeks, pedicels and rachises, and that they were deposited as single cells, and not in pairs or groups on the different morphological parts of the bunches. Conidia germinated readily on the different tissues, but germination rates varied substantially and ranged between 58-88%. Conidial viability on the different tissues 24 h post inoculation differed substantially, but the propotion viable structures mostly exceeded 45%.

Infection and disease expression. Analysis of variance for effects of season, phenological stage, cultivar and treatment on decay development is given in Table 1. Incubation on the two media showed that seasons significantly affected disease expression at

the different developmental stages (Table 2). At pea size stage, disease expression levels on both media were significantly higher in 1998 than in the 1999 season. This difference was also found at bunch closure and at harvest on Kerssies', but not on the paraquat medium. Furthermore, in 1998 on both media, disease expression levels were significantly higher at pea size than at the following stages. This difference was not found in 1999.

Disease expression in the different parts was significantly influenced by phenology (Table 3). On Kerssies' medium, disease expression in all parts, except for the pedicel, remained more or less constant during the three stages. On pedicels, disease expression was significantly lower at bunch closure than at the two other stages. Regarding the disease reaction of the individual parts, two distinct trends were found. Firstly, rachises and laterals corresponded in their disease reaction at the different stages and had the highest disease levels. Secondly, disease in the berry cheek was significantly lower at all three stages than in most of the other parts. A different disease expression pattern was found on paraquat medium. All parts, except for the pedicel, showed significantly more disease at pea size than at the other two stages. There was furthermore at each stage significantly less disease in the berry cheek than the other parts.

Disease expression in the different parts was also influenced by cultivar (Table 4). On Kerssies' medium, disease levels were significantly higher in laterals and pedicels of Merlot than Dauphine. These differences were not found on paraquat medium.

Disease resistance and inoculum levels. Mean decay levels for both cultivars, based on the data recorded in the different parts during two seasons, are given in Table 5-6. Descriptions of disease resistance and of inoculum levels are given in Table 7-8. The two cultivars did not differ in resistance of the berry cheek, which was at all stages classified as resistant. However, in Dauphine, inoculum levels in berry cheeks declined from intermediate at pea size to low at the following stages, whereas in Merlot, levels were intermediate during pea size and at harvest. Some differences between cultivars were found in the resistance of the structural bunch parts, and of their inoculum levels. In Dauphine, the rachis reacted susceptible at pea size, and was classified moderately resistant later in the season. Laterals and pedicels were moderate resistant at pea size, and resistant at later stages. Inoculum levels in rachises, laterals and pedicels were high at pea size, but intermediate at bunch closure and

at harvest. In Merlot, the structural bunch parts were at all stages classified as moderate resistant, with the exception of the pedicel, which reacted resistant at bunch closure. Inoculum levels in rachises and laterals followed a similar pattern to Dauphine, whereas pedicels at all stages carried intermediate inoculum levels.

DISCUSSION

In this study different parts of grape bunches, inoculated with airborne conidia of *B. cinerea,* were kept under conditions that facilitated disease expression by latent mycelia under the influence of host resistance, or when resistance was terminated. The resistance of rachises and laterals of Dauphine increased from pea size to harvest stage, and the amount of latent infection declined. On Merlot, no change in resistance was noted, but latent infection declined. The two cultivars also differed in the level of pedicel infection. On Dauphine, pedicels showed an increase in resistance, and a decline in latent infection. On Merlot, resistance did not change and latent infection stayed at one level. In both cultivars the berry cheek reacted resistant from pea size to harvest, and mostly carried low latent infection levels. These trends found in the laboratory with airborne conidia, corresponded with those reported for natural *B. cinerea* infection in Dauphine and Merlot vineyards (Part 2). However, in the laboratory study, pedicels of Merlot did not show the change from resistant to susceptable as reported for Merlot in the field. Based on the trends showed by airborne *B. cinerea* conidia in the laboratory, and of natural infection (Part 2), it can be concluded that in Dauphine and Merlot bunches, berry cheeks are the most resistant sites and carry the lowest levels of latent infection. Rachises, laterals and pedicels are less resistant than the berry cheek, and mostly carry higher latent infection. Furthermore, latent infection usually peaked at pea size.

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 (Parts 2 and 4) 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 conducive to *B. cinerea* infection from bloom to pea size stage of 1998, but less favourable in 1999. In the present study in the 1998 season, when natural infection in these parts were high at pea size, incubation of artificially inoculated parts on both Kerssies' and paraquat medium also revealed high infection levels. In 1999, when the climatic conditions were less favourable for natural infection, lower disease expression levels were recorded in artificially inoculated material. These differences 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, gives 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.

On grapevine, most studies with *B. cinerea* on various aspects such as host resistance, timing of fungicide application, biological control, control by cultural practises and disease prediction models, comprised investigations on mature berries. In most studies where grapes were artificially inoculated, berries were atomised with (Coertze and Holz, 1999; Jarvis, 1962b; Kosuge and Hewitt, 1964; McClellan and Hewitt, 1973), dipped in (Bessis, 1972), or injected with (Avissar and Pesis, 1991; Hoos and Blaich, 1988; Pezet and Pont, 1986) conidial suspensions, or suspension droplets were placed onto the berry cheek (Bulit and Verdu, 1973; Holz *et al.*, 1995). These methods allowed for the deposition of groups of conidia on berries, and differ from primary natural infection in the vineyard. The finding that *B. cinerea* infected and naturally occurred commonly in the structural parts of immature bunches, that these parts carried more *B. cinerea* than the berry cheek, and that these infections may be important in *B. cinerea* bunch rot, suggest that emphasis should be placed on the disease reaction of the pedicel and related parts of immature bunches rather than on the berry.

LITERATURE

Avissar, I. & 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 ultrastructure of mesquite mesophyll cells. *Weed Research* 9: 81-85.

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 detection of fungi in soybean tissues. *Phytopathology* 70: 1036-1038.

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.

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.

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

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

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.

Harvey, J.M. 1955. A method of forecasting decay in California storage grapes. *Phytopathology* 45: 229-232.

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

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.

Holz, G. 1999. Behaviour and infection pathways of diverse fungal pathogens on fruit. Page 257 In: Conference Handbook. Bien. Aust. Plant Pathol. Soc. Conf., 12th. Canberra, Australia.

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

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:S88.

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

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. 1962. Splash dispersal of spores of *Botrytis cinerea* Pers. *Nature (London)* 193:599.

Jarvis, W.R. 1980. Epidemiology. Page 219 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.

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

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.

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. & 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* 119:52-63.

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 Diseases of International Importance. Vol III: Diseases of Fruit Crops.

J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

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

Nair, N.G., Guilbaud-Oulton, S., Barchia, I. & Emmett, R. 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. *Austrialian Journal of Experimental Agriculture* 35:1177-1180.

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.

O'Brien, T.P., and McCully, M.E. 1981. The study of Plant Structure Principles and Selected Methods. Termarcarphi Pty. Ltd. Melbourne, Australia.

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.

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 les grappes de *Vitis vinifera* (var. Gamay). *Revue suisse Viticulture Arboric. Hortic.* 18:317-322.

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

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 Bot*any 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.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance (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 grape and plum fruit surfaces. *Plant Disease* 80:688-691.

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.

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.

Table 1. Analysis of variance for effects on percentage decay in *Botrytis cinerea* infected grapevine tissue

Source of variation	Df [a]	MS [b]	SL [c]
Season (S)	1	21313.611	0.0001
Phenological Stage (P)	2	11590.000	0.0001
S x P	2	11674.444	0.0001
Error (S x P)	24	414.444	
Cultivar (C)	1	1480.278	0.0552
S x C	1	0.278	0.9789
P x C	2	1254.444	0.0453
S x P x C	2	751.111	0.1538
Time (T)	2	2147.500	0.0055
S x T	2	1818.611	0.0118
P x T	4	2810.000	0.0001
S x P x T	4	844.444	0.0802
C x T	2	71.944	0.8337
S x C x T	2	1325.278	0.0382
P x C x T	4	33.611	0.9869
S x P x C x T	4	498.611	0.2886
Error (S x P x C x T)	120	394.944	
Medium (M)	1	12840.278	0.0001
S x M	1	722.500	0.1347
P x M	2	2721.111	0.0003
C x M	1	902.500	0.0949
T x M	2	353.611	0.3332
S x P x M	2	5053.333	0.0001
S x C x M	1	1173.611	0.0572
P x C x M	2	30.000	0.9104
S x P x K	2	847.778	0.0737
S x T x M	2	772.500	0.0926
P x T x M	4	344.444	0.3693
S x P x T x M	4	543.333	0.1528
C x T x M	2	1172.500	0.0278
S x C x T x M	2	653.611	0.1329
P x C x T x M	4	237.500	0.5637
S x P x C x T x M	4	880.278	0.0302
Error (S x P x C x T x M)	144	319.306	
Morphological Part (MP)	3	14264.722	0.0001
S x MP	3	778.056	0.0255
P x MP	6	771.111	0.0054
S x P x MP	6	1051.111	0.0003
C x MP	3	242.500	0.4056
S x C x MP	3	33.611	0.9394
P x C x MP	6	153.333	0.7192
S x P x K x MP	6	1103.333	0.0002
T x MP	6	268.611	0.3751
S x T x MP	6	248.611	0.4268
P x T x MP	12	420.000	0.0658
S x P x T x MP	12	253.333	0.4329
K x T x MP	6	753.056	0.0077
S x C x T x MP	6	219.722	0.5089
P x C x T x MP	12	344.722	0.1691
S x P x C x T x MP	12	361.944	0.1379
M x MP	3	509.167	0.1068
S x M x MP	3	189.907	0.5163
P x M x MP	6	773.333	0.0052
S x P x M x MP	6	401.852	0.1413
C x M x MP	3	870.648	0.154
S x C x M x MP	3	175.833	0.5496
P x C x M x MP	6	250.370	0.4221
S x P x C x M x MP	6	217.778	0.5147
T x M x MP	6	188.056	0.6070
S x T x M x MP	6	518.796	0.0535
P x T x M x MP	12	308.889	0.2523
S x P x T x M x MP	12	494.074	0.0233
C x T x M x MP	6	312.870	0.2768
S x C x T x M x MP	6	270.278	0.3710
P x C x T x M x MP	12	120.093	0.9265
S x P x C x T x M x MP	12	204.722	0.6300
Error	864	279.676	

[a] Degrees of freedom
[b] Mean square
[c] Significance level

Table 2. Mean decay incidences[v,w] recorded in grape bunches inoculated at three phenological stages with airborne *Botrytis cinerea* conidia

	Kerssies medium		Paraquat medium	
Stage	**1998**	**1999**	1998	**1999**
Pea size	17.50 a A	7.17 c B	37.67 d C	9.83 f B
Bunch closure	8.33 b D	5.33 c E	11.33 e D	10.17 f D
Harvest	12.00 b F	6.50 c G	11.00 e F	12.67 f F

[v] Bunches (table grape cultivar Dauphine, wine grape cultivar Merlot) were dusted with dry conidia in a settling tower and incubated for 24 h at high relative humidity (±93%). Following incubation, bunches were surface sterilised to eliminate the pathogen on the bunch surface and to determine the development of latent infections established during moist incubation. Sections from different sites (rachises, laterals, pedicels, berry cheek) were incubated in Petri dishes on Kerssies' *B. cinerea* selective medium, or on water agar medium supplemented with paraquat. Disease expression was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues.

[w] Values in each column followed by the same small letter, and in rows followed by the same capital letter are not statistically different according to the Student's *t* – test ($P = 0.0001$).

Table 3. Mean decay incidences[v,w] recorded at different sites in grape bunches inoculated at three phenological stages with airborne *Botrytis cinerea* conidia

	Pea Size		Bunch closure		Harvest	
Site	**Kerssies[z]**	**Paraquat[z]**	**Kerssies**	**Paraquat**	**Kerssies**	**Paraquat**
Rachis	18.33 a A	33.67 c B	13.00 e A	17.67 g A	14.33 j A	14.67 l A
Lateral	13.67 a C	32.67 c D	10.00 e C	11.33 h C	9.00 j C	15.33 l CE
Pedicel	15.33 a F	19.00 c FG	3.33 f H	12.00 h F	10.00 j F	12.00 l F
Berry cheek	2.00 b I	9.67 d J	1.00 f I	2.00 i I	3.67 k I	5.33 m IJ

[v] See Table 2.

[w] Values in each column followed by the same small letter, and in rows followed by the same capital letter are not statistically different according to the Student's t – test ($P = 0.0052$).

[z] Kerssies = Kerssies' medium; paraquat = paraquat medium

Table 4. Mean decay incidences[v,w] recorded at different sites in bunches of two grape cultivars inoculated with airborne *Botrytis cinerea* conidia

	Dauphine		Merlot	
Site	**Kerssies[z]**	**Paraquat[z]**	**Kerssies**	**Paraquat**
Rachis	15.33 a A	20.89 e B	15.11 g A	23.11 i B
Lateral	7.11 b C	19.11 e DE	14.67 g D	20.44 l E
Pedicel	6.44 c F	16.44 e G	12.67 g G	12.22 j G
Berry cheek	1.78 d H	4.44 f IH	2.67 h HI	6.89 k I

[v] See Table 2.

[w] Values in each column followed by the same small letter, and in rows followed by the same capital letter are not statistically different according to the Student's t – test ($P = 0.0154$).

[z] Kerssies = Kerssies' medium; paraquat = paraquat medium

Table 5. Mean decay incidences recorded in Dauphine bunches inoculated with airborne *Botrytis cinerea* conidia

	Structural bunch parts						Berry parts	
	Rachis		Lateral		Pedicel		Cheek	
Stage	K[u]	PQ[u]	K	PQ	K	PQ	K	PQ
Pea	22.00	32.00	11.33	32.67	14.00	23.33	0	9.33
Bunch closure	12.67	18.00	6.00	10.67	2.67	14.00	2.00	2.67
Harvest	11.33	12.67	4.00	14.00	2.67	12.00	3.33	1.33

[u] K = Kerssies' medium; PQ = paraquat medium.

Table 6. Mean decay incidences recorded in Merlot bunches inoculated with airborne *Botrytis cinerea* conidia

	Structural bunch parts						Berry parts	
	Rachis		Lateral		Pedicel		Cheek	
Stage	K[u]	PQ[u]	K	PQ	K	PQ	K	PQ
Pea	14.67	35.33	16.00	32.67	16.67	14.67	4.00	10.00
Bunch closure	13.33	17.33	14.00	12.00	4.00	10.00	0	1.33
Harvest	17.33	16.67	14.00	16.67	17.33	12.00	4.00	9.33

[u] K = Kerssies' medium; PQ = paraquat medium

Table 7. Description of disease resistance [x] assigned to various sites in bunches and leaves of table grape cultivar Dauphine, and of *Botrytis cinerea* inoculum levels [y]

	Structural bunch parts			Berry parts
Stage	**Rachis**	**Lateral**	**Pedicel**	**Cheek**
Pea	S +++	MR +++	MR +++	R ++
Bunch closure	MR ++	MR ++	R ++	R +
Harvest	MR ++	R ++	R ++	R +

[x] Disease resistance: R = resistant (<5% decay on Kerssies' medium); MR = moderately resistant (6-20% decay on Kerssies' medium); S = susceptible (21-40% decay on Kerssies' medium); HS = highly susceptible (> 41% decay on Kerssies' medium).

[y] Latent inoculum levels: + = low infection levels (<5% decay on Paraquat medium); ++ = intermediate infection levels (6-20% decay on Paraquat medium); +++ = high infection levels (21-40% decay on Paraquat medium); ++++ = very high infection levels (> 41% decay on Paraquat medium).

Table 8. Description of disease resistance [x] assigned to various sites in bunches and leaves of table grape cultivar Merlot, and of *Botrytis cinerea* inoculum levels [y]

	Structured bunch parts			Berry parts
Stage	**Rachis**	**Lateral**	**Pedicel**	**Cheek**
Pea	MR +++	MR +++	MR ++	R ++
Bunch closure	MR ++	MR ++	R ++	R +
Harvest	MR ++	MR ++	MR ++	R ++

[x] Disease resistance: R = resistant (<5% decay on Kerssies' medium); MR = moderately resistant (6-20% decay on Kerssies' medium); S = susceptible (21-40% decay on Kerssies' medium); HS = highly susceptible (> 41% decay on Kerssies'medium).

[y] Latent inoculum levels: + = low infection levels (<5% decay on Paraquat medium); ++ = intermediate infection levels (6-20% decay on Paraquat medium); +++ = high infection levels (21-40% decay on Paraquat medium); ++++ = very high infection levels (> 41% decay on Paraquat medium).

4. INFECTION AND DISEASE EXPRESSION IN VEGETATIVE PARTS OF GRAPEVINE INOCULATED WITH AIRBORNE *BOTRYTIS CINEREA* CONIDIA

ABSTRACT

Shoots on young vinelets prepared from cuttings, or shoots obtained from vineyards (table grape cultivar Dauphine, wine grape cultivar Merlot) were dusted with dry conidia of *Botrytis cinerea* in a settling tower and incubated for 24 h at high relative humidity (±93%). Following incubation, shoots were surface sterilised in 70% ethanol for 5 s to elminate the pathogen on the tissue surface and to determine the development of latent infections established during moist incubation. From each shoot, leaf blades, petioles, internodes and inflorescenses were removed. The different segments were placed in Petri dishes on Kerssies' *B. cinerea* selective medium, or water agar medium supplemented with paraquat. Disease expression was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues. In the case of vinelets, leaf blades, petioles, internodes and inflorescenses were all classified susceptible to highly susceptible. The different parts, furthermore all carried very high latent inoculum levels. In the vineyard shoots, petioles and inflorescences showed resistance, and carried intermediate to high latent inoculum levels. This finding suggests that leaf blades are not an appropriate medium for studying the behaviour of inoculum of *B. cinerea* and host responses in grape bunches. Instead, petioles and inflorescenses of vineyard shoots can be used for this purpose.

INTRODUCTION

Botrytis cinerea Pers.:Fr., a pathogen of grapevine (*Vitis vinifera* L), can attack most of the plant's organs. It maintains itself in grapevines as sclerotia (Nair and Nadtotchei, 1987), conidia (Corbaz, 1972; Bulit and Verdu, 1973) and mycelia (Gessler and Jermini, 1985; Northover, 1987) and is associated with early-season latent infections (Nair and Hill, 1992). Vegetative organs are not normally classified as susceptible but heavy infection during periods of prolonged wetness, may lead to colonisation of leaf tissue. Young leaves

are susceptible whereas matured ones are resistant (Hill *et al.*, 1981). These infections can produce conidia later in season during wet periods. Healthy grape stalks undergo little risk from direct infection by conidia of *B. cinerea* but can occasionally be invaded by mycelial material growing from flower debris or attached berries (Hill, 1985). In autumn *B. cinerea* sometimes invades nodes of shoots through the grape stalks and occasionally colonise the grape shoots (Agulhon *et al.*, 1971). Berries, on which the most prominent symptom of the disease is found (Nair and Nadtotchei, 1987), are considered resistant to infection when immature, and susceptible when mature (Hill *et al.*, 1981; Nair and Hill, 1992; Nelson, 1956). In spite of this differential susceptibility, infection of flowers and berries may destroy immature fruit (McClellan and Hewitt, 1973; Nair and Parker, 1985). In addition, colonised senescent floral tissues and aborted berries can serve as conidial and mycelial inoculum (Gessler and Jermini, 1985; Hill, 1985; Northover, 1987; Nair and Nadtotchei, 1987) for late-season infections of sound berries.

On grapevine, studies with *B. cinerea* on various aspects such as host resistance, timing of fungicide application, biological control, control by cultural practises, disease prediction models, usually comprised investigations on mature berries. In most studies where grapes were artificially inoculated, berries were atomised with (De Kock and Holz, 1991; Nair, 1985; Nair *et al.*, 1988; Nelson, 1951), dipped in (Broome *et al.*, 1995), or injected with (Avissar and Pesis, 1991; Marois *et al.*, 1986; Thomas *et al.* 1988) conidial suspensions, or suspension droplets were placed onto the berry cheek (Chardonnet, 1997; Marois *et al.*, 1987). These methods allowed for the deposition of groups of conidia on berries, and differ from primary natural infection in the vineyard. The finding that *B. cinerea* infected and naturally occurred commonly in the structural parts of immature bunches, that these carried more *B. cinerea* than the berry cheek, and that these infections may be more important in *B. cinerea* bunch rot (Part 2, 3), suggest that more emphasis should be placed on the disease reaction of the structural bunch parts rather than on the berry.

It was recently showed (Part 2) that leaf blades and petioles on vine shoots of grape cultivars Dauphine and Merlot reacted similarly to natural *B. cinerea* infection as the structural bunch parts. The aim of this study is to find a morphological part which corresponds to the structural bunch parts in its disease reaction to *B. cinerea*. In breeding programmes today, researchers have to wait for bunches to develop before conclusions

regarding resistance against *B. cinerea* can be made. It would be of great value to the grape industry if a faster and more effective screening procedure could be developed. This study will compare the resistance reaction of leaf blades, petioles, internodes and inflorescenses on cuttings to those on older shoots from the vineyard.

MATERIALS AND METHODS

Grapevine material. Infection studies were conducted on young vinelets prepared from cuttings, or on shoots obtained from vineyards. Material was obtained from two vineyards (table grape cultivar Dauphine, wine grape cultivar Merlot) with a history of low *B.cinerea* incidences. Cuttings were obtained during July and August and left overnight in a captab (500 WP) solution before cold storage (4°C) in moist perlite in plastic bags. These measures ensure budding and prevent decay. When needed, cuttings were removed from the plastic bags and placed in warm water (50°C) for 30 minutes (Goussard & Orffer, 1979). The cuttings were then cut into 5-6 cm lenghts each with one dormant eye and placed into foamalite trays with holes. The trays with the cuttings (later reffered to as vinelets) were placed in large plastic containers filled with tap water and kept in a growth room at high relative humidity (85%) and temperature (25°C) until budding. Young vinelets were used for infection studies approximately two weeks after budding had commenced, or one month after budding. Older shoots were obtained from the vineyard when shoot length was approximately 25 cm. Vinelets and shoots were surface sterilised for 2 min in 20% sodium hypochlorite, rinsed in distilled water and air-dried. This treatment elminated the pathogen on the leaf surface (Sarig *et al.*, 1997) and promoted the development of latent infections established during moist incubation. The vinelets were replaced into clean foamalite trays and positioned in stainless steel containers with distilled water. The shoots were placed in flasks containing 20% sucrose solution to maintain turgidity.

Inoculation. A virulent isolate of *B. cinerea* obtained from a naturally infected grape berry was maintained on potato dextrose agar (PDA) at 5°C. For the preparation of inoculum, the isolate was first grown on canned apricot halves. Conidiophores from the colonised fruit were transferred to PDA in Petri dishes and incubated at 22°C under a diurnal regime (12h near-ultraviolet light; 12h dark light). Conidia were harvested dry with a suctiontype collector from 14 day old cultures and stored dry at 5°C until use (1 to 16 weeks).

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 20 min to settle onto the vinelets or shoots that were positioned on two screens. Petri dishes with water agar (WA) and PDA were placed on the floor of the settling tower at each inoculation and percentage germination of conidia was determined after 6 h incubation at 22°C (100 conidia per Petri dish, three replicates). Following inoculation, the screens were placed in 12 ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionized water to establish high relative humidity (≥93% RH). Each chamber contained one screen carrying 20 vinelets, or 10 shoots. Each chamber was considered as a replicate. These conditions provided circumstances commonly encountered in nature by the pathogen on grape leaves, namely dry conidia on dry leaves under high relative humidity (humid leaves). The chambers were incubated at 22°C with a 12 h photoperiod daily. After 24 h, the screens were removed from the chambers and placed in dry chambers (≤60% RH) for 48 h before the material was used for the determination of infection and disease expression.

Infection and disease expression. Following incubation vinelets or shoots were surface sterilised in 70% ethanol for 5 s. This treatment elminated the pathogen on the berry surface (Sarig *et al.*, 1997) and promoted the development of latent infections established during moist incubation (Coertze and Holz, 1999). The vinelets or shoots were then divided in two groups consisting of five vinelets, or five shoots each. From each vinelet or shoot, 10 leaf blades, 10 petioles, 10 internodes (approximately 20 mm each) and 10 inflorescenses were removed. Five each of the different parts were placed in Petri dishes on Kerssies' *B. cinerea* selective medium (Kerssies, 1990), and five on a water agar medium supplemented with paraquat (Grindrat and Pezet, 1994). The plates were incubated at 22°C under diurnal light. Disease expression was recorded for each sample, and incidences for each morphological part calculated after 14 days. These treatments provided conditions that facilitated the development of disease expression by latent infections established during moist incubation (Coertze and Holz, 1999). On Kerssies' medium, disease expression was the result of latent infection as influenced by host resistance. Previous studies showed that no superficial mycelial growth developed on the leaves during the early phases of incubation on

Kerssies' medium (Kerssies, 1990). Fungal structures that penetrated the skin during the period of moist incubation, therefore grew further under the influence of active defence. Incidences therefore described infection levels of the morphological part as regulated by host resistance. Paraquat terminated host resistance in the cells of the cuticular membrane without damaging host tissue (Baur *et al.*, 1969; Cerkauskas and Sinclair, 1980; Pscheidt and Pearson, 1989; Grindrat and Pezet, 1994). On paraquat medium, disease expression was the result of latent infection developing after surface sterilisation and the termination of host resistance. Incidences therefore described infection levels of a morphological part when host resistance was negated.

Disease resistance and inoculum levels. At each developmental stage, parts of a cultivar were catagorised for disease resistance according to the mean decay incidences recorded on Kerssies' medium. Sites showing decay of ≤5%, 6-20%, 21-40% and ≥41% were classified respectively as resistant, moderately resistant, susceptible and highly susceptible to infection. The sites were also catagorised into different sub-classes according to decay development on paraquat medium to describe their latent inoculum level. Sites showing decay of ≤5%, 6-20%, 21-40% and ≥41% were classified respectively as carrying low, intermediate, high and very high latent inoculum levels.

Statistical analysis. A split plot experimental design was used in all experiments. 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 and Wilk, 1965). The data was examined further by using the analysis of variance (ANOVA) and the treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor and Cochran, 1980).

RESULTS

Conidial germination on media. Conidia used at each inoculation were highly viable and germinated freely on PDA and WA. Germination on PDA and WA varied between 77-87%.

Infection and disease expression. Analysis of variance for effects of season, phenological stage, cultivar and treatment on decay development is given in Table 1. On

both cultivars, significantly more parts yielded the pathogen on paraquat than on Kerssies' medium (Table 2). Disease expression was furthermore significantly influenced by tissue age (Table 3). On all the parts used, disease expression for both cultivars was at a significantly higher level on the young than the old vinelets, and at significantly lower levels on the shoots than the vinelets. For both cultivars, leaf blades consistently yielded the highest, and petioles the lowest number of infected parts.

Disease resistance and inoculum levels. Mean decay levels for both cultivars, based on the data recorded in the different parts in two seasons, are given in Table 4-5. Descriptions of disease resistance and of inoculum levels are given in Table 6-7. In the case of vinelets, leaf blades, internodes and inflorescenses were all classified susceptible to highly susceptible. Only the petiole of the older vinelet was classified as moderately resistant. The different parts furthermore all carried very high inoculum levels. The shoots, petioles, internodes and inflorescences showed resistance, and carried high to intermediate inoculum levels. Leaf blades were susceptible.

DISCUSSION

This study, which was conducted in the laboratory with airborne conidia of *B. cinerea*, confirmed that solitary conidia readily penetrated leaf tissue and that latent infection was established at very high levels in leaf blades. Young leaves from vinelets and older leaves from vineyard shoots were furthermore classified as highly susceptible and susceptible, respectively. It was recently shown (Part 2) that although blades of mature grape leaves do not develop grey mould, they normally carried high levels of latent natural *B. cinerea* inoculum. These findings indicate that leaf blades are not appropriate parts for studying the behaviour of inoculum of *B. cinerea* and host responses in grape bunches.

Pezet and Pont (1986) showed in their histological studies of laboratory-inoculated bunches that *B. cinerea* colonises the stamens and invades their base situated on the receptacle. From there it spreads to the pedicel and vascular tissue in berries. My study (Part 2) on natural *B. cinerea* infection and disease expression in parts of grapevine bunches confirmed the role of this infection pathway in *B. cinerea* bunch rot. Based on the combined data for the different treatments, decay levels were the highest in the pedicels and the pedicel-

end of the berry. Overall, approximately 30% of these sites yielded *B. cinerea.* Levels were lower in leaf blades, rachises and laterals, of which approximately 20% yielded *B. cinerea.* The pathogen less often caused decay of petioles (10%) and berry cheeks (5%). The style ends of the berries, on the other hand, were virtually free (≤0.02%) from *B. cinerea* decay. Careful observation furthermore 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. According to this pattern of natural occurrence of the pathogen in grape bunches, incipient infections can cause both mid- or late-season bunch rot following a period of fungal latency in the rachises, laterals or pedicels, and not in berry cheeks and style ends. In this study, petioles and inflorescenses reacted more resistant and carried lower latent infection levels after inoculation with airborne conidia. Petioles were previously (Part 2) classified resistant and carried low to intermediate natural inoculum levels. It is therefore suggested that petioles and inflorescenses of vineyard shoots are appropriate parts for studying the behaviour of inoculum of *B. cinerea* and host responses in grape bunches.

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.

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

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.

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 detection of fungi in soybean tissues. *Phytopathology* 70: 1036-1038.

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.

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.

Corbaz, R. 1972. Etudes des spores fongiques captèes dans l'air. *Phytopatologische Zeitschrift* 74: 314-328.

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

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

Goussard, P. G. & Orffer, C. J. 1979. The propagation of Salt Creek. *Deciduous Fruit Grower* 29: 56-62.

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.

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

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.

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

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.

Marois, J.J., Bledsoe, A.M., Gubler, W.D. & 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.

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

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. & 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* 119:52-63.

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 Diseases of International Importance. Vol III: Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

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. & 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 grape. *Plant Disease* 73: 829-833.

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

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance (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 grape and plum fruit surfaces. *Plant Disease* 80: 688-691.

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.

Table 1. Analysis of variance for effects on percentage decay in *Botrytis cinerea* infected grapevine tissue

Source of variation	Df [a]	MS [b]	SL [c]
Season (S)	1	67513.611	0.0059
Phenological Stage (P)	2	329152.500	0.0001
S x P	2	35543.611	0.1068
Error (S x P)	24	5074.306	
Cultivar (C)	1	7380.278	0.0059
S x C	1	1033.611	0.2960
P x C	2	1368.611	0.2367
S x P x C	2	1011.944	0.3434
Time (T)	2	32.500	0.9660
S x T	2	1021.944	0.3398
P x T	4	3468.750	0.0071
S x P x T	4	3500.694	0.0067
C x T	2	410.278	0.6468
S x C x T	2	551.944	0.5569
P x C x T	4	1437.361	0.1972
S x P x C x T	4	1331.528	0.2318
Error (S x P x C x T)	120	938.306	
Medium (M)	1	113422.500	0.0001
S x M	1	9713.611	0.0001
P x M	2	4877.500	0.0006
C x M	1	146.944	0.6275
T x M	2	1300.833	0.1271
S x P x M	2	1168.611	0.1563
S x C x M	1	4340.278	0.0091
P x C x M	2	293.611	0.6245
S x P x K	2	1416.944	0.1060
S x T x M	2	566.944	0.4040
P x T x M	4	854.583	0.2455
S x P x T x M	4	873.194	0.2353
C x T x M	2	678.611	0.3384
S x C x T x M	2	596.944	0.3852
P x C x T x M	4	96.528	0.9603
S x P x C x T x M	4	607.361	0.4220
Error (S x P x C x T x M)	144	621.528	
Morphological Part (MP)	3	54118.056	0.0001
S x MP	3	4023.241	0.0001
P x MP	6	5310.278	0.0001
S x P x MP	6	2811.019	0.0001
C x MP	3	79.537	0.0798
S x C x MP	3	1058.241	0.9051
P x C x MP	6	1797.870	0.0214
S x P x K x MP	6	1046.944	0.0003
T x MP	6	426.019	0.0227
S x T x MP	6	612.083	0.4220
P x T x MP	12	499.213	0.1415
S x P x T x MP	12	144.352	0.2963
K x T x MP	6	378.981	0.9513
S x C x T x MP	6	533.657	0.4999
P x C x T x MP	12	478.565	0.2397
S x P x C x T x MP	12	4769.167	0.3343
M x MP	3	321.019	0.0001
S x M x MP	3	1395.278	0.5191
P x M x MP	6	1142.685	0.0034
S x P x M x MP	6	299.537	0.0136
C x M x MP	3	1830.648	0.5491
S x C x M x MP	3	643.981	0.0050
P x C x M x MP	6	202.870	0.1697
S x P x C x M x MP	6	888.611	0.8253
T x M x MP	6	317.685	0.0519
S x T x M x MP	6	650.139	0.6112
P x T x M x MP	12	1343.935	0.1075
S x P x T x M x MP	12	567.870	0.0002
C x T x M x MP	6	512.870	0.2379
S x C x T x M x MP	6	512.870	0.3000
P x C x T x M x MP	12	243.565	0.8646
S x P x C x T x M x MP	12	755.509	0.0474
Error	864	424.769	

[a] Degrees of freedom
[b] Mean square
[c] Significance level

Table 2. Mean decay incidences[v,w] recorded in vinelets and grape shoots inoculated with airborne *Botrytis cinerea* conidia

	Dauphine		Merlot	
Medium	**1998**	**1999**	**1998**	**1999**
Kerssies	25.89 g	46.56 de	31.56 f	48.67 cd
Paraquat	51.67 bc	55.00 b	51.67 bc	65.33 a

[v] Vinelets, developed from cuttings, and shoots, obtained from vineyards (table grape cultivar Dauphine, wine grape cultivar Merlot) were dusted with dry conidia in a settling tower and incubated for 24 h at high relative humidity (±93%). Following incubation, the material was surface sterilised to eliminate the pathogen on the bunch surface and to determine the development of latent infections established during moist incubation. Sections from different sites (leafblades, petioles, internodes, inflorescences) were incubated in Petri dishes on Kerssies' *B. cinerea* selective medium, or on water agar medium supplemented with paraquat. Disease expression was positively identified by the formation of sporulating colonies of *B. cinerea* on the different tissues.

[w] Values of each column or row followed by the same letter are not statistically different according to the Student's t – test ($P = 0.0091$).

Table 3. Mean decay incidences[v,w] recorded at different sites in vinelets and grape shoots inoculated with airborne *Botrytis cinerea* conidia

	Dauphine			Merlot		
Site	**Vinelet (2wk)**	**Vinelet (4wk)**	**Shoot**	**Vinelet (2wk)**	**Vinelet (4wk)**	**Shoot**
Leaf blade	84.67 a A	72.67 d B	22.67 h C	82.33 j A	68.00 m B	32.33 o D
Leafpetiole	48.00 b J	30.67 f M	8.00 i N	50.67 k J	34.33 n K	21.00 p L
Internode	79.33 a E	52.33 e F	14.33 i G	79.67 j E	67.00 m H	23.33 p I
Infloressence	65.00 c O	46.67 g Q	13.00 i P	68.66 l O	51.67 n Q	12.67 q P

[v] See Table 2.

[w] Values of each column that received the same small letter and rows that received the same capital letter are not statistically different according to the Student's t – test ($P = 0.0214$).

Table 4. Mean decay incidences recorded in vinelets and shoots of table grape cultivar Dauphine inoculated with airborne *Botrytis cinerea* conidia on Kerssies and paraquat medium

	Leaf blade		Petiole		Internode		Inflorescence	
Material	**K**[u]	**PQ**[u]	**K**	**PQ**	**K**	**PQ**	**K**	**PQ**
Vinelet (2wk)	78.67	90.67	68.67	90.00	27.33	68.67	52.67	77.33
Vinelet (4wk)	66.67	78.67	45.33	59.33	18.00	43.33	37.33	56.00
Shoot	20.67	24.67	7.33	21.33	5.33	10.67	6.67	19.33

[u] K = Kerssies' medium; PQ = paraquat medium.

Table 5. Mean decay incidences recorded in vinelets and shoots of wine grape cultivar Merlot inoculated with airborne *Botrytis cinerea* conidia on Kerssies and paraquat medium

	Leaf blade		Petiole		Internode		Inflorescence	
Material	**K**[u]	**PQ**[u]	**K**	**PQ**	**K**	**PQ**	**K**	**PQ**
Vinelet (2wk)	76.67	88.00	70.67	88.67	30.67	70.67	56.67	80.67
Vinelet (4wk)	56.67	79.33	60.67	73.33	21.33	47.33	46.00	57.33
Shoot	35.33	29.33	12.00	34.67	8.67	33.33	6.00	19.33

[u] K = Kerssies' medium; PQ = paraquat medium.

Table 6. Description of disease resistance [x] assigned to various sites in vinelets and shoots of table grape cultivar Dauphine, and of *Botrytis cinerea* inoculum levels [y]

Material	Leaf blade	Petiole	Internode	Inflorescence
Vinelet (2wk)	HS ++++	S ++++	HS ++++	HS ++++
Vinelet (4wk)	HS ++++	MR ++++	HS ++++	S ++++
Shoot	S +++	R ++	MR +++	MR +

[x] Disease resistance: R = resistant (<5% decay on Kerssies' medium); MR = moderately resistant (6-20% decay on Kerssies' medium); S = susceptible (21-40% decay on Kerssies' medium); HS = highly susceptible (> 41% decay on Kerssies' medium).

[y] Latent inoculum levels: + = low infection levels (<5% decay on Paraquat medium); ++ = intermediate infection levels (6-20% decay on Paraquat medium); +++ = high infection levels (21-40% decay on Paraquat medium); ++++ = very high infection levels (> 41% decay on Paraquat medium).

Table 7. Description of disease resistance[x] assigned to various sites in vinelets and shoots of wine grape cultivar Merlot, and of *Botrytis cinerea* inoculum levels[y]

Material	Leaf blade	Petiole	Internode	Inflorescence
Vinelet (2wk)	HS ++++	S ++++	HS ++++	HS ++++
Vinelet (4wk)	HS ++++	S ++++	HS ++++	HS ++++
Shoot	S +++	MR +++	MR +++	MR ++

[x] Disease resistance: R = resistant (<5% decay on Kerssies' medium); MR = moderately resistant (6-20% decay on Kerssies' medium); S = susceptible (21-40% decay on Kerssies medium); HS = highly susceptible (> 41% decay on Kerssies' medium).

[y] Latent inoculum levels: + = low infection levels (<5% decay on Paraquat medium); ++ = intermediate infection levels (6-20% decay on Paraquat medium); +++ = high infection levels (21-40% decay on Paraquat medium); ++++ = very high infection levels (> 41% decay on Paraquat medium).