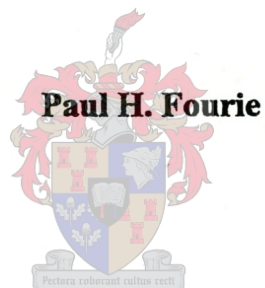


**EPIDEMIOLOGY OF *MONILINIA LAXA* ON NECTARINE AND PLUM:
INFECTION OF FRUITS BY CONIDIA**



**Dissertation presented for the degree of Doctor of Philosophy in Agriculture at the
University of Stellenbosch**

**Promoter: Prof. G. Holz
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DECLARATION

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

EPIDEMIOLOGY OF *MONILINIA LAXA* ON NECTARINE AND PLUM: INFECTION OF FRUITS BY CONIDIA

SUMMARY

Postharvest decay of stone fruit in the Western Cape province of South Africa is caused primarily by *Botrytis cinerea* (grey mould) and *Monilinia laxa* (brown rot). Little is known about the relative importance and seasonal occurrence of the two pathogens in nectarine and plum orchards, the mode of penetration of fruits by *M. laxa*, latency and subsequent disease expression by the latter pathogen. These aspects were investigated in this study.

By sampling from the Unifruco Quality Evaluation Scheme and from 11 stone fruit orchards, observations were made over a 3-year period of the occurrence of grey mould and brown rot in the major stone fruit regions. *Botrytis cinerea* was found to be the most important pathogen causing blossom blight and postharvest decay on stone fruit. The pathogen was most prominent on early- and mid-season cultivars. Brown rot was exclusively caused by *M. laxa* and no evidence was found that *M. fructicola* had been introduced into the region. *Monilinia laxa* was most prominent on the later maturing cultivars. *Botrytis cinerea* blossom infection did not contribute directly to postharvest decay. Both surface inoculum and latent infection consistently occurred on fruit in each orchard, although at fluctuating levels. Disease expression on developing fruit was not governed by the amount of *B. cinerea* occurring on fruit surfaces, but by the ability of fruit to resist disease expression. The amount of *B. cinerea* on fruits was generally higher during spring than during summer. *Monilinia laxa* occurred sporadically on the blossoms of late-maturing cultivars. Immature fruit were generally pathogen-free and disease expression occurred on maturing fruit only. These findings suggest that conidia of *M. laxa* are generally produced in orchards when fruits are approaching maturity and can penetrate and infect maturing fruit only.

The behaviour of airborne *M. laxa* conidia was subsequently studied on nectarine (cultivar Flamekist) and plum (cultivar Laetitia) fruit. For these studies, an inoculation method that simulates natural infection by airborne conidia was used. Fruit at pit hardening,

2 wk before harvest, harvest stage and after cold storage (nectarines 4 wk at -0.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH; plums 10 days at -0.5°C , 18 days at 7.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH) were dusted with dry conidia of *M. laxa* in a settling tower. The fruits were incubated for periods ranging from 3 to 48 h at high relative humidity ($\geq 93\%$, humid fruit) or covered with a film of water (wet fruit). Behaviour of the solitary conidia was examined with an epifluorescence microscope on skin segments stained in a differential stain containing fluorescein diacetate, aniline blue and blankophor. The ability of solitary conidia to colonise the fruit surface, penetrate fruit skins and to induce disease expression was determined by using a differential set of tests. For these tests, fruit were surface-sterilised (30 s in 70% ethanol) or left unsterile. From each group, fruit were selected for isolation (skin segment test), immersed in a 3% paraquat solution (paraquat-treated fruit test) or left untreated (sound fruit test). The findings demonstrated that solitary conidia of *M. laxa* behaved consistently on plum and nectarine fruit surfaces: appressorium formation and direct penetration was not observed on any of the fruit surfaces and germ tubes penetrated fruit predominantly through stomata, lenticels and microfissures in the fruit skin. The monitoring of airborne conidia revealed subtle effects of the fruits on the behaviour of solitary germlings, which could not be seen when using conidial suspensions. On both fruit types, no deleterious effect was seen on conidial and germling survival when fruit were kept humid at pit hardening, 2 wk before harvest and harvest. However, conidial and germling survival were drastically reduced by prolonged wet incubation of fruits. The findings on disease expression in the skin segment, paraquat-treated fruit and sound fruit tests clearly showed that the skin of both nectarine and plum fruits were not penetrated at the pit hardening stage, latent infections were not established and fruits reacted resistant to disease expression. These facets on both fruit types were furthermore unaffected by wetness. The barrier capacity of the fruit skin of the two stone fruit types however differed drastically later in the season. On nectarine, fruit skins were more readily penetrated and disease expression became more pronounced when fruit approached maturity. Penetration and disease expression on ripening nectarine fruit were furthermore greatly influenced by wetness. Maturing plum fruit, on the other hand, did not display the drastic change in the barrier capacity of fruit skins as observed on nectarine. The influence of wetness on infection and disease expression was also less pronounced than on nectarine. In fact, plum fruit remained asymptomatic in the sound fruit test after inoculation and humid incubation at the 2 wk before harvest stage, harvest stage and after cold storage. Plum fruit at these stages only developed disease after a prolonged period

(≥ 12 h) of wet incubation. The paraquat fruit test revealed that these fruits became more susceptible to latent infection, but they were not as susceptible as nectarine. Collectively, these findings indicate that *M. laxa* fruit rot epidemics on plum and nectarine are driven by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period. However, the barrier capacity of plum skins is considerably more effective than that of nectarine fruit. Wounds would therefore play an important role in the epidemiology of *M. laxa* on plum fruit.

Infection of fresh wounds by airborne *M. laxa* conidia, and by conidia and germlings that have established on fruits, was therefore investigated. Plum fruit (cultivar Laetitia) at pit hardening, 2 wk before harvest, harvest stage and after cold storage were dusted with dry conidia of *M. laxa* in a settling tower. Infection of nonwounded fruit and of fresh wounds by the airborne conidia on dry, humid and wet plum fruit surfaces, and by conidia and germlings that have been established on fruits under the wetness regimes was then investigated. Nonwounded immature and mature fruit remained mostly asymptomatic, whereas nonwounded cold stored fruit decayed readily. Wounding drastically increased infection by airborne conidia. Immature fruits were less susceptible to wound infection by the airborne conidia than mature fruits. Conidia dispersed freshly were more successful in infecting fresh wounds than conidia that were deposited, or germlings that established, on fruit surfaces 4 days prior to wounding. This decrease in infectivity was especially pronounced on humid and even more on wet incubated fruit. This study clearly showed that in order to reduce the incidence of brown rot, inoculum levels on fruit approaching maturity should be reduced by sanitation practices and fungicide applications. Furthermore, it is essential to protect fruits, especially near-mature fruits, from being wounded.

EPIDEMIOLOGIE VAN *MONILINIA LAXA* OP NEKTARIEN EN PRUIM: INFEKSIE VAN VRUGTE DEUR KONIDIA

OPSOMMING

Naoesverrotting van steenvrugte in die Wes-Kaap provinsie van Suid-Afrika word hoofsaaklik veroorsaak deur *Botrytis cinerea* (vaalvrot) en *Monilinia laxa* (bruinvrot). Min is bekend oor die relatiewe belang en seisoenale voorkoms van hierdie patogene in nektarien- en pruimboorde, asook oor die infeksieweg, latensie en daaropvolgende siekte-uitdrukking van *M. laxa*. Hierdie aspekte is in dié studie nagevors.

Monsters is oor 'n 3-jaar periode van die Unifruco Kwaliteitsevalueringskema, en ook van 11 steenvrugboorde verkry. Die voorkoms van vaalvrot en bruinvrot in die hoof steenvrugareas is so bepaal. *Botrytis cinerea* was die belangrikste patogeen wat betref bloeiselversenging en naoesverrotting. Verder was hierdie patogeen ook meer prominent op die vroeë- en middel-seisoen kultivars. Bruinvrot is uitsluitlik deur *M. laxa* veroorsaak en geen aanduiding omtrent die moontlike voorkoms van *M. fructicola* in Suid-Afrika is waargeneem nie. *Monilinia laxa* was meer prominent op die laat-seisoen kultivars. *Botrytis cinerea* bloeiselinfeksie het nie direk bygedra tot naoesverrotting nie. Beide oppervlakkige inokulum en latente infeksie het deurgaans, maar wel teen wisselende hoeveelhede, op vrugte in die onderskeie boorde voorgekom. Siekte-uitdrukking op ontwikkelende vrugte is egter nie beïnvloed deur die hoeveelheid *B. cinerea* op die vrug nie, maar eerder deur die vermoë van die vrug om siekte-uitdrukking te onderdruk. Die hoeveelheid *B. cinerea* op vrugte was verder hoër gedurende lente as gedurende somer. *Monilinia laxa* het slegs sporadies op die bloeisels van laat-seisoen kultivars voorgekom. Groen vrugte was in die algemeen vry van die patogeen en siekte-uitdrukking het slegs op ryp vrugte plaasgevind. Hierdie bevindinge dui daarop dat *M. laxa* in boorde hoofsaaklik op ryp vrugte geproduseer word. Hierdie swam infekteer ook net ryp vrugte.

Die gedrag van luggedraagde *M. laxa* konidia is bestudeer op nektarien- (kultivar Flamekist) en pruimvrugte (kultivar Laetitia). 'n Inokulasie-metode wat natuurlike infeksie deur luggedraagde konidia simuleer, is vir hierdie studies gebruik. Vrugte van die

pitverharding-, twee weke voor oes-, oesstadium, asook koud-opgebergde vrugte (nektariene, 4 weke by -0.5°C gevolg met 1 week by 23°C en $\pm 56\%$ RH; pruime, 10 dae by -0.5°C , 18 dae by 7.5°C gevolg deur 1 week by 23°C en $\pm 56\%$ RH), is met droë konidia in 'n inokulasietoring geïnkuleer. Die vrugte is vir periodes wat gewissel het van 3 tot 48 h geïnkubeer by hoë relatiewe humiditeit ($\geq 93\%$ RH, vogtige vrugte), of dit is bedek met 'n film water (nat vrugte). Die gedrag van die enkelspore (konidia) op die vrugoppervlak is met 'n epifluorisensiemikroskoop bestudeer. Skilsegmente is gekleur in 'n kleurstof, bevattende fluorisein diasetaat, analien-blou en blankofoor. Die vermoë van die enkelspore om die vrugoppervlak te koloniseer, te penetreer en om siekte-uitdrukking te induseer, is met 'n differensiële stel toetse bepaal. Vir hierdie toetse is die vrugte oppervlakkig gesteriliseer (30 s in 70% etanol), of nie-steriel gelaat. In elke groep is vrugte geneem vir isolasie (skilsegment-toets), of gedoop in 'n 3% parakwat-oplossing (parakwat vrugtoets), of onbehandeld gelaat (onbehandelde vrugtoets). Die bevindinge het op die soortgelyke gedrag van *M. laxa* enkelspore op die verskillende vrugsoorte gedui: appressoria en direkte penetrasie is nie waargeneem nie, en kiembuise het die vrugte hoofsaaklik deur huidmondjies, lentiselle en mikro-krakies in die vrugskil gepenetreer. Deur luggedraagde spore te bestudeer, is sekere subtiele effekte van die vrug op die gedrag van enkelspore op die vrugoppervlak waargeneem. Op beide vrugtypes is geen nadelige effek op konidium- en kiembuisoorlewing opgemerk wanneer die vrugte onder hoë vogtoestande geïnkubeer is. Konidium- en kiembuisoorlewing is egter drasties verlaag hoe langer die vrugte onder nat toestande geïnkubeer is. Die bevindinge van die skilsegment-, parakwat en onbehandelde vrugtoetse het duidelik daarop gewys dat die vrugskil van nektarien en pruim nie gepenetreer is tydens die pitverhardingstadium nie, latente infeksies is nie gevorm nie, en die vrugte was bestand teen siekte-uitdrukking. Hierdie fasette op beide vrugtypes is ook nie beïnvloed deur inkubasie-natheid nie. Die beskermingskapasiteit van die vrugskil van hierdie steenvrugtypes het egter drasties verskil later in die seisoen. Nektarien-vrugskille is meer geredelik gepenetreer en siekte-uitdrukking het toegeneem met rypwording. Penetrasie en siekte-uitdrukking is verder in 'n groot mate deur inkubasie-natheid bevoordeel. Rypwordende pruime het egter nie so 'n drasties verandering in die beskermingskapasiteit van die vrugskil getoon nie. Die invloed van inkubasie-natheid op infeksie en siekte-uitdrukking was ook minder opsigtelik as op nektarien. Pruimvrugte van die twee weke voor oes-, oesstadium, en koud-opgebergde pruime, wat onder hoë vog geïnkubeer is, het simptoombloos in die onbehandelde vrugtoets gebly. Vrugte van hierdie stadia het slegs simptome ontwikkel na

periodes van langer as 12 h onder nat toestande. Die parakwat-behandelde vrugtoets het egter gewys dat die pruimvrugte meer vatbaar vir latente infeksies raak, maar steeds nie so vatbaar soos die nektarienvrugte nie. Gesamentlik dui hierdie bevindinge daarop dat *M. laxa* bruinvrot epidemies op pruim en nektarien afhanklik is van inokulumvlakke op rypwordende vrugte, asook die weerstoestande gedurende die vooroes- en oesstadia. Die beskermingskapasiteit van pruim vrugskille was egter aansienlik meer effektief as dié van nektarien vrugte. Wonde op vrugte sal dus 'n groter rol speel in die epidemiologie van *M. laxa* op pruim.

Infeksie van vars wonde deur luggedraagde *M. laxa* konidia, en deur konidia en kiembuise wat reeds op die vrugoppervlak gevestig is, is gevolglik bestudeer. Pruimvrugte (kultivar Laetitia) van die pitverharding-, twee weke voor oes-, oesstadium, asook koud-opgebergde vrugte is in 'n inokulasie-toring geïnokuleer met droë *M. laxa* konidia. Infeksie van nie-gewonde vrugte en van vars wonde deur luggedraagde konidia op droë, vogtige en nat pruim vrugoppervlaktes, asook deur konidia en kiembuise wat reeds op die vrugoppervlak onder hierdie toestande gevestig is, is bepaal. Nie-gewonde groen tot ryp vrugte het meestal simptoemloos gebly, terwyl koud-opgebergde ryp vrugte wel verrot het. Wonde het die hoeveelheid infeksie deur luggedraagde spore drasties vermeerder. Konidia wat geïnokuleer is op vrugte met vars wonde, was meer in staat om hierdie wonde te infekteer as konidia en kiembuise wat 4 dae voor wonding gevestig is. Hierdie afname in infektiwiteit was meer sigbaar op die vogtige, maar veral die nat vrugte. Hierdie studie het duidelik gewys dat inokulumvlakke op rypwordende vrugte verlaag moet word deur sanitasie-praktyke en fungisiedtoedienings. Dit is verder belangrik om vrugte, veral rypwordende vrugte, teen wonding te beskerm.

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CONTENTS

1. Etiology and control of the brown rot fungi.....	1
2. Occurrence of <i>Botrytis cinerea</i> and <i>Monilinia laxa</i> on nectarine and plum in Western Cape orchards.....	68
3. Behaviour of solitary conidia of <i>Monilinia laxa</i> and disease expression on nectarine fruit	102
4. Behaviour of solitary conidia of <i>Monilinia laxa</i> and disease expression on plum fruit	134
5. Wound infection of plum fruit by airborne conidia of <i>Monilinia laxa</i>	159

1. ETIOLOGY AND CONTROL OF THE BROWN ROT FUNGI

INTRODUCTION

Towards the end on the nineteenth century serious losses to the cherry crops of Europe occurred, at first attributed to frost damage. However, investigation proved the involvement of *Monilia* fungi, causing blossom wilts, death to young twigs and often branches, due to the girdling and obstruction of the xylem tissue, as well as fruit rots (Wormald, 1919). According to Wormald (1919) the first report of *Monilia* was by Persoon in 1796, finding a fungus producing tufts of moniliform chains of conidia on decaying fruit of *Pyrus communis*, *Prunus domestica* and *Amygdalus persica*. He originally named the fungus *Torula fructigena*, but changed the name to *Monilia fructigena* five years later. Several workers renamed the fungus, however, before Aderhold found the apothecial stage of *M. fructigena* in 1904, calling it *Sclerotinia cinerea* in 1905. In 1928 Honey renamed *S. cinerea* to *Monilinia fructicola* and described *M. laxa* and *M. fructigena* in 1946 (Batra, 1991).

CAUSAL ORGANISMS

Brown rot of stone fruit is caused by several species of *Monilinia*. This genus belongs to a family, Sclerotiniaceae, of the Discomycetes, a sub-division of Ascomycotina. The genus *Monilinia* is characterised as forming long branched chains of lemon-shaped macroconidia, developing from simple conidiophores, usually occurring in cushion-like sporodochia on the surface of infected fruit. In the event of sexual reproduction, apothecia are formed, bearing inoperculate asci, containing ellipsoidal ascospores. The presence or absence of disjunctors in the conidial chain divides the genus *Monilinia* into two groups: the junctoriae and the disjunctoriae. The brown rot causing species are grouped in the former group, with conidia joined directly in the conidial chain (Byrde & Willetts, 1977; Willetts & Bullock, 1993). Three *Monilinia* species can cause brown rot: *Monilinia fructicola* (Wint.) Honey, *Monilinia fructigena* (Aderh. and Ruhl.) Honey and *Monilinia laxa* (Aderh. and Ruhl.) Honey. *Monilinia fructicola* originated from America, but also occurs in East Asia and Australasia. *Monilinia fructigena* and *M. laxa* are essentially Old World species with a

wide distribution. *Monilinia fructigena* attacks mainly apples, pears and occasionally stone fruit, while *M. laxa* and *M. fructicola* attack mainly stone fruit (Batra, 1985; Willetts & Bullock, 1993).

Distinction between species, in particular between *M. laxa* and *M. fructicola*, is of great importance, especially since the more virulent species, *M. fructicola*, does not occur in Europe (Willetts & Bullock, 1993) and South Africa (Fourie & Holz, 1985a; Schlagbauer & Holz, 1987). No clear distinction exists between morphological characters *in vivo*, hence the use of cultural characteristics and molecular techniques. Cultural growth of *M. laxa* on potato dextrose agar (PDA) is characteristically lobed with poor spore production, compared with the abundant spore production and entire colony margins of *M. fructicola* (Hewitt & Leach, 1939; Calavan & Keitt, 1948; Ogawa *et al.*, 1954; Jenkins, 1965a; Heyns, 1968; Penrose *et al.*, 1976; Ogawa *et al.*, 1978; Sonoda *et al.*, 1982b; Corazza *et al.*, 1998; Leeuwen & Kesteren, 1998). Calavan and Keitt (1948) however, observed *M. fructicola* cultures with similar lobed growth on PDA to *M. laxa* at low temperatures. *In vitro* growth of *M. fructicola* was also shown to be faster than that of *M. laxa* (Leeuwen & Kesteren, 1998). Sonoda *et al.* (1982b) distinguished between *M. laxa* and *M. fructicola* by the characteristic interactions between these species on oatmeal agar. Distinct black lines formed between these colonies within 10 days of incubation, whereas light, double lines were occasionally observed between *M. laxa* isolates. Spore germination has also been used to distinguish between these two species. Spores germinated quicker (75 min) in the case of *M. fructicola*, compared with the 4 h it took for the conidia of *M. laxa* to germinate. Once germinated, germ tubes of *M. laxa* on PDA were branched and crooked, compared with the straight, unbranched germ tubes of *M. fructicola* (Hewitt & Leach, 1939; Calavan & Keitt, 1948; Ogawa *et al.*, 1954; Jenkins, 1965a; Heyns, 1968; Leeuwen & Kesteren, 1998). Other criteria used to distinguish between these species are the more frequent formation of hyphal anastomosis by *M. fructicola* (Ogawa & English, 1964) and electrophoretic studies (Penrose *et al.*, 1976). More recent techniques for the distinction between *Monilinia* spp. include the use of total protein profiles (Belisario *et al.*, 1998), a species-specific primer for *M. fructicola* (Corazza *et al.*, 1998) and ELISA (Hughes *et al.*, 1998).

Clear distinction between the brown rot fungi and the correct identification thereof, in particular between *M. laxa* and *M. fructicola*, has far-reaching consequences. The latter

fungus is a quarantine organism in Europe, thus preventing stone fruit export from *M. fructicola*-inhabiting countries to this major export destination. Heyns (1967; 1968) and Matthee (1970) reported that brown rot of peaches in South Africa was caused by *M. fructicola*. Later studies however confirmed that South African brown rot is in fact caused by *M. laxa* and that *M. fructicola* does not occur in this country, rendering *M. fructicola* an important quarantine pathogen in South Africa (Fourie & Holz, 1985a; Schlagbauer & Holz, 1987).

DISEASE CYCLE

The brown rot fungi are temperate fungi with a fairly specific host range, causing moderate to severe amounts of disease. Reproduction is mainly asexual and the conidia that are formed can infect blossoms to cause blossom and twig blight, infect green fruit to cause latent or quiescent infections and infect ripening or harvested fruit to cause the characteristic brown rot. The following section will summarize various aspects of the disease cycle, also focusing on host and environmental factors that influence these fungi.

Environmental requirements

Germination. Optimum conidial germination occurred at 15 to 25°C under free moisture conditions, with germ tubes visible after 2 to 4 h. In the absence of free moisture, *M. laxa* germinated at relative humidity of 98 to 100% (Tamm & Flückiger, 1993; Tamm, 1994). Under laboratory conditions, Good and Zathureczky (1967) found that a small proportion of *M. fructicola* germlings died when a dry spell interrupted the period of high moisture required to initiate germination. They commented on the considerable ability of the germlings to tolerate severe drying and noted the possibility that successful infection in the field might occur after several days of intermittent growth of the germ tube. Naqvi and Good (1957) reported that fresh spores germinated after 3 to 4 h, whereas old spores that have been stored developed a lag of 36 h, in some cases up to 60 h, before germination occurred. This delay in germination was independent of temperature and was attributed to the metabolism of the dormant spore, which is in part controlled by physical limitations imposed by the extreme dehydration of the cytoplasm.

Ascospore discharge and germination are influenced by weather conditions such as temperature, rain, moisture, relative humidity and light. The optimal temperature for ascospore discharge and germination for *M. fructicola* is 15 to 16°C. Higher temperatures of up to 25°C did not affect germination, but caused a reduction in ascospore discharge, mainly due to the faster disintegration of the apothecia (Hong & Michailides, 1998).

Mycelial growth. The brown rot fungi are well adapted to moderate weather conditions. *In vitro* mycelial growth of *M. laxa* was observed at 2.5°C up to 31°C, with optimum at 25°C. Diurnal cycles did not stimulate or decrease mycelial growth under these conditions (Tamm & Flückiger, 1993).

Sporulation. Incubation temperature influenced the size and consequently also certain characters affecting the virulence of developing conidia. *Monilinia fructicola* colonies incubated at 15°C, rather than 20 or 25°C, produced larger quantities of bigger conidia with better germination percentages and infective ability (Phillips, 1982a; Phillips, 1984; Margosan & Phillips, 1985). Increased glucose concentration significantly influenced the aggressiveness, spore volume, nuclear number and germination of *M. fructicola* conidia produced on PDA. Aggressiveness was positively correlated with spore volume and nuclear number (Phillips & Margosan, 1985; Phillips *et al.*, 1989). Temperature affected spore volume, nuclear number and aggressiveness considerably more when produced at lower concentrations of glucose. Spore volume and aggressiveness were greatest when spores were grown at 15°C with 15% glucose in the medium, and nuclear number greatest at 15°C in 30% glucose and 25°C in 15% glucose (Phillips & Margosan, 1985). A later study showed that glucose affected spore size, but not mannose or fructose, indicating that glucose might be more easily utilised (Margosan & Phillips, 1989). Spore production on fresh nectarine or peach fruit in the laboratory or in the orchard was similarly influenced (Phillips, 1984). Tamm and Flückiger (1993) found *in vitro* sporulation of *M. laxa* enhanced by low temperatures, producing the highest number of conidia at 10°C. Weather conditions are a major determinant of the inoculum level reached by harvest. This was especially the case in humid areas where desiccated infections could produce a substantial amount of inoculum following repeated wetting (Kable, 1969a). Although high humidity was a prerequisite for sporulation, Byrde and Willetts (1977) noted that an increase in humidity often suppressed conidial production since it stimulated vegetative growth. The septa that separate conidia in

the spore chains did not develop under moist conditions and relatively low humidity was needed for fragmentation of conidial chains (Byrde & Willetts, 1977).

Blossom infection. Temperature and wetness duration are important environmental factors determining the infection incidence of *M. laxa* (Calavan & Keitt, 1948; Weaver, 1950; Corbin, 1963; Biggs & Northover, 1988b; Northover & Biggs, 1995; Tamm *et al.*, 1995). Calavan and Keitt (1948) found that moderate to high temperatures were favourable for cherry blossom blight by *S. laxa*, with infection and disease development most rapid at 24°C. Infection occurred at lower temperatures (11°C), further favoured by the slower abscission of diseased flowers. The pathogen was also greatly favoured by constant conditions of abundant moisture, such as rain or heavy dew. Once the pathogen has rotted the pedicels, the moisture requirements declined, mainly because dry weather advanced the abscission of diseased flowers (Calavan & Keitt, 1948). Tamm *et al.* (1995) observed that *M. laxa* was able to cause sweet cherry blossom infection at temperatures as low as 5°C with short periods of wetness duration, with increased infection as temperature and wetness period increased. Weaver (1950) reported similar results for *M. fructicola* on peach blossoms and Wilcox (1989) for *M. fructicola* on sour cherry blossoms. Blossom blight incidence was proportional to incubation temperature (optimum 20°C) and wetness duration, the latter being especially important, since it provided the requisite environment for spore germination and germ tube development (Wilcox, 1989). High relative humidity during the post-infection incubation period increased *M. fructicola* blight incidence, rate of colonisation and sporulation on peach blossoms (Weaver, 1950) and sour cherry blossoms (Koball *et al.*, 1997) proportionally to the number of hours at high humidity.

Fruit infection. Infection incidence by *M. fructicola* of peach and sweet cherry fruit increased with an increase in temperature and wetness duration, with the incubation period decreasing with increased wetness. Optimum temperatures for cherry fruit decay were 20 to 22.5°C and for peach 22.5 to 25°C (Biggs & Northover, 1988b). Corbin (1963) found that post-infection humidity did not affect sporulation intensity on fruit. Abbas *et al.* (1981) attributed the occurrence of *M. laxa* in northern Iraq, as well as the absence of this fungus from central Iraq, to the higher average rainfall in the north, especially during bloom. Ogawa *et al.* (1983) supported this attribution by stating that crop loss in California in the absence of rain during the harvesting period is negligible.

Survival

The brown rot fungi overwinter mainly as mycelium on mummified fruit, infected fruit peduncles or cankers on trees (Jenkins, 1965b; Sutton & Clayton, 1972; Byrde & Willetts, 1977; Ogawa *et al.*, 1983; Willetts & Bullock, 1993). Sutton and Clayton (1972) furthermore isolated *M. fructicola* from discoloured xylem tissue in peach branches within 3 cm of infected peduncles. The development of vascular discolouration and subsequent establishment of *M. fructicola* in peach branches was attributed to the absence of an abscission layer, which also permitted the infected blossom or fruit to remain attached to the branch. Cankers played an important role in the overwintering of brown rot fungi, especially in areas where sexual reproduction occurred infrequently (Batra, 1985). Hewitt and Leach (1939) found sporodochia of *S. laxa* in abundance mostly on blighted twigs, cankers and mummies in California and reported that *S. fructicola* rarely produced conidial tufts on these twigs or cankers, but were repeatedly isolated from mummified fruit. In a study by Shepherd in 1968 on the survival of *M. fructicola* conidia, it was observed that 0.1 to 6% of the spores survive on the mummies in the trees (Byrde & Willetts, 1977). The reason for this loss in viability was ascribed to several factors: desiccation, extremes of temperature, radiation, starvation and competition from other microorganisms (Byrde & Willetts, 1977). Smith *et al.* (1965) observed from *in vitro* studies that exposure to low temperatures (10 days at -1.1 to 4.4°C) did not affect the germination of *M. fructicola* conidia, whereas the exposure of conidia to hot air was lethal at relative humidity of 80 or 90%, but not at 50% RH. Kable (1969b) attributed the poor overwintering ability of *M. fructicola* in apricot orchards in the Murrumbidgee irrigation areas in Australia to the length of the survival time (at least 8 months), very high temperatures and low humidities during the midsummer months. Naqvi and Good (1957) however remarked on the considerable resistance to desiccation of *M. fructicola* conidia during storage at temperatures ranging from 5 to 35°C and concluded that dehydration was not a significant factor in the inactivation of these spores. They found that very moist conditions were more detrimental to conidium survival than very dry conditions.

Melanin, associated with the cell walls of conidia and the outer rind of the stroma, is of great importance to the survival of these structures. Rehnstrom and Free (1996) observed that the conidia of melanin-deficient mutants of *M. fructicola* were more readily killed by

high temperature, desiccation, freezing, ultra-violet irradiation and digestion with hydrolytic enzymes. The mutant stroma also had reduced tensile strength.

Sexual reproduction

In the event of sexual reproduction, ascospores are produced in apothecial ascomata that develop from mummies that have overwintered on the orchard floor. Ascospore discharge usually coincides with the emergence of young shoots and blossoms. The sexual stage has rarely been reported for *M. fructigena* and *M. laxa* (Wormald, 1921; Calavan & Keitt, 1948; Jenkins, 1965a; Ogawa *et al.*, 1983), but is frequent, although production is erratic, for *M. fructicola* (Byrde & Willetts, 1977; Tate & Corbin, 1978; Willetts & Harada, 1984; Willetts & Bullock, 1993; Hong *et al.*, 1996; Hong & Michailides, 1998). Willetts and Harada (1984) divided apothecial production into four stages: stromatal development, stroma maturation, apothecial initiation and apothecial differentiation. The vegetative hyphae of *Monilinia* spp., under optimum conditions, formed a sclerotial stroma that differentiated into an outer, melanin-pigmented rind, which protected a central tissue or medulla (Whetzel, 1945). The stroma matured under fair environmental conditions (20 to 30°C), allowing apothecial initiation, which required low temperatures (0 to 15°C), but no light. Incomplete stroma maturation of *M. laxa*, *M. fructigena* and to a lesser extent *M. fructicola*, infecting mainly late-ripening fruits, might be the reason why apothecial development was not often found in the life cycles of these species. Holtz and Michailides (1994) could only induce ascospore formation from stromatised mummies and never from non-stromatised mummies. Apothecial differentiation occurred when temperatures rise in early spring (10 to 15°C), either in diffused sunlight or daylight. Apothecia developed only in moist soils, protected from desiccation by foliage or other covering (Willetts & Harada, 1984). Complete hydration was essential for apothecial production and soaking of mature stromata probably stimulated sexual structures to develop (Sanoamuang *et al.*, 1995). Hong *et al.* (1996) found apothecia only in orchards with either a cover crop or natural vegetation between rows. When the relative humidity was high, the discs of mature apothecia of *M. fructicola* were fully expanded, giving a characteristic disc-like appearance and maximum exposure to the atmosphere. Under dry conditions the disc became cup-shaped, preventing water loss from the fertile elements of the fruiting body (Willetts & Harada, 1984).

In a study on the population structure of *M. fructicola* in nectarine tree canopies, Sonoda *et al.* (1991) demonstrated the genetic variability by the variation in vegetative compatibility groups from different lesions on the same fruit, as well as from different fruit and trees. Studies on the mating behaviour in field populations of *M. fructicola* by means of vegetative compatibility tests indicated that individual apothecia generated populations of genetically diverse progeny. Certain apothecia segregated a 1:1 ratio of benomyl resistant and sensitive progeny, proving that *M. fructicola* was capable of outcrossing and generating new genotypic combinations (Free *et al.*, 1996). Sanoamuang *et al.* (1995) reported similar results.

Inoculum sources

The first rain in spring triggers sporulation from overwintering mycelium when temperature, relative humidity and day-length are suitable. Tufts of conidia develop on mummies, infected twigs, peduncles, cankers or any infected area of the host. A direct dependence exists between the number of effective precipitations and the number of conidial tuft generations (Karova, 1974). Macro-conidia produced on these tufts served as the primary inoculum for infection of blossoms (Matthee, 1970; Byrde & Willetts, 1977; Tate & Corbin, 1978; Landgraf & Zehr, 1982; Ogawa *et al.*, 1983; Biggs & Northover, 1985). However, ascospores as the major source of primary inoculum for *M. fructicola* were reported from New Zealand (Tate & Corbin, 1978) and California (Hong *et al.*, 1996; Hong & Michailides, 1998).

Mummies on trees were found to be a significantly greater source of overwintering inoculum than mummies found on the orchard floor, with infected peduncles, cankers and twigs also contributing to the primary inoculum source (Biggs & Northover, 1985). Wilcox (1989) observed that mummified fruit in sour cherry orchards in New York state yielded only 2 to 18% as many conidia of *M. fructicola* during the sample periods before shuck fall as during the preharvest period. Thus, under New York conditions, mummies were often a more important inoculum source for initiating fruit rot than blossom blight. Poor sporulation from mummies was attributed to the lack of rainfall during the blossom period in the years studied. Blighted blossoms in South Carolina peach orchards were not a major contributor to the secondary inoculum source in the preharvest period, because sporulation on blighted

blossoms declined as the season progressed and did not increase drastically during rainy periods. Under these conditions sporulation on fruit peduncles, twig cankers and mummified fruit were not epidemiologically important (Landgraf & Zehr, 1982). Hong *et al.* (1997) observed the desiccation of blighted blossoms and non-abscised, aborted fruit and the consequential prevention of sporulation in the dry weather conditions of California. They therefore concluded that non-abscised, aborted fruit and blighted blossoms were unlikely to be important sources of secondary inoculum for fruit brown rot in California orchards. However, thinned fruit on the orchard floor were often wetted during irrigation and consequently enhanced the sporulation of *M. fructicola*. This resulted in an exponential increase in decay with the increased density of thinned fruit on the orchard floor sporulating with *M. fructicola*, highlighting the significance of thinned fruit as secondary inoculum source for pre- and postharvest fruit decay (Hong *et al.*, 1997). Biggs and Northover (Biggs & Northover, 1985) cited thinned fruit and non-abscised, aborted fruit in the tree as the main inoculum sources during the fruit ripening stages. Another important inoculum source was conidia produced on early-maturing cultivars, wind-dispersed to the later maturing cultivars. Landgraf and Zehr (1982) made similar conclusions and found that the later the fruit were thinned, the higher the percentage sporulating fruit during the preharvest period.

Alternative hosts might also play an important role in the etiology of brown rot fungi. Landgraf and Zehr (1982) reported that conidia from wild plum mummies and infected wild plum tissue and ascospores from wild plum thickets were important primary inocula.

In general the inoculum dose is of great importance for successful infection and several studies have shown increased infection with an increase in inoculum dose (Roberts & Dunegan, 1926; Corbin, 1963; Hall, 1971; Fourie & Holz, 1985b; Biggs & Northover, 1988a; Brown & Wilcox, 1989; Wilcox, 1989; Northover & Biggs, 1990; Northover & Biggs, 1995; Hong *et al.*, 1998). An increase in the inoculum dose would increase the likelihood of the increased number of germ tubes encountering sites susceptible to penetration. However, conidia act independently and synergism might only occur at high doses (Hall, 1971). Northover and Biggs (1990) found in a study on sweet and sour cherries that host resistance against *M. fructicola* was overcome when inoculum dose was increased. In a similar study on mature sweet and sour cherries, Northover and Biggs (1995) found that increasing the inoculum dose of *M. fructicola* advanced initial lesion appearance, increased infection

incidence and increased the percentage of fruit with sporodochia. Hong *et al.* (1998) inoculated peaches, nectarines and plums with different conidial concentrations of *M. fructicola* and observed decay of wounded fruit at spore loads as low as two spores per wound and even in some cases infection of nonwounded nectarines and peaches with this low inoculum dose. However, the increase in lesion diameter and decay incidence was substantial when the spore load was increased. Incubation periods were furthermore decreased with increase in spore concentration.

Spore dispersal

Primary inocula, macro-conidia and/or ascospores, are wind or rain dispersed (Byrde & Willetts, 1977; Ogawa *et al.*, 1983). Conidiophores are short and unspecialised, but still elevate the spore chains well above the infected host surface, exposing it to wind currents (Byrde & Willetts, 1977). Infected blossoms, twigs, peduncles or mummies in the tree with sporulating lesions are ideally positioned for optimum dispersal of conidia by air currents or by water splash from rain or overhead irrigation. Batra (1985) also listed birds and humans as potential dispersing agents of the brown rot fungi.

According to Byrde and Willetts (1977), P.F. Kable used the Hirst automatic volumetric spore trap to study conidial dispersal of *M. fructicola* in a peach orchard in the Murrumbidgee irrigation areas of Australia. The author reported one major dispersal period each year that started about 4 wk before harvest and peaked at the harvest stage. Dispersal continued up to 8 wk after the fruit were harvested and declined to relatively low levels for the remainder of the year. Dispersal was favoured by low humidity and high wind speeds, conditions frequently experienced during early afternoons (Byrde & Willetts, 1977). Ogawa *et al.* (1983) however reported that wind speeds as low as 2 mph were sufficient to dislodge and spread conidia from mummies and blighted blossoms. After a period of optimum dispersal, inoculum levels depleted, but renewed spore production occurred following rain showers (Byrde & Willetts, 1977). Similar results were obtained by Jenkins (1965b) on the conidial dispersal of *M. fructicola* in a peach orchard in Victoria, Australia, and by Corbin *et al.* (1968) of *M. laxa* in an apricot orchard in California. Phillips and Harvey (1975) washed stone fruit and plated the wash water on a selective medium for *Monilinia* spp.. The authors reported spore densities of 0 to 170 000 spores per fruit and noted a detectable increase in the

amount of inoculum present on the fruit late in the harvest period. Since conidia on the fruit surface might not remain viable for extended periods (Corbin *et al.*, 1968), it was hypothesised that these spore densities were not the result of long-term inoculum build-up, but rather from current sporulation from infected fruit in the orchard (Phillips & Harvey, 1975).

Although wind dispersal allowed the spread of conidia over a large area, it was believed that splash dispersal was more effective. Splash dispersal not only liberated the conidia from the sporulating colony, but also supplied moisture essential for germination and subsequent mycelial development. Conidia were spread in the presence of free moisture to other parts of the tree, in some cases adjacent trees, and were not subjected to the extreme environmental conditions associated with wind dispersal (Jenkins, 1965b; Byrde & Willetts, 1977; Tate & Corbin, 1978). Due to this efficient inoculation of fruit, Tate and Corbin (1978) ruled splash dispersal as the main mechanism of spore dispersal for quiescent infections early in the season.

Insect vectors. Several insect species that may or may not be considered as pests might act as vectors for plant pathogenic propagules. Louis *et al.* (1996) reported the persistence of *Botrytis cinerea* in the vinegar fly, *Drosophila melanogaster*. Conidia adhering externally to the fly cuticle (heads or legs) were eliminated within 2 days by mechanical loss and the cleaning behaviour of the flies. Microscopic examination immediately after dissection of flies that fed on sporulating fungal cultures however showed rectums filled with non-germinated conidia that germinated *in situ* after 24 h at 20°C under a cover slip. Cultivation of isolated faeces resulted in *B. cinerea* development. A long-term relationship between vector and fungus was obtained in the fly life cycle when *B. cinerea* conidia germinated, produced mycelium and differentiated into microsclerotia.

Dissemination of brown rot spores by the dried fruit beetle (*Carpophilus hemipterus*) was first reported by Ogawa (1957). Vinegar flies (*D. melanogaster*) and nitidulid beetles (*C. hemipterus* and *C. freemani*) captured in Californian nectarine and peach orchards were contaminated with *Mucor piriformis*, *Rhizopus stolonifer*, *Cladosporium* spp., *Penicillium* spp. and *M. fructicola*, but transmitted mainly the fast-growing *M. piriformis* to wounded or nonwounded fruit (Michailides & Spotts, 1990). It is possible that *M. fructicola* was also

transmitted to healthy fruit and subsequent brown rot decay contaminated by *Mucor* decay. Tate and Ogawa (1975) demonstrated the vectoring capabilities of nuttallid beetles in late-maturing peach and nectarine orchards in California. *Carpophilus mutilatus* and *Haptonchus luteolus* were the most important vectors of *M. fructicola* conidia, since they were active visitors of oriental fruit moth exit holes in healthy fruit and when contaminated, transmitted viable conidia to fungicide-treated or untreated fruit. Other nuttallid species, *C. freemani* and *C. hemipterus*, were less important vectors, since they preferred decaying fruit and rarely visited injured, healthy fruit (Tate & Ogawa, 1975). Kable (1969a) observed the vectoring capabilities of *C. hemipterus* and *C. davidsoni* in Australian peach orchards, with or without association with the oriental fruit moth, but concluded that the relative roles of the various *Carpophilus* species were essentially the same. Lack (1989) accentuated the role of insect vectors in the spread of *M. fructigena* in apple orchards, especially under conditions unsuitable for wind or splash dispersal. He noted that insects had clear advantages over wind dispersal, since they acted selectively when picking up and depositing inoculum at suitable sites, also reducing the passage time from source to new substrate and in some cases afforded physical protection to spores being carried. Garic *et al.* (1990) demonstrated the effect of *Cydia molesta* on the incidence of *Monilinia* spp. on quince fruit. The first and second generations of this important pest of peach and quince in Yugoslavia develop on peach, while the third and fourth generations develop on quince. Of the total number of brown rot decayed quince fruit in 1987 and 1988, more than 80% had been injured by *C. molesta*. The authors concluded that timely control of *C. molesta* was the most effective measure against the incidence of *Monilinia* spp. on quince fruit.

Batra and Batra (1985) reported the fascinating floral mimicry induced by *Monilinia* spp. in order to exploit pollinators of blueberries and huckleberries as vectors. Leaves and shoots of the host, when infected, became ultraviolet reflective, fragrant and also secreted sugars at their lesions, thus attracting insect pollinators to the discoloured leaves. The insects digested the sugars and also transmitted the conidia from the lesions to the host's flowers, depositing the conidia on stigmas or other flower parts, which resulted in infected ovaries and consequently mummy-berry disease.

Despite vectoring inoculum, insects also play an important role in the brown rot disease cycle by causing wounds on healthy fruit that might act as penetration sites for

inoculum dispersed by wind, water splash or by other insects (Croxall *et al.*, 1951; Poulos & Heuberger, 1952; Kable, 1969a; Tate & Ogawa, 1975; Michailides & Spotts, 1990). Ogawa *et al.* (1983) described this scenario for nitidulid beetles vectoring *M. fructicola* conidia from diseased fruit to healthy fruit wounded by oriental fruit moth or twig borer larvae. Kable (1969a) reported a similar association in Australian peach orchards with dried fruit beetles vectoring spores to wounds caused by the oriental fruit moth.

Ascospore dispersal. Ascocarp initials are phototropic, producing discs orientated at right angles to the light source. Ascospores are discharged in the general direction of the light and are picked up by the air currents. Apothecia are mostly produced from the stroma of mummified fruit lying beneath the tree, resulting in ascospore discharge in close vicinity of susceptible host tissue in early spring. Ascospores are discharged in large numbers following a turgor build-up in the fruiting bodies, usually associated with an increase in temperature and air movement (Byrde & Willetts, 1977).

Infection and colonisation

Monilinia fructicola and *M. laxa* are the brown rot species mostly associated with brown rot of stone fruit. Both species occur in Australasia and America, resulting in several studies on their relative pathogenicity. *Monilinia laxa* is mostly associated with blossom and twig blight, whereas *M. fructicola* causes extensive fruit rot (Hewitt & Leach, 1939; Ogawa *et al.*, 1954; Ogawa & English, 1960; Ogawa *et al.*, 1975; Byrde & Willetts, 1977; Ogawa *et al.*, 1983). Despite this observation, *M. fructicola* was found to be more virulent on blossoms than *M. laxa* (Ogawa & English, 1960). In a later report, Ogawa *et al.* (1983) stated that blossom blight of peaches and nectarines was caused almost exclusively by *M. fructicola*. Penrose *et al.* (1976) inoculated apricot and peach fruit with *S. fructicola* and *S. laxa* and found that after 3 days the rot caused by *S. fructicola* was more extensive than that caused by *S. laxa*. *Sclerotinia fructicola* sporulated profusely, while the lesion caused by *S. laxa* only showed signs of sporulation after 4 days. Hewitt and Leach (1939) reported similar results.

Host resistance. On all hosts, successful infection and colonisation by brown rot fungi became more eminent with increasing fruit maturity (Corbin, 1963; Hall, 1971; Jones, 1983; Ogawa *et al.*, 1983; Biggs & Northover, 1988a; Northover & Biggs, 1990; Emery *et al.*, 2000). Long lag phases on green fruit existed before establishment of infection, lessening

the possibility of epiphytotics during the green fruit stages. Lag phases shortened as fruit matured, thus increasing the possibility of epiphytotics. Low incidences of brown rot in green fruit could thus be attributed to host resistance as well as low spore loads (Corbin, 1963). Ogawa *et al.* (1983) reported that *M. fructicola* required a wetting period of 30 h on green almond fruit, whereas ripe fruit could be infected with 2 to 4 h wetness at 20°C. Northover and Biggs (1990) found host resistance of sweet and sour cherries against *M. fructicola* to rise at the onset of pit hardening, but it declined 3 weeks before harvest. In another study on peach fruit, the authors found fruit before pit hardening as susceptible as mature fruit (Biggs & Northover, 1988a). Hall (1971) found infection of ripe peach fruit by *M. fructicola* to be more rapid than infection of unripe fruit.

Cuticle thickness was an important characteristic of host resistance (Adaskaveg *et al.*, 1991). The behaviour of these fungi on different hosts was also influenced by other fruit surface characteristics such as pubescence, epidermal cell wall thickness, presence and distribution of stomata and also certain physiological differences like the presence of germination inhibitors or phytoalexins (Byrde & Willetts, 1977). Biggs and Northover (1989) found that thicker epidermal cell walls of sweet cherries caused delayed infection by *M. fructicola*, therefore increasing host resistance. Higher levels of nitrogen fertilisation in nectarine orchards resulted in higher levels of *M. fructicola* fruit decay. This was partially ascribed to the consequential reduction in cuticle thickness (Michailides *et al.*, 1992). Gradziel (1994) reported the epidermis to be the site of brown rot resistance in peach, the resistance of green fruit to infection and the decline thereof with ripening, which was initiated at colouring. Pioneering work by Curtis (1928) demonstrated the morphological aspects of brown rot resistance in a variety of plum, nectarine, peach and apricot cultivars. The author concluded that several factors attributed to host resistance: stoma morphology, number of stomata, cuticle thickness, presence of pubescence and the resistance of the hypoderm.

Bostock *et al.* (1999) associated the suppressive action of surface phenolics on cutinase production with host resistance of peach fruit to *M. fructicola*. The concentration of these phenolics, in particular chlorogenic and caffeic acids, was especially high in resistant peach genotypes and declined with fruit maturity, resulting in increased susceptibility. Direct toxicity to *M. fructicola* was not observed, but cutinase activity was reduced at low levels of either phenolic acid. An earlier report by Ogawa (1958) stated that green peach fruit

produced inhibitory effects on spore germination of *M. fructicola*, whereas ripe peach, apricot and cherry fruit produced stimulatory effects on spore germination of *M. laxa* and *M. fructicola*. The author furthermore observed less germination and shorter germ tubes on the surface of green fruit compared with that on ripe fruit.

Host resistance varies amongst different fruit types. The infection incidence of two *M. fructicola* conidia per inoculation droplet on nonwounded peaches and nectarines was 30 and 25% respectively, whereas no decay was observed on nonwounded plums. On wounded fruit, *M. fructicola* infected 54% of the plums and 100% of the peaches and nectarines (Hong *et al.*, 1998).

Blossom and twig blight. Germ tubes from conidia of *M. laxa* and *M. fructicola* are capable of penetrating any part of the blossom. Entry occurred mainly through the stigma and also the stamens or petals, thus more frequently through open flowers (Roberts & Dunegan, 1926; Ogawa & English, 1960; Byrde & Willetts, 1977; Willetts & Bullock, 1993). Calavan and Keitt (1948) noted stigma infection, but found *S. laxa* penetrated cherry blossoms more readily through the anthers, which were found in greater numbers in each flower. Weaver (1950) observed the germination of dry conidia on anthers at 96% RH and on the stigma at as low as 80% RH. Floral parts were moist and abundant in exudates providing exogenous nutrients for growth of conidial germ tubes. External exudates on the host surface were necessary as conidia of the brown rot fungi contained insufficient reserves for germ tube differentiation and penetration of the host surface. Spore germination and germ tube length were greater in water exposed to leachates from stigmas, anthers or the insides of floral tubes, than in water drops exposed to other floral parts (Ogawa & English, 1960). Ogawa and English (1960) monitored natural blossom infection and concluded that most infections occurred through the stigma. The pathogen grew through the stigma and penetrated the ovary before it moved into the peduncle, followed by the sudden withering of blossoms, associated with blossom blight (Calavan & Keitt, 1948; Ogawa & English, 1960). Weaver (1950) reported a similar pathway of infection, but noted that the fungus did not spread into the peach fruit or peduncle after a period of 24 h at 15°C in saturated atmosphere because of the abscission of the calyx from the peduncle and desiccation of the floral organs. The author did however observe peduncle rot on small green fruits 9 and 12 days after pollination when the incubation period at saturated atmosphere was increased to 40 h and the temperature to 19°C.

Shinners and Olson (1996) followed the infection of lowbush blueberry gynoecia by *M. vaccinii-corymbosi* after penetration of the stigma through the stylar canal and adjacent tissue down to the ovules (similar to pollen tube growth), colonising the entire fruit wall.

Following successful infection of the flower, browning of tissue and blossom blight developed, the infected blossoms hanging, with gumming at the point of attachment (Weaver, 1950; Jenkins, 1965a). The presence of mycelium in the flower prevented the formation of an abscission layer and infected blossoms and young fruits remain on the tree (Willetts & Bullock, 1993). Calavan and Keitt (1948) reported the abscission of cherry blossoms infected by *S. laxa*. Less abscission occurred at lower temperatures (11°C), resulting in higher levels of spur blight. The fungus grew into the woody tissue and the infection might develop into a sunken, elliptical canker and the consequent death of the spur (Jenkins, 1965a; Heyns, 1967; Heyns, 1968; Matthee, 1970; Batra, 1985). The fungus might also grow from killed spurs into larger branches, where its invasion often led to the formation of elliptical, gumming cankers. These branches might furthermore be girdled, the xylem tissue blocked, facilitating the death of the distal portions within weeks (Wormald, 1919; Calavan & Keitt, 1948). These cankers could predispose trees to infection by certain wood-rotting fungi, as these incipient lesions might serve as penetration sites (Batra, 1985). Mycelium from infected blossoms could also spread to fruit peduncles and young fruit (Heyns, 1968; Byrde & Willetts, 1977; Willetts & Bullock, 1993). Mycelium of *M. fructicola* remained latent or quiescent in unripe fruit until ripening (Wade, 1956a; Jenkins & Reinganum, 1965; Kable, 1969a; Kable, 1969b; Tate & Corbin, 1978; Wade & Cruickshank, 1992a; Wade & Cruickshank, 1992b; Willetts & Bullock, 1993).

Latent and quiescent infections. Infection of undamaged fruit at a stage not susceptible to decay might lead to the establishment of latent or quiescent infections. Quiescent infections are arrested, visible infections, while latent infections are not macroscopically visible (Jenkins & Reinganum, 1965; Byrde & Willetts, 1977; Swinburne, 1978). Tate and Corbin (1978) described quiescent infections by *S. fructicola* on peach, apricot and plum fruit as “small, superficial, halo-shaped blemishes, quite distinct from other fungal infections”. Northover and Cerkauskas (1994) noted that latent infections had considerable epidemiological significance and were not completely inactivated by host response or fungicide treatment. On plums grown in Ontario, latent infections of *M.*

fructicola were presumed to be the origin of many vigorously sporulating lesions that developed on maturing plums and served as inoculum source for potential infection of susceptible maturing fruit (Northover & Cerkauskas, 1994). Quiescent infections were considered to be the main origin of decay during dry harvest periods (Jenkins & Reinganum, 1965) and might also explain inconsistency of fungicidal sprays (Wade, 1956a; Jenkins & Reinganum, 1965; Kable, 1971). Kable (1971) reported short-term latent infections (i.e. latent infections initiated during ripening prior to fungicide applications) as the cause for this inconsistency. However, in an earlier study, Kable (1969a; 1969b) observed latent and quiescent infections of peaches and apricots by *M. fructicola* in the Murrumbidgee irrigation areas of Australia, but concluded that it did not cause the loss of large quantities of fruit. Emery *et al.* (2000) found significant correlations between the incidence of blossom blight and latent infections on immature fruit and also between brown rot at harvest and the incidence of latent infections earlier in the growing season. The authors thereby demonstrated the importance of latent infections in the etiology of *M. fructicola* in peach orchards, especially as carry-over inoculum from spring to the ripening stages.

Latency might be the result of nutritional insufficiency in the infected cells through competition of the actively metabolising neighbouring cells. Additionally, oxidised phenolic compounds present in green fruit might also inhibit enzymes secreted by the fungus or reduce fungus growth through direct toxicity (Swinburne, 1978; Wade & Cruickshank, 1992b). Swinburne (1978) also reported that fungistatic compounds, formed by the host after infection, mediated latency by providing temporary resistance. The toxicity of these compounds was reduced by the physiological changes in the ripening host or was degraded by the host or pathogen, allowing further infection development.

Early-season latent infection of peach, apricot and plum by *M. fructicola* was initiated in the period between full bloom and shuck fall (Wade, 1956a; Jenkins & Reinganum, 1965; Tate & Corbin, 1978). Earlier infections resulted in the total destruction of the flower so that no fruit was set (Wade, 1956a). Lesions of quiescent infections on peaches were mostly confined to the stylar end of the fruit, indicating infection between petal fall and shuck fall (Jenkins & Reinganum, 1965). Latent infection was initiated by inoculating fruit with spore suspensions of *M. fructicola* at full bloom and at shuck fall, but not when the fruit were half grown. *Monilinia fructicola* penetrated the immature fruit through stomata and latent

infections of apricots were confined to the epidermis (Wade, 1956a). Wade and Cruickshank (1992b) also reported stomatal penetration after inoculation of green apricot fruit with *M. fructicola* at shuck fall and 43 and 63 days after shuck fall. Host response occurred: cells around the infection site died, walls of surrounding cells suberised and cells up to 20 cells distant accumulated phenolic compounds. Periderm formed at shuck fall, but was absent from fruit inoculated 65 days later. Jenkins and Reinganum (1965) also found periderm formation in peaches, although not as extensive as in apricots. Suberisation around latent infections on apricots functioned as a barrier to further fungal decay and impeded diffusion of nutrients to the infected cells (Wade & Cruickshank, 1992b). The authors showed that at fruit ripening, 100 days after shuck fall, viable hyphae in latent infections escaped from lesions by forcibly growing out through the sub-epidermal cell layers or between the cuticle and epidermis. These hyphae now utilised nutrients from tissue lacking active host defence mechanisms due to ripening. The outbreak of latent infections was more efficient when the infection occurred later in the growing season (Wade & Cruickshank, 1992b). Latent infections were activated at ripening by the presence of the fruit volatiles, acetaldehyde and ethanol, produced by the ripening apricot fruit. Production of volatiles was first detected after chlorophyll breakdown during the development of orange colouration in the fruit. Artificial activation of latent infections on green apricots by exposure to acetaldehyde and/or ethanol vapours resulted in outbreak and limited growth by the fungus, but general invasion of the fruit was prevented by host defence reactions and/or by insufficient inoculum potential of the outbreaking hyphae (Wade & Cruickshank, 1992a). Northover and Cerkauskas (1994) used ethephon to generate ethylene, a volatile hormone associated with fruit ripening, to advance ripening in order to effect the development of latent infections in plum fruit. Tate and Corbin (1978) however attributed the reactivation of quiescent infections of peaches, apricots and plums from any maturity stage to the occurrence of warm, wet periods.

Schlagbauer and Holz (1989a) did not observe any quiescent *M. laxa* infections on peaches, plums and apricots. However, latent *M. laxa* infections of peaches and apricots occurred frequently, while plums were highly resistant throughout the growing season. Light and scanning electron microscopy (SEM) of latent infections on immature plum fruit revealed extensive periderm formation in the cortex beneath the necrotic tissue, with evidence of gum deposits and the presence of suberin or lignin. The inability of these lesions to yield *M. laxa* indicated that necrotic lesions on plums were due to host defence reactions leading to loss of

pathogen viability. Consequently the authors concluded that long-term latent infections of plum fruit by *M. laxa* were unimportant.

Jerome (1958) reported a different perspective on latency from field investigations on stone fruits in Canberra, Australia. She ascribed resistance to *M. fructicola* of green peach fruit to the mechanical resistance of the epidermis to penetration and suggested that resistance of fruit to penetration reduced with ripening, enabling spores on the fruit surface to infect the fruit. Viable spores on the fruit surface invaded wounds rapidly, irrespective of the fruit maturity, thus adding to the spore population. Consequently she attributed the rotting of ripe fruit to latent contamination, rather than latent infections. Studies by Powell (1951) and Phillips and Harvey (1975) also attributed brown rot epidemics on ripening fruit to the presence of conidia on the fruit surfaces. The ability of conidia to survive for long periods on fruit surfaces in the orchard, as well as its ability to remain dormant during wet periods was however questioned (Naqvi & Good, 1957; Jenkins & Reinganum, 1965; Kable, 1969b; Byrde & Willetts, 1977).

Fruit infection. According to Ogawa *et al.* (1983) and Emery *et al.* (2000) there was a direct correlation between the severity of blossom blight and the subsequent fruit rot. Powell (1951) also reported that effective blossom blight control retarded the development of preharvest brown rot. Green fruit rot and subsequent mummification has been observed in the case of *M. fructicola* (Ogawa *et al.*, 1983). The author furthermore attributed shoot blight subsequent to mummification to toxins produced by the fungus-fruit interaction. The green fruit stages were however fairly resistant to infection by *Monilinia* spp. and the brown rot fungi must therefore bridge the period between blossom and fruit ripening. It accomplished this by surviving as conidia or mycelium on infected blossoms or twigs, as well as mummies still hanging in the trees. Conidia produced from these infections act as secondary inoculum that infects the ripening fruit. Kable (1969a) concluded from studies on the etiology of *M. fructicola* in canning peach orchards, that blighted blossoms were not major contributors of inoculum during the fruit ripening stage, but were important links in the infection chain, since they provided inoculum for the first infections of injured fruit. Latent infections of *M. fructicola* on green peach fruit also acted as links in the inoculum chain from the blossom to ripening stages (Emery *et al.*, 2000).

Penetration occurs through stomata, lenticels, micro-cracks, hair sockets or directly through the cuticle, but successful infection mostly occurs through mechanical or insect wounds (Curtis, 1928; Smith, 1936; Hall, 1971; Byrde & Willetts, 1977; Willetts & Bullock, 1993). Although stomata were mentioned as a possible penetration site by various authors (Curtis, 1928; Smith, 1936; Hall, 1971; Byrde & Willetts, 1977; Willetts & Bullock, 1993), very little has been reported on the actual dynamics surrounding stomatal penetration. Concluding from an ultrastructural study of nectarine surfaces, Fogle and Faust (1975) deemed it unlikely that stomata were primary infection sites for brown rot spores. They noted that stomatal penetration would lead to infection of immature fruit and might not be the penetration site during the infection boom occurring on maturing fruit since these stomata appeared suberised and were probably less receptive.

The ability of *M. fructicola* to form appressoria was demonstrated by Adams *et al.* (1962) on *Ginkgo biloba* leaves. Local swellings in the epidermal cell wall directly beneath the appressoria were however induced and the formation of infection pegs was not observed. Hall (1971) found that the proportion direct penetrations of peach leaves declined with an increase in thickness and hardness of epidermal cell wall and cuticle, therefore concluding that direct penetration of peach leaves by *M. fructicola* was largely a mechanical process. However, appressoria or other specialised forms of attachment were not observed. The production of thin-walled, hyaline, multinucleate appressoria by *M. fructicola* on green and ripe apricot fruit as well as plum petals was however observed by Cruickshank and Wade (1992b). They also observed the simultaneous incidence of anastomoses, which would increase the inoculum potential and lead to a synergistic effect in pathogenesis. This synergism was even more increased when spore concentration and sugar content in the suspension droplet increased.

Smith (1936) observed conidia of *S. fructicola* suspended above the fruit surface by hairs on peach fruit, germinating in no particular direction. The germ tubes infected the fruit via the side of the hair into the V-shaped depression at the base of the hair socket. On brushed peaches, infection occurred mainly through the broken hair stubs.

Micro-cracks occurring naturally with fruit swelling of nectarines might be an important site for infection (Fogle & Faust, 1975; Fogle & Faust, 1976). Nguyen-The *et al.*

(1989) found micro-cracks on the nectarine surface to be the main natural penetration site for *M. laxa* and observed no lenticel penetration or direct penetration through the intact cuticle. However, mycelium frequently grew over micro-cracks without any visible attraction or penetration, hence their conclusion that micro-cracks on nectarine fruit could not be considered as wounds and its frequency on fruit was insufficient explanation for susceptibility or resistance. Nguyen-The and Chamel (1991) observed a marked deterioration of the epidermis of nectarine fruit when subjected to culture filtrates of *M. laxa*, indicating probable pectinolytic enzyme action aiding penetration at cuticular micro-cracks. They furthermore concluded that cuticle degradation was not involved in the infection of nectarine fruit by *M. laxa*. In general, intact fruit surfaces were fairly resistant to direct penetration by the brown rot fungi. Light microscopy and SEM by Schlagbauer and Holz (1989b) revealed no successful penetrations of uninjured plum fruit by *M. laxa* after drop-inoculation with a spore density that would normally ensure infection (Fourie & Holz, 1985b), neither did they observe stomatal penetration on immature or mature fruit. *Monilinia laxa* entered mature fruit through cracks present in the cuticle next to stomata of hard- and full-ripe plums.

Fruit-to-fruit contact surfaces predisposed prune fruit to infection by *M. fructicola*. Michailides and Morgan (1997) found that these surfaces had cracked and thin cuticles with larger micro-cracks surrounding the lenticels, less epicuticular wax, a higher carbohydrate content in the exudates allowing better conidial germination and consequently higher rates of infection. Additionally, fruit-to-fruit contact surfaces dried off much slower than single fruit surfaces with a thicker hydrophobic wax layer.

Bruised cherry fruit were more susceptible to *Monilinia* and other fungal decay pathogens (Ogawa *et al.*, 1962). Most successful infections by brown rot fungi occurred at mechanical or insect wounds (Poulos & Heuberger, 1952). Infection of injured fruit did not necessarily require free water, since the injured tissue or gum produced by the wound reaction provided the moisture needed for germination. Sound, immature fruit were highly resistant to infection, but once injured it became more susceptible (Kable, 1969a). Wounds of immature tissue produced metabolites associated with ripening and senescence (Williamson, 1950). It has been shown that volatiles produced by ripening apricots contributed to the activation of latent infections (Wade & Cruickshank, 1992b) and might thus also contribute to the susceptibility of wounds on green fruit. Wade and Cruickshank (1992a) found fresh wounds

on the surface of green apricot fruit were susceptible to infection by *M. fructicola*, but rapidly became resistant within 6 h. This increase in wound resistance was strongly correlated to the depleting concentration of free nutrients on the surface due to diffusion and absorption by underlying living cells. This effect was not observed when ripe fruit were wounded. Periderm, suberin and phenolic compounds were formed after the wounds on green apricots became resistant. It was concluded that readily available carbon sources, such as glucose, were most important for successful infection (Wade & Cruickshank, 1992a). Successful infection at a wound site could be achieved with lower inoculum doses and shorter incubation periods than required for the penetration of intact fruit (Corbin, 1963; Hong *et al.*, 1998).

Once an infection was established, hyphae colonised the host tissue. Hyphae of *M. laxa* grew mainly in intercellular spaces, between cells, often forming conspicuous tunnels. Hyphae were also observed growing intracellularly in cells with collapsed protoplasts. In the latter case, hyphae frequently ruptured and then penetrated the wall, with thin intracellular hyphae growing from thick, vacuolated intercellular hyphae, which occurred mainly in heavily colonised tissue (Schlagbauer & Holz, 1989b). Extracellular enzymes that degrade host cell walls play an important role in the pathogenesis of brown rot fungi (Byrde & Willetts, 1977). Nguyen-The *et al.* (1989) observed an enzymatic breakdown of nectarine epidermis, but concluded that it more likely involved cell wall degradation than hydrolysis of the cuticle. Schlagbauer and Holz (1989b) found no clear evidence of host tissue degenerating in advance of penetrating hyphae. *Monilinia laxa* usually colonised the epidermal tissue and only colonised the hypodermis via vascular tissue in the vicinity of the pedicel (Schlagbauer & Holz, 1989b). Browning of the host tissue occurred around the infection site, spreading circularly outward. As the disease progressed, conidiophores ruptured the epidermis and formed small tufts on the fruit surface. Sporogenous hyphae produced macro-conidia in chains. Sporodochia were often arranged in concentric circles around the infection site, but with the development of colonisation, the total surface could be enveloped by a mass of brown spore-bearing sporodochia. In the event of adverse conditions, or unripe fruit, the infection would not spread as radically as on soft, ripe fruit during moist conditions and considerably less sporodochia would be observed. Once the fruit was colonised, water loss occurred, resulting in discolouration, shrivelling and eventual mummification of the fruit (Wormald, 1919). Most of these mummies remained hanging from the tree, since the formation of an abscission layer was prevented by the presence of

fungus mycelium (Byrde & Willetts, 1977; Willetts & Bullock, 1993). These infected fruit and mummies facilitated an inoculum boom at fruit ripening reported by several researchers (Jerome, 1958; Jenkins, 1965b; Corbin *et al.*, 1968; Byrde & Willetts, 1977). Conidia were wind and splash dispersed to ripening fruit. These fruits were either infected in the orchard or during the postharvest stage.

INTEGRATED CONTROL

When conditions are favourable for disease development, control of brown rot requires an integration of all control measures (Mappes, 1990). It is obvious from the disease cycle of brown rot fungi that several diverse factors might play a role in the establishment of the disease. Integrated control measures should target these factors and manage it in such a way that conditions unfavourable for brown rot development are created. The following section gives a brief account of the various cultural, chemical, biological and postharvest control measures that can be implemented to control this disease.

Cultural practices

Brown rot development and epiphytotics on more resistant cultivars would be slower than on more susceptible cultivars. Fruit genotypes have been evaluated for brown rot resistance in order to determine the genotypes and morphological characteristics needed for long term breeding programmes (Curtis, 1928; Biggs & Northover, 1989; Brown & Wilcox, 1989). Differences in host susceptibility were observed, but resistant cultivars have not been bred and given the etiology of this disease, it would seem to be unlikely.

Ogawa *et al.* (1983) reported that cultural practices like sanitation and cultivation of the orchard floor do not appear to have much impact on disease control. Cultivation of the orchard floor to hasten decomposition of fruit and mummies would however reduce the chance for apothecial development. No apothecia were found in orchards that were disked or rototilled (Hong *et al.*, 1996). Hong and Michailides (1998) suggested the possible suppressive effect of herbicides on apothecium formation, after observations that no apothecia of *M. fructicola* developed in the herbicide-treated zones in plum orchards in California.

Several other authors however have stated that sanitation constitutes a major element of brown rot control, since it effects the reduction of inoculum sources and consequently inoculum potential (Hewitt & Leach, 1939; Heyns, 1967; Matthee, 1970; Zehr, 1982; Zehr, 1983; Mappes, 1990; Biggs *et al.*, 1997). Blighted shoots and spurs should be removed before blossoming and newly blighted spurs after blossoming. Mummified fruit from trees and orchard floor should be removed as soon as possible after harvest (Heyns, 1967). Infected, thinned fruit is an important inoculum source and thinning should be done as early as possible to minimize the attribution from this inoculum source (Landgraf & Zehr, 1982; Zehr, 1982).

Removal of alternative hosts of brown rot fungi in close vicinity of stone fruit orchards was also identified as a notable control measure (Ogawa *et al.*, 1983). Wild plums (*Prunus angustifolia* and other *Prunus* spp.) were identified as continuous reservoirs for brown rot inoculum to nearby stone fruit orchards (Landgraf & Zehr, 1982; Zehr, 1982).

General orchard practices should be aimed at creating conditions unsuitable for disease development. Dense tree foliage should be prevented by summer pruning and avoiding excessive irrigation and nitrogen fertilising. This would promote good aeration, quicker drying of fruit and would minimize humidity (Heyns, 1967). Close spacing of trees induced environmental conditions favourable for infections and should be avoided (Ogawa *et al.*, 1983). Excessive nitrogen fertilisation of nectarine trees resulted in fruit more susceptible to decay by *M. fructicola* (Michailides *et al.*, 1992). Other orchard management programmes directed at altering the environment in the orchard, include reduction of weeds during bloom to minimize apothecial development, avoiding sprinkler irrigation and planting orchards in areas conducive to good air movement that would facilitate quicker drying and lower humidity (Ogawa *et al.*, 1983).

Insect pests that cause wounds on the fruit should be controlled (Heyns, 1967). The use of effective insecticides in conjunction with a regular brown rot control programme will aid significantly in controlling the fruit rot phase of this disease (Poulos & Heuberger, 1952). It would furthermore minimize the possibility of insects vectoring inoculum of the brown rot fungi (Kable, 1969a; Lack, 1989; Michailides & Spotts, 1990). Kable (1969a) noted that effective control of oriental fruit moth and dried fruit beetles in Australian orchards would

reduce brown rot decay and might even break the infection chain in seasons when no blossom blight or quiescent infection occurred.

Orchards where cultural practices were implemented in order to reduce pesticide use required regular scouting for emerging disease or insect problems. When problems were observed, producers could either spray corrective to remedy the problem or protective before harvest (Zehr, 1983).

Chemical control

Chemical control is the most relied upon measure for brown rot control. Ogawa *et al.* (1983) estimated a total of US\$ 43 million spent annually on chemical brown rot control during the 1982 season in California.

Method of application. Fungicides to control blossom blight and brown rot were mostly applied as sprays or dusts by means of ground spray rigs, but some producers reverted to aerial sprays during very wet periods without much success (Ogawa *et al.*, 1972; Ogawa *et al.*, 1983). Earlier reports recommended the application of benzimidazole fungicides during blossom and before harvest by means of overhead sprinklers (Ogawa *et al.*, 1975). Mappes (1990) reported no systemic effect of fungicides in petals, and highlighted the general importance of good spray coverage. Good control (99%) was achieved when using 550 l/ha water, whereas only 73% control was achieved with 45 l/ha water.

Timing of applications. Proper timing of fungicide applications is very important. Full-season, reduced or minimum fungicide schedules were proposed for stone fruit orchards in South Carolina, depending on the history of brown rot decay, inoculum sources in and around orchards and fungicide resistance (Zehr, 1982). The author highlighted the importance of good cultural practices (orchard sanitation and weed control), as well as careful monitoring for the disease in reduced and minimum schedule orchards.

Ogawa *et al.* (1983) attributed extensive blossom blight and quiescent infections to improper timing of blossom applications during a wet blossom period. However, based on a monograph on systemicity by Shephard in 1985, Osorio *et al.* (1994b) concluded that the translocation of certain fungicides could be enhanced under prolonged wetness periods. Lo *et al.* (1998) attributed brown rot problems in New Zealand orchards to growers not heeding to

early bloom infection warnings, poor spray timing, not countering twig blight by pruning or applying mid-season brown rot sprays and by not applying sufficient sprays during the preharvest period.

Chemical control of blossom and twig blight was most successful when applying protectant fungicides during flowering (Powell, 1951; Matthee, 1970; Ogawa *et al.*, 1983; Mappes, 1990). Fungicide treatment during blossom must protect the anthers and could be achieved by applying protectant fungicides during full bloom or systemic fungicides before full bloom (Ogawa *et al.*, 1983). Mappes (1990) advised application of fungicides as early as the pink bud stage, reasoning that infection might occur at this stage and once the fungus has penetrated the stigma the fungicide cannot affect it. Matthee (1970) also advised benomyl or dichlofluanid application when 10% of the blossoms were in pink to balloon stage, with follow-up applications every 3 to 5 days until the petal-fall stage. This would assure good spray coverage of all new blossoms. Wilcox (1990) tested vinclozolin, iprodione, tebuconazole, propiconazole, myclobutanil, flusilazole, triforine, fenarimol and captan for the ability to prevent infection 1 to 3 days after inoculation of sour cherry blossoms with different inoculum doses of *M. fructicola*. At relatively low inoculum dosages, all fungicides provided good control (86 to 100%) when applied 24 h after inoculation. By increasing the dosage and incubation period before application of the fungicides, decay increased proportionally. Mappes (1990) also advised a second application at full bloom and possible further applications when flowering was prolonged due to weather conditions or the presence of different varieties in an orchard. In Michigan, *M. fructicola* brown rot was a serious disease and therefore an extensive spray programme was advised during bloom with sprays at white bud, bloom, petal fall and shuck split, using the conventional protectant fungicides like sulphur to also control other diseases. Less applications were however needed when using the modern brown rot fungicides (Jones, 1983). In almond, a pink bud and full bloom spray of non-systemic fungicides provided protection of petals and internal floral tissue, whereas one application of a systemic fungicide, like benomyl, at pink bud stage resulted in the translocation of the chemical to the non-exposed blossom parts, providing effective protection (Osorio *et al.*, 1994b).

In the South-Eastern United States, where brown rot was not often severe, test plots were not sprayed for blossom blight. Severe blossom blight infection that affected fruit yield

occurred only once in 14 years. Despite the low occurrence of blossom blight, producers still applied two to four blossom applications regardless of weather conditions. In order to reduce pesticide use, minimize production costs and reduce fungicide resistance build-up, a spray prediction system must be implemented (Zehr, 1983). Hogmire and Biggs (1994) proposed a variable reduced-rate pesticide programme based on tree phenology, with early-season applications made at 25% normal rate, mid-season 50%, and late-season at full rate. This programme utilised the contribution of spray drift to produce a more uniform pesticide deposit as the tree grows, in stead of excessive deposits early in the season that typify application of the same rate throughout the year. The control of brown rot in this programme did not differ significantly from the full-rate programme, although there was an increase in fruit rot and postharvest brown rot in the second year tested. This increase was attributed to possible disease carry-over and more favourable weather conditions for infection in the second season (Hogmire & Biggs, 1994).

Peach fruit became more resistant to infection by *M. fructicola* at the pit hardening stage and increasingly susceptible about two weeks before full ripeness. A post-shuck fall application would protect the fruit against brown rot infection and also limit sporulation on non-abscised, aborted fruit (Biggs & Northover, 1988a).

Fungicide application on maturing fruit was not recommended until about 4 weeks before harvest (Ogawa *et al.*, 1983). Ogawa *et al.* (1983) proposed one application 3 to 4 weeks before harvest, a follow-up application 1 to 2 weeks before harvest and a protectant spray a few days from harvest. Similar recommendations were made by Heyns (1967) to control brown rot of peaches with compounds such as captan, wettable sulphur or dichloran. In South Africa iprodione is registered for use in brown rot control 10 and 3 days before harvest (Combrink *et al.*, 1996). A single application of iprodione, 12 or 5 days before harvest reduced brown rot of stored sweet cherries (Spotts *et al.*, 1998).

A dormant or "presporodochial" spray of benomyl and oil reduced the numbers and size of *M. laxa* sporodochia formed on almond twigs (Ramsdell & Ogawa, 1973a). Ogawa *et al.* (1983) proposed dormant sprays to reduce or eradicate the fungus from blighted blossom, twigs and mummies on the trees, as a feasible practice when the only source of inoculum was

on the tree. Sporulation during late winter could be suppressed by the application of monocalcium arsenite, lime sulphur or carboxin (Matthee, 1970).

The best method to manage stone fruit diseases with minimal fungicides was to target applications according to infection period forecasts. Protective sprays should be applied before infection periods and if these were missed, infections eradicated immediately with fungicides with post-infection activity (Lo *et al.*, 1998). Northover and Cerkaskas (1998) demonstrated the comparable eradivative efficacy of several sterol inhibiting (tebuconazole, flusilazole, myclobutanil, fenbuconazole and triforine) and dicarboximide (iprodione) by doing postharvest dip treatments with harvest ripe plum fruit with a high incidence of latent *M. fructicola* infections. Multi-site protectant fungicides, captan, chlorothalonil and dichlone, gave temporary suppression of latent infection development in soft-ripe, but not firm-ripe fruit. Orchard application in general was less effective in eradicating latent infections than post harvest dip treatment (Northover & Cerkaskas, 1998).

Multi-site protectant fungicides. Effective control of blossom blight can be obtained with properly timed captan applications when anthers start to show and at 80% bloom (Ogawa *et al.*, 1983). Copper compounds can be applied before full bloom (Ogawa *et al.*, 1983). Poulos and Heuberger (1949) demonstrated the inefficacy of various dithiocarbamates to control peach brown rot in the orchard. In a later study, the authors concluded from three years' data that preharvest sprays with wettable sulphur were not effective in controlling brown rot by *M. fructicola* on late-maturing varieties (Poulos & Heuberger, 1952).

Benzimidazole fungicides. Ogawa *et al.* (1968) reported on the efficacy of a new benzimidazole fungicide in 1968 (1-(butylcarbonyl)-2-benzimidazole carbamic acid), showing very effective mycelial inhibition of *M. fructicola*, *M. laxa* and *B. cinerea* and inhibition of spore germination of *M. laxa*. This compound was also effective in controlling brown rot blossom blight and fruit rot (Ogawa *et al.*, 1968). Methyl 2-benzimidazolecarbamate (MBC), a derivative of benomyl, was translocated into internal almond blossom tissue when the fungicide was applied at green and pink bud stages (Ramsdell & Ogawa, 1973b). The authors furthermore showed that benomyl was broken down to MBC under field conditions and that these compounds displayed similar degrees of

fungitoxicity to *M. laxa*. Benomyl has been very effective in blossom blight and brown rot control, mainly due to its systemic and eradivative action. Osorio *et al.* (1994b; 1994a) demonstrated the eradivative action of benomyl by achieving effective suppression of infection of almond blossoms following application 24 h after inoculation with *M. laxa* or *M. fructicola*. A single application of a systemic benzimidazole fungicide, like benomyl or thiophanate-methyl, at pink bud stage provided control equal to two applications of a protectant fungicide.

Dicarboximide fungicides. In general, the dicarboximide (DC) fungicides inhibit spore germination and mycelial growth. A specific mechanism of action has not been identified, but Ellner (1996) provided evidence of a possible dual mechanism of action of the dicarboximides in *B. cinerea*: initiation of lipid peroxidation by the generation of reactive oxygen and the reduction of glutathione concentration by reducing equivalents and co-substrate of membrane-protecting and other glutathione-dependent enzymes. Ellner (1996) also noted that enhanced levels of glutathione synthetase with reduced sensitivity in resistant strains of *B. cinerea* might be a mechanism of resistance to the DC fungicides.

Iprodione exhibited some systemic activity when applied to blossoms or fruit. It showed better systemic activity in almond blossoms when applied to sepals rather than petals and when applied to closed blossoms, provided stamen protection similar to the systemic fungicide, benomyl (Osorio *et al.*, 1989; Osorio *et al.*, 1994b; Osorio *et al.*, 1994a). Iprodione effectively suppressed infection up to 24 h after inoculation with benomyl sensitive or resistant strains of *M. laxa* or *M. fructicola* (Osorio *et al.*, 1994a). Wilcox (1990) remarked on the pronounced post-infection and anti-sporulant infection of iprodione and vinclozolin. Ritchie (1983a) observed no sporulation by dicarboximide resistant or sensitive strains of *M. fructicola* inoculated on iprodione treated peach fruit. Elmer and Gaunt (1988) also reported significantly reduced spore production of a dicarboximide resistant strain inoculated on iprodione sprayed peach blossoms, despite the evident decay of the sprayed blossoms.

Iprodione effectively controlled *B. cinerea* and *M. laxa* on peaches, but is less effective against brown rot on plums (Fourie, 1984). Combrink *et al.* (1996) reported the high stability of iprodione residues on nectarine and plum fruit. Preharvest iprodione sprays

on plums never realised residue levels above 0.85 mg/kg, whereas on nectarines it was as high as 2.5 mg/kg. The low residue levels on plums were attributed to the hydrophobic wax layer on plum surfaces, resulting in quicker run-off and drying (Combrink *et al.*, 1996). Osorio *et al.* (1993) reported that sufficient amounts of iprodione penetrated the mesocarp tissue of peach fruit to reduce internal decay caused by *M. fructicola* when conidia were injected 1 cm deep into the mesocarp. Adaskaveg and Ogawa (1994) also attributed the effective prevention or suppression of *M. fructicola* and *B. cinerea* decay by iprodione to the penetration of the fungicide into the mesocarp tissue of sweet cherry fruit. Iprodione furthermore suppressed *M. fructicola* or *B. cinerea* infections established 24 h prior to application and provided complete control over a 9-day period (Adaskaveg & Ogawa, 1994).

Ergosterol biosynthesis inhibitor fungicides. Ergosterol biosynthesis inhibitor (EBI) fungicides are a diverse class of modern systemic fungicides. The fungicides in this class have a similar mode of action, which involves the inhibition of C-14 demethylation, a biosynthetic step occurring during the conversion of lanosterol to ergosterol, the final product of fungal sterol synthesis (Köller & Scheinpflug, 1987). These fungicides gave good control of brown rot and were also effective against leaf spot and powdery mildew (Jones, 1983). A single pink-bud spray with prochloraz effectively controlled *M. fructicola* blossom blight in an orchard with benomyl-sensitive and -resistant populations (Dijkhuizen *et al.*, 1982). Wilcox (1990) tested nine fungicides (captan, fenarimol, flusilazole, iprodione, myclobutanil, propiconazole, tebuconazole, triforine, and vinclozolin) for post-infection and anti-sporulant activities when applied 1 to 3 days after inoculation of sour cherry blossoms with different inoculum doses of *M. fructicola*. At the dosage rate tested, the dicarboximides, vinclozolin and iprodione, and the EBI fungicides, tebuconazole and propiconazole, were the most effective in post-infection and anti-sporulant modes, with captan the least effective. However, the EBIs provided poor control when applied more than 48 h after inoculation. Szkolnik (1981) however demonstrated excellent post-infection activity of prochloraz, fenarimol and triforine on sour cherry blossoms and fruit when applied 24 h after inoculation with *M. fructicola*. Intraspecific variation in EBI sensitivity amongst isolates of *M. laxa* and *M. fructigena* has been reported, with EC_{50} -values 3 to 10 times lower for cyproconazole and difenoconazole than those of myclobutanil and triadimenol (Zhang *et al.*, 1991). Triforine effectively controlled *M. laxa* on stone fruit and is registered in South Africa for use until 3 days before harvest (Fourie, 1984). Hildebrand and McRae (1995) tested the protectant and

post-infection activity of triforine against ascospore infections of *M. vaccinii-corymbosi* in lowbush blueberries. Very effective post-infection activity was observed when applied up to 96 h after inoculation, but triforine did however not provide very good protection when applied to plants 4 days before inoculation. They furthermore observed a larger reduction in conidial production in the post-infection treatments compared with the protectant treatments. Triforine however penetrated plant tissue relatively quickly, thus providing good protection and eradication when applied shortly before simulated rain. Given these characteristics of triforine, a post-infection spray strategy using this fungicide was proposed to producers (Hildebrand & McRae, 1995).

Other fungicide classes. An experimental fungicide E-0858 in a new class, pyridyl fungicides, was translocated in almond blossoms similar to translocation reported for benomyl (Ramsdell & Ogawa, 1973b) and provided similar protection against blossom blight when applied to closed blossoms (Osorio *et al.*, 1994b; Osorio *et al.*, 1994a). E-0858 effectively suppressed infection up to 24 h after inoculation with benomyl sensitive or resistant strains of *M. laxa* or *M. fructicola*, and gave consistently better control than iprodione (Osorio *et al.*, 1994a). In an associated study on peach fruit Osorio *et al.* (1993) found E-0858 consistently more effective than iprodione and benomyl. This efficacy was attributed to its high activity against *M. fructicola*, penetration into mesocarp tissue of peach fruit and longer residual activity under field conditions.

Non-fungicidal chemicals. Adaskaveg *et al.* (1992) compared the efficacy of nutritional materials (calcium formate and calcium silicate) and film-forming anti-transpirants (di-l-p-menthene and an acrylic resin) to control brown rot caused by *M. fructicola* with that of iprodione. None of the afore-mentioned chemicals showed *in vitro* fungitoxicity, except di-l-p-methene, which inhibited conidial germination. Preharvest applications significantly reduced the severity and incidence of brown rot compared with the untreated fruit, while only calcium formate provided control similar to that of iprodione. The authors concluded that materials that may strengthen epidermal tissue or enhance the cuticular layer might supplement or provide alternatives to fungicides (Adaskaveg *et al.*, 1992).

Fungicide resistance

Benzimidazole fungicides. Tate *et al.* (1974) found no benomyl resistant isolates in California two years after benomyl was first used. Whan (1976) reported the presence of benomyl tolerant strains in cherry orchards where benomyl was ineffective. In the same year, Jones and Ehret (1976) reported total crop loss in a Michigan sweet cherry orchard, despite several benomyl applications as the only brown rot fungicide applied. *Monilinia fructicola* strains tolerant to benomyl were isolated from these orchards where this compound was used exclusively for 3 years. The authors demonstrated cross-resistance to other benzimidazoles, resistance stability and *in vitro* fitness comparable to sensitive strains. In 1977, Szkolnik and Gilpatrick (1977) reported that benomyl failed to control *M. fructicola* in two commercial sweet cherry orchards in western New York State. Following *in vitro* laboratory and *in vivo* glasshouse trials, control failure was attributed to *M. fructicola* strains tolerant to benomyl. Zehr (1982) found resistance build-up after routine applications with benomyl-captan mixtures. In a later study Ogawa *et al.* (1983) demonstrated practical resistance in *M. laxa* and *M. fructicola* to benomyl in Californian stone fruit orchards after severe blossom blight following continuous rain showers throughout the blossom stage. With the onset of resistance development, control failure was observed (Jones, 1983; Ogawa *et al.*, 1983; Ritchie, 1983b) and producers started using protectant fungicides, like triforine, iprodione and chlorothalonil, to provide good blossom blight control. More applications were however required (Ogawa *et al.*, 1983). Poor control of brown rot by benomyl was reported from the Murrumbidgee Irrigation Areas in Australia during disease favourable weather conditions and was attributed to the high frequency (93% of isolates tested) of benomyl resistance (Watson *et al.*, 1992).

Resistance to benzimidazole fungicides is governed by a single dominant gene that is inherited in Mendelian fashion in meiotic progeny, resulting in a 1:1 segregation ratio of sensitive and resistant ascospore progeny. This relationship between a single dominant resistant gene and heterokaryosis is important for the evolution of resistance in populations of *M. fructicola*, but will also maintain the sensitive genotype in heterokaryotic populations exposed to benzimidazole fungicides (Sanoamuang *et al.*, 1995). Sonoda *et al.* (1982a) drew similar conclusions from an earlier study, providing evidence for heterothallism and monogenic resistance to benomyl in *M. fructicola*.

Distribution studies on *M. fructicola* in peach and nectarine orchards showed that resistant brown rot isolates were most likely to develop within an orchard, rather than be carried in from adjacent orchards (Penrose *et al.*, 1979). Michailides *et al.* (1987) observed an increase of benomyl resistance in California prune and apricot orchards, despite the low number of benomyl applications per season. The authors attributed this increase in resistance frequency to the possible movement of benomyl resistant *M. fructicola* strains from adjacent peach or nectarine orchards. This brought about a shift in the brown rot populations to predominantly more *M. fructicola* than *M. laxa*, since the *M. laxa* isolates were mostly sensitive to benomyl, which was the main fungicide used to control brown rot. Benomyl resistance in *M. fructicola* was very stable (Adaskaveg *et al.*, 1987) and resistant isolates did not have any competitive disadvantage compared with the sensitive strains, as was seen in equal virulence on peach fruit, and equal growth, sporulation and germination percentage *in vitro* (Jones & Ehret, 1976; Penrose *et al.*, 1979). Sonoda *et al.* (1983) also observed equal competitiveness of benomyl-resistant and -sensitive *M. fructicola* as incitants of blossom blight on peach. In a later study, Penrose (1990) concluded from the prolonged field persistence of benomyl resistant *M. fructicola* strains that once benomyl resistance has been detected in an orchard, it may never be possible to resume the effective use of benzimidazole fungicides. Adaskaveg *et al.* (1987) however used benomyl and iprodione to control blossom blight in an orchard with 82% incidence of benomyl resistant isolates and found similar control by both these compounds. Reduced efficacy of the benzimidazole can however be anticipated, since it controlled *M. laxa* blossom blight markedly better than iprodione in orchards where benomyl resistance was absent. The superior efficacy of benomyl over iprodione in sensitive orchards was also observed by Osorio *et al.* (1989). Resistant isolates of *M. fructicola* exhibiting a significantly reduced growth rate on peach fruit compared with sensitive strains (Sonoda & Ogawa, 1982) and low-level benomyl resistant isolates of *M. laxa* with reduced ability to infect almond and prune blossoms (Canez & Ogawa, 1982; Ogawa *et al.*, 1984) were also reported.

Osorio *et al.* (1994a) found that benomyl resistant isolates of *M. laxa* and *M. fructicola* were sensitive to iprodione and E-0858, thus proving no cross-resistance between these compounds. The authors furthermore confirmed that benomyl reduced twig blight in almond orchards with benomyl resistant populations, but did not find the reduction economically sufficient, compared with the control obtained by iprodione.

Dicarboximide fungicides. Demonstrating the potential for fungicide resistance in *M. fructicola*, Jones (1983) reported high frequencies of spontaneous dicarboximide resistant mutant strains with no apparent cross-resistance to benomyl resistant isolates. Dual resistance in certain isolates to both dicarboximides and benzimidazoles were however observed (Jones, 1983; Ritchie, 1983b; Elmer & Gaunt, 1986; Braithwaite *et al.*, 1991). As can be expected, cross-resistance between dicarboximide compounds were observed (Ritchie, 1982; Jones, 1983; Ritchie, 1983a; Penrose *et al.*, 1985; Elmer & Gaunt, 1986), with resistance to both groups proven to be stable through several generations grown in the absence of fungicides (Jones, 1983; Elmer & Gaunt, 1994). Elmer and Gaunt (1994) did however find a resistant strain that produced a mixture of sensitive and resistant conidia on fungicide-amended media, which also showed a significant increase in sensitivity after 9 generations. Dicarboximide resistant strains of *M. fructicola* produced darker pigmented mycelium, compared with the brown mycelium of sensitive strains (Ritchie, 1982; Jones, 1983; Penrose *et al.*, 1985).

Strains of *M. fructicola* resistant to dicarboximide fungicides were isolated from a New South Wales nectarine orchard where dicarboximides were used for four consecutive seasons. Severe losses from this orchard were reported in the fourth season, despite five vinclozolin sprays to control brown rot (Penrose *et al.*, 1985). Dicarboximide resistant isolates of *M. fructicola* were detected in New Zealand stone fruit orchards in 1985, but in contrast to benzimidazole resistance, no control failure was observed at that time. The resistant isolates were classified as sensitive (EC_{50} -value $<0.7 \mu\text{g a.i. iprodione/ml}$), low-level resistant (EC_{50} -value 0.7 to $10 \mu\text{g/ml}$), or high-level resistant (EC_{50} -value $>70 \mu\text{g/ml}$), with the majority of resistant isolates classified as high-level resistant (Elmer & Gaunt, 1994). Jones (1983) also found that resistant strains were highly resistant, with *in vitro* growth on PDA amended with up to $1000 \mu\text{g/ml}$ of fungicide. This differs from dicarboximide resistance in *B. cinerea* populations in vineyards, where the majority of resistant isolates were classified as low-level resistant (Pommer & Lorenz, 1987; Beever *et al.*, 1989; Fourie, 1996). This can be attributed to increased reduction in fitness with increased level of resistance (Fourie, 1996), which was also substantiated by a gradual shift in dicarboximide resistant *B. cinerea* sub-populations in New Zealand kiwi fruit orchards from low-level to ultra-low-level resistant isolates (Pak *et al.*, 1995). A decline in resistance incidence was observed when dicarboximide applications were reduced or absent, suggesting a reduction in fitness of the

resistant *M. fructicola* isolates (Elmer & Gaunt, 1993). Elmer and Gaunt (1990) furthermore observed a reduction in the level of resistance over time in the absence of dicarboximides. The reduced fitness of resistant isolates on nectarine fruit, but not on peach blossoms, emphasised the need for investigation on all relevant tissue in epidemiological studies of resistance (Elmer & Gaunt, 1990; Elmer & Gaunt, 1994). Elmer and Gaunt (1988) reported that a proportion of resistant strains were unable to colonize wounded nectarine fruit and attributed it to abnormal osmotic sensitivity, which in preliminary tests was found in one strain. Beever (1983) reported that the inability of resistant strains to rot fruit might be related to abnormal sensitivity, since high osmotic pressures are commonly associated with ripening fruit. Resistant isolates were furthermore significantly less competitive than sensitive isolates when mixed inocula were applied to flowers or fruit (Ritchie, 1983a; Elmer & Gaunt, 1988; Elmer & Gaunt, 1990; Elmer & Gaunt, 1994). Ritchie (1983a) found that dicarboximide resistant strains of *M. fructicola* produced smaller lesions and/or sporulated less than sensitive strains on fungicide-treated or untreated peach fruit. Resistant strains caused decay on treated fruit, although the onset of decay was delayed by 1 to 2 days compared with decay on untreated fruit (Jones, 1983). By studying the spatial and temporal characteristics of dicarboximide resistant strains of *M. fructicola* in New Zealand stone fruit orchards, Elmer *et al.* (1998) observed only slight spatial distribution and no temporal distribution. In other words, spread of resistant strains was mostly restricted to the vicinity of the original focus and there was no carry-over of inoculum from resistant strains from previous seasons. The lack of significant spatio-temporal correlation between seasons suggested that selection of resistant sub-populations occurred within each growing season. Hence the authors concluded that the resistant strains have not acquired the necessary characteristics to remain in, or dominate field populations.

Ergosterol biosynthesis inhibitor fungicides. EBI fungicides have a high risk of resistance development, mainly due to its single-site mode of action and extensive use as broad-spectrum fungicides. Resistant laboratory mutants have also been obtained easily. Contrary to the disruptive selection of a resistant sub-population, as is the case with benzimidazoles and dicarboximides, continuous selection pressure by EBIs causes a directional selection pattern in the pathogen population (Köller & Scheinpflug, 1987). Zehr *et al.* (1999) demonstrated a shift in the propiconazole sensitivity of *M. fructicola* isolates in an experimental peach orchard after three season's use (29 applications). A broader range of

EC₅₀-values was observed in the third season (0.02 to 2.16 µg/ml) compared with that of the initial population (0.02 to 0.15 µg/ml). Effective disease control was however maintained. In a survey of New Zealand orchards in 1989/90, resistance to triforine and bitertanol was not detected (Braithwaite *et al.*, 1991). However, upon investigation of disease control failures in certain New Zealand orchards, Elmer *et al.* (1992) reported significant reduced triforine sensitivity in these *M. fructicola* populations. The resistant isolates were less virulent than the sensitive isolates on untreated nectarines, but were pathogenic on triforine treated segments.

Fungicide resistance management. The basic objective of fungicide resistance management is to prevent unexpected crop losses and to prolong the efficacy and lifetime of a fungicide (Köller & Scheinpflug, 1987). According to Ritchie (1983b) strategies for resistance management should adhere to three general premises: (a) adequate control of the sensitive wild-type population must be provided, (b) development or increase of a resistant sub-population must be prevented or delayed, and (c) adequate protection to prevent crop loss in case of fungicide resistance must be provided. The number of fungicide applications should be minimised in order to reduce the selection pressure on the fungus population (Elmer & Gaunt, 1990; 1993). This can be achieved by applying fungicides with different modes of action in alternation and/or mixtures with multi-site protectant fungicides (Jones, 1983; Ogawa *et al.*, 1983; Skylakakis, 1983; Köller & Scheinpflug, 1987; Elmer & Gaunt, 1990). Several models have been developed to predict the effect of alternations or mixtures on the resistant sub-population (Kable & Jeffery, 1980; Skylakakis, 1981; Levy *et al.*, 1983). These models all predict the risk of resistance development to highly active fungicides. The use of mixtures are considered to be more effective in reducing resistance build-up than using the at-risk fungicide in alternation or alone. At-risk fungicides should be applied protectively to prevent the onset of an epidemic, rather than be applied eradically when disease pressure is high and therewith the risk of increased resistance development. In this case a multi-site protectant fungicide should rather be used in an attempt to slow down the epidemic by protecting uninfected tissue (Ritchie, 1983b).

Reduced ecological competence of resistant strains would provide additional strategies that can be implemented to manage fungicide resistance. This was found to be the case with dicarboximide resistant isolates of *B. cinerea* in South African table grape

vineyards. Due to reduced fitness of the resistant strains, resistance incidences in the *Botrytis* population declined in the absence of the selection pressure from dicarboximide applications, especially over the winter period, resulting in low resistance incidences at the flowering stage when *Botrytis* control is initiated (Fourie, 1996; Fourie & Holz, 1998). Elmer and Gaunt (1990; 1993) and Elmer *et al.* (1998) reported a similar decline in the incidence of dicarboximide resistant *M. fructicola* in New Zealand stone fruit orchards.

Cultural practices aimed at reducing blossom blight and brown rot should be optimised, in order to facilitate the reduction of fungicide applications. In these cases at-risk fungicides should be applied at critical periods for disease control (Ritchie, 1983b). Improved orchard hygiene would reduce the brown rot population and also the risk of resistant strains carrying over from one season to another (Elmer & Gaunt, 1986; 1993).

Biological control

Successful biological control depends on the use of an organism exhibiting some form of antagonism or competition to the pathogen while being able to flourish in the pathogen's environment. Brief accounts of various organisms studied for the biological control of brown rot fungi follows.

Naturally occurring epiphytic fungi from apple leaves were screened for their efficacy in controlling *M. fructigena*, *B. cinerea* and *Penicillium expansum*. Isolates of *Aureobasidium pullulans*, *Epicoccum purpurascens*, *Sordaria fimicola* and *Trichoderma polysporum* provided good protection of wounded apples, with efficacy increased when mixtures of these antagonists were applied (Falconi & Mendgen, 1994). Conidia of *A. pullulans*, *E. purpurascens* and *Gliocladium roseum* were applied to sweet cherry blossom that were subsequently inoculated with conidia of *M. fructicola*. *Aureobasidium pullulans* and *E. purpurascens* significantly reduced blossom blight and latent infections, although control with iprodione proved to be significantly better (Wittig *et al.*, 1991; Wittig *et al.*, 1997).

Stevens *et al.* (1998) reported more rapid progress of brown rot in thoroughly washed peaches than in non-washed peaches. The predominant yeast species from the washings was *Debaryomyces hansenii*, which proved to be antagonistic to *M. fructicola*. They ventured that

the removal of an antagonistic yeast may play a role in the host peripheral defence system. Hong *et al.* (1996) made a survey of the resident fungi of peach, nectarine, plum and prune fruit mummified by *M. fructicola*, and found that *Trichoderma* spp., *Trichothecium roseum*, *Penicillium* spp. and *Epicoccum nigrum* suppressed the brown rot pathogen *in vitro*, indicating the occurrence of natural biocontrol, and the possibility of utilising these antagonists as biocontrol agents.

Epicoccum nigrum, a natural epiphyte of peach twigs, produces an antibiotic, flavipin, which inhibits mycelial growth of *M. laxa* and has the potential for biological control of brown rot (Madrigal *et al.*, 1991; Madrigal & Melgarejo, 1995). Various preparations of spores or mycelium of *E. nigrum*, alone or in combination with captan, reduced twig blight caused by *M. laxa*, although incomplete control was obtained in some years. Discrepancies in control can be attributed to climatic conditions and to the timing of applications (Madrigal *et al.*, 1994). The effect of flavipin was visible as hyphal coilings, swellings, deformations and frequent branching. Hyphae, germ tubes and spores treated with flavipin furthermore showed intense cytoplasmic vacuolisation, disorganisation and coagulation (Madrigal & Melgarejo, 1995). Madrigal and Melgarejo (1994) studied the mechanism of action of flavipin in *M. laxa* and concluded that flavipin inhibited ATP and protein synthesis independently, but did not primarily affect the cellular membrane. Flavipin furthermore has a highly reactive chemical structure, and could thus act as a multisite inhibitor with pleiotropic effects on fungus cells. *Monilinia laxa* was however able to metabolise flavipin within a relative short incubation time (6 h), resulting in its degradation to a metabolite of lower toxicity (Madrigal *et al.*, 1993).

The efficacy of *Penicillium frequentans*, a component of mycoflora frequently isolated from peach twigs, to control *M. laxa* was attributed to the production of two antibiotic substances with ED₅₀ values for germ tube growth ranging from 0.02 to 0.13 mg/ml (De Cal *et al.*, 1988). Application of spore and/or mycelium preparations of the antagonist *P. frequentans*, alone, in alternation or combination with captan, gave similar control of peach twig blight (*M. laxa*) compared with that of the chemical alone. The efficacy of the biocontrol agent was enhanced by the addition of nutrients in some form (bran, malt, yeast extracts or nutrient agar) to the application. It was furthermore concluded that control could be more effective when the antagonist is applied before blossom infection occurs (De Cal *et*

al., 1990). Pascual *et al.* (2000) attempted to increase the efficacy of this biocontrol agent by growing it at reduced water potential, thus reducing the imbalance in water potential with the phyllosphere compared with inoculum produced at high water availability. Similar reductions in lesion length were however observed, leading the authors to conclude that *P. frequentans* is xerotolerant, rapidly adapting to the dry environment in the phyllosphere.

Trichoderma atroviride and *T. viride* reduced infections of peach, nectarine and plum fruit by *M. fructicola* and also suppressed sporulation on plum fruit. This is an important aspect of disease control and implies that *Trichoderma* spp. could also suppress secondary infections (Hong *et al.*, 1998). From the same study the authors reported the biocontrol potential of an antagonistic yeast from the *Rhodotorula* species.

Pusey and Wilson (1983) demonstrated the potential of a *Bacillus* spp. to control *M. fructicola* on peaches, nectarines, apricots and plums. Antibiotic substances, extracted from *Bacillus subtilis* cultures, proved to be fungistatic and not fungicidal to *M. fructicola* conidia, but provided almost complete suppression of this pathogen on peach fruit at 1 mg/ml (McKeen *et al.*, 1986). Pusey *et al.* (1988) proved the potential of *B. subtilis* as postharvest biocontrol agent for *M. fructicola* in commercial pilot trials. Brown rot control on spray- or dip-inoculated peaches by the bacterium was equal to that obtained by benomyl (1 to 2 µg/ml residue in fruit).

Antibiotic-producing *Pseudomonas corrugata* and *P. cepacia* significantly reduced decay of nectarines and peaches when applied up to 12 h after wound-inoculation with *M. fructicola*. Both bacteria controlled wound-decay better than thiabendazole and similar to triforine. However, decay control on commercial fruit, not artificially inoculated, was poor, indicating that field-infection was not accurately simulated by the inoculation methods used. Latent and quiescent infections might furthermore have been protected from the antagonistic bacteria by the host tissue (Smilanick *et al.*, 1993).

Endophytic bacteria, isolated from the sub-epidermis of various vegetables and fruits, were tested for antagonistic activity against *M. laxa* and *R. stolonifer* in stone fruit. *Monilinia laxa* was more susceptible to control than *R. stolonifer*, with some of the tested isolates affording complete protection against *M. laxa* for up to 6 days at 20°C. The main antagonistic action is thought to be competition for nutrients (Pratella *et al.*, 1993).

Hong *et al.* (2000) recorded the mycoflora occurring on stone fruit mummies in California orchards. The relative recovery of *M. fructicola* from the inner carpocrostromasphere tissues of *Prunus* mummies was negatively correlated with *Botrytis*, *Penicillium* and *Rhizopus*. The mechanisms by which these fungi suppress *M. fructicola* were thought to be through substrate competition, niche exclusion and/or antibiosis. The authors concluded that it might be possible to promote naturally occurring colonising fungi by modifying cultural practices to favour biological control of brown rot. Pesticides applied in orchards affect the quantity as well as the quality of mycoflora. Populations of non-target epiphytic microorganisms were reduced by the application of fungicides. However, *Penicillium* spp., including the antagonistic *P. frequentans*, although sensitive to the fungicides applied, were stable in field populations under fungicide regimes. This stability was attributed to their highly competitive nature and ability to exploit any ecological niche left vacant as a result of the fungicide application (De Cal & Melgarejo, 1992).

Postharvest

Latent or quiescent infections established during the green fruit stages or shortly before harvest caused a significant proportion of the amount of postharvest decay by *M. fructicola* (Swinburne, 1978; Northover & Cerkauskas, 1994; Northover & Biggs, 1995). Ogawa *et al.* (1983) reported that postharvest decay in California caused by *M. fructicola* is of greater concern than that caused by *M. laxa*.

Injuries that occur during harvesting, processing, packaging or transport are often the origin of postharvest decay. Propagules of decay bacteria or fungi are abundant on fruit surfaces and free moisture and nutrients exuding from wounds provide an ideal environment for pathogen propagule germination, germ tube growth and penetration. Successful penetration and infection through wounds depend on many factors, such as the physiological changes that occur at the site of injury which affect susceptibility to infection. Eldon Brown (1989) reviewed the instances where physiological changes in injured tissue influenced host susceptibility and discussed how it can be manipulated through the use of cultural and handling practices. Phenylpropanoid metabolism was initiated in unbroken cells adjacent to the injury site. This led to the formation of certain phenolic compounds (quinones, melanin, chlorogenic and caffeic acid, isocoumarins, related chromones, coumarins, lignin and

suberin) that play a role in protecting the injury from invasion by decay pathogens. These compounds might be involved with wound healing and/or might be toxic to the postharvest pathogen. Temperature and relative humidity are very important factors affecting the wound healing process. Optimum temperature for the relevant metabolic processes involved in wound healing is higher than 10°C, and adequate moisture (RH > 85%) must prevent desiccation and death of the tissue surrounding the damaged cells. However, optimum temperatures for wound healing might also favour the pathogen and infection might occur before healing can convey resistance. Free water at the wound site would also favour the pathogen. Various chemicals such as sanitising agents, bactericides and fungicides are often applied to harvested fruit. Several of these chemicals might inhibit the wound healing process (resorcinol, cyclohexamide, dichlorophen, ferbam, sulphur, mercuric chloride, phenylmercury acetate, streptomycin and captan-rhodamine), whereas others might be beneficial (chlorogenic acid, catechol, metiram, hydrated lime, chloranil and chlorine). Growth regulators such as ethylene, abscisic acid and traumatic acid stimulated phenolic compound accumulation, lignification and/or suberisation. Lower oxygen and higher carbon dioxide levels during storage progressively inhibited suberin and periderm formation. Effective management of all the factors that influence wound healing should be combined with other postharvest control measures to assure good keeping quality of harvested crops (Eldon Brown, 1989).

Fruit should be harvested when mature, but not yet soft-ripe, since it extends shelf life and lessens decay (Ogawa *et al.*, 1983). Careful handling of fruit during harvesting, packaging and transport would minimize the amount of wounds. During the packing process, overmature, bruised or wounded fruit and fruit with insect punctures should be discarded (Heyns, 1967). Ogawa *et al.* (1963) demonstrated that mechanical harvesting of peaches and apricots resulted in more fruit decay compared with hand harvesting. Fruit should also be cold stored within 24 h of packing (Heyns, 1967). The removal of pubescence of peaches by means of brushing machines predisposed the peach fruit to quicker infection by *M. fructicola* (Smith, 1936).

During the postharvest stage fruit can be treated with fungicides alone or in mixtures. Successful control has been achieved with benomyl alone or with captan in mixture (Ogawa *et al.*, 1983). Ethanol at relatively low concentrations (30%) improved the efficacy of a

benomyl-DCNA mixture for control of postharvest decay caused by *M. fructicola*. High concentrations were more effective in reducing decay, but dehydration and consequent shrivelling of fruit increased. This increase was ascribed to the fungitoxicity of ethanol and the increased solubility of benomyl (Feliciano *et al.*, 1992). Iprodione can be applied by atomiser on the packing line or in a drenching system before fruit are graded and sorted (Combrink *et al.*, 1996). After obtaining effective control with phosphonic acid in different host-pathogen systems, Heaton and Dullahide (1990) tested this compound for the control of postharvest *M. fructicola* of peaches. They observed good fungistatic control up to 4 days, but it broke down rapidly after 6 days.

Peracetic acid and chlorine dioxide was effective in reducing *M. laxa* decay of nectarines and plums. The efficacy of these compounds was related to concentration of the product used and duration of treatment (Mari *et al.*, 1999). Calcium salts, in particular calcium propionate, calcium hydroxide, calcium oxide, calcium silicate and calcium pyrophosphate, reduced *in vitro* growth of *M. fructicola* significantly compared with the control. Incidence and severity of decay after spray-inoculation on peach fruit, which were dipped in calcium salts, were reduced and were least when the fruit were dipped in solutions of calcium phosphate or calcium silicate. Calcium oxide and calcium hydroxide were the most effective in reducing brown rot severity on wound-inoculated peaches. The efficacy of calcium salts in inhibiting *M. fructicola* was attributed to the inhibition of polygalacturonase activity, thus effecting reduced virulence. An additional value of calcium salts is that it has no activity against yeasts and can therefore be used to supplement biological control by yeasts (Biggs *et al.*, 1997).

Natural substances have been utilised in postharvest pathology. The glucosinolates in plant cells are hydrolysed under catalysis of the myrosinase enzyme to a series of fungitoxic compounds. Isothiocyanates produced from the hydrolysis of the glucosinolates, glucoraphenin and sinalbin, showed consistent antifungal activity against *M. laxa* and *B. cinerea*. High concentrations of glucoraphenin-isothiocyanates (3.6 mg/ml) afforded pathogen control at high inoculum levels (10^6 conidia/ml) after 6 days at 20°C and had curative effect up to 40 h after inoculation. This compound proved to be stable at room temperature and active on pears against *M. laxa* at 0°C, therefore warranting further investigations into its possible use in postharvest control (Mari *et al.*, 1996). Several sugar

analogs were also tested for potential fungicides for postharvest decay of apples (*B. cinerea* and *Penicillium expansum*) and pears (*M. fructicola*) (El Ghaouth *et al.*, 1995). Only 2-deoxy-D-glucose was effective in controlling decay in inoculated apple and peach fruit. Results from *in vitro* studies suggested that the observed inhibition was due to direct antifungal properties of the sugar analog.

The proven fungistatic activity of several fruit aroma compounds associated with natural ripening, like benzaldehyde vapours, against *M. laxa* and *Rhizopus stolonifer*, increased the possibility of the use of naturally occurring chemicals for postharvest decay control (Caccioni *et al.*, 1995). Acetic acid is an effective postharvest fumigant. Fumigation with 1.4 mg/l acetic acid reduced postharvest decay by *M. fructicola* compared with the untreated control, without any deleterious effect on the internal quality of the fruit. Higher concentrations (2.7 mg/l) however controlled decay by *Rhizopus stolonifer*, but slight indications of phytotoxicity was observed (Sholberg & Gaunce, 1996). In a later study, Sholberg (1998) also reported the effective control of *M. fructicola*, *Penicillium expansum* and *R. stolonifer* on sweet cherry by fumigation with short-chain organic acids, acetic, formic and propionic acid.

Storage of packaged sweet cherries wound-inoculated with *M. fructicola* in increased carbon dioxide levels reduced the onset of decay development and overall fruit decay. De Vries-Paterson *et al.* (1991) showed that at 50% CO₂ brown rot development was completely inhibited for the 7-day storage period at 20°C. Fruit did however develop decay within 2 to 4 days when returned to normal air at 25°C, indicating that CO₂-treatments are fungistatic and not fungicidal.

Low hormetic doses of radiation with ultraviolet light (254 nm, UV-C) reduced postharvest diseases, including brown rot. Pretreatment of fruit with low doses of UV-C, followed by artificial inoculation, reduced postharvest decay, indicating induced resistance of the fruit to decay (Stevens *et al.*, 1996). In a later study Stevens *et al.* (1998) re-affirmed the beneficial effect of low doses of UV-C on host resistance after this treatment controlled latent brown rot infection. Their results showed a negative relationship between UV-C doses, colony forming units and the number of brown rot lesions. UV-C light thus has a germicidal and hormetic effect on reducing brown rot, and furthermore increased phenylalanine

ammonia-lyase activity, delayed ripening and suppressed ethylene production. The population of an epiphytic yeast, *Debaryomyces hansenii*, proven antagonistic against *M. fructicola* was also increased.

Thermotherapy is also a successful means of preventing postharvest decay. Moist air treatment at 52°C for 15 min prevented decay in nectarine fruit puncture-inoculated with *M. fructicola*. Each fruit is individually wrapped in plastic to reduce or eliminate undesirable skin browning associated with heat treatment. Although shorter treatments controlled decay of naked fruit, it only slowed decay development of wrapped nectarines and eventually resulted in increased decay. The combined effect of heat treatment and wrapping resulted in better quality fruit, not only because of reduced decay, but also slower softening and reduced ethylene production and respiration (Anthony *et al.*, 1989).

Brown rot and *Rhizopus* rot of inoculated peaches was significantly reduced when the fruit were treated with hot water (Smith, 1962) and/or hot suspensions of 2,6-dichloro-4-nitroaniline (DCNA) or benomyl (Wells & Harvey, 1970; Smith, 1971; Jones & Burton, 1973). Phillips (1982b) however examined the injuries to peaches that were immersed in hot water. The author observed increased weight loss, surface browning and increased staining with fast green dye of the hot-water treated fruit.

In other early attempts to control postharvest peach brown rot, Ogawa and Lyda (1960) found propanol to be the most toxic alcohol against *M. fructicola* spores, followed by isopropanol, ethanol and methanol. Fifty percent ethanol inactivated *M. fructicola* spores on the fruit surface within 5 s and spores on the flesh within 2 minutes. The authors concluded that when fruit were rinsed immediately after a 1 min immersion in 60% ethanol, no phytotoxicity was observed and the treatment also produced firmer fruit with more intense colour.

Hydrocooling of fruit in chlorinated water before fungicide treatment cleaned the fruit, reduced viable inoculum of micro-organisms on fruit surfaces and removed latent heat, thereby slowing the ripening process and growth of decay organisms (Osorio *et al.*, 1993). Chlorine at relatively low concentrations (5 to 10 ppm ClO⁻) was fungicidal to *M. fructicola* conidia when suspended in water, but low temperature treatments reduced this effect (Phillips & Grendahl, 1973). Hydrocooling similarly reduced the efficacy of iprodione and benomyl,

but not of E-0858. This was attributed to the better systemicity of E-0858, which resulted in less wash-off of fungicidal residue (Osorio *et al.*, 1993).

Postharvest decay control is most effective when several control measures are integrated in one programme. Spotts *et al.* (1998) reported the more effective control of *M. fructicola* brown rot of sweet cherry by preharvest treatment with iprodione, postharvest treatment with the antagonistic yeast, *Cryptococcus infirmo-miniatus*, followed by modified atmosphere packaging (initial in-package atmosphere of 50% CO₂ and 50% N₂), compared with these treatments alone. Although iprodione residues on harvested fruit were sufficient to control *M. fructicola*, it did not inhibit the antagonistic yeast. Synergism was furthermore observed between the yeast and modified atmosphere treatment (Spotts *et al.*, 1998). Pusey *et al.* (1986) demonstrated the compatibility of *Bacillus subtilis* with commercial fruit waxes, postharvest dichloran treatment and cold storage conditions and the retained efficacy of the biocontrol agent against *M. fructicola*.

CONCLUSION

Unpublished research reports from South Africa (Dept. of Plant Pathology, University of Stellenbosch) have shown that due to the similarity of brown rot and grey mould symptoms shortly after cold storage, a significant proportion of *M. laxa* postharvest decay is misidentified as grey mould, caused by *B. cinerea*. The relevance of *M. laxa* as an important pre- and postharvest pathogen in South Africa might consequently be underestimated. Little is also known about the etiology of *M. laxa*. Most research on the brown rot fungi involved the more virulent species, *M. fructicola* (Wormald, 1919; Curtis, 1928; Smith, 1936; Hewitt & Leach, 1939; Weaver, 1950; Wade, 1956a; Wade, 1956b; Ogawa & English, 1960; Corbin, 1963; Jenkins & Reinganum, 1965; Corbin *et al.*, 1968; Kable, 1969a; Kable, 1969b; Hall, 1971; Kable, 1971; Byrde & Willetts, 1977; Tate & Corbin, 1978; Ritchie, 1983a; Biggs & Northover, 1988a; Biggs & Northover, 1988b; Biggs & Northover, 1989; Brown & Wilcox, 1989; Northover & Biggs, 1990; Adaskaveg *et al.*, 1991; Cruickshank & Wade, 1992a; Wade & Cruickshank, 1992a; Cruickshank & Wade, 1992b; Wade & Cruickshank, 1992b; Willetts & Bullock, 1993; Elmer & Gaunt, 1994; Northover & Cerkaskas, 1994; Northover & Biggs, 1995; Biggs *et al.*, 1997; Hong *et al.*, 1998; Bostock *et al.*, 1999). *Monilinia laxa* rarely forms apothecia (Willetts & Harada, 1984). The fungus overwinters on mummified fruits, the

fruit stalks and scars and on buds, as well as in cankerous lesions (Batra, 1985). *Monilinia laxa* infects by means of conidia, which may be produced throughout the year. There is however only limited quantitative information on the importance of the different inoculum sources and infection of blossoms and fruit by this pathogen. In stone fruit orchards of the Western Cape province of South Africa, brown rot, and not blossom and spur blight, is the most destructive phase of the *M. laxa* disease syndrome (Fourie & Holz, 1985a; 1985b; 1987a; 1987b; Schlagbauer & Holz, 1989a; 1989b). *Botrytis cinerea* has, on the other hand, been associated with blossom blight of stone fruit (Ogawa & Lyda, 1960; Fourie & Holz, 1994) and pistachio (Michailides, 1991). Information about the relative occurrence of *M. laxa* and *B. cinerea* from the flower to the fruit stages of different stone fruit types is therefore lacking. Research concerning the etiology and epidemiology of *M. laxa* on stone fruit is furthermore needed in order to facilitate the recommendation of scientifically based control measures.

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2. OCCURRENCE OF *BOTRYTIS CINEREA* AND *MONILINIA LAXA* ON NECTARINE AND PLUM IN WESTERN CAPE ORCHARDS

ABSTRACT

Observations were made over a 3-year period of the occurrence of grey mould (*Botrytis cinerea*) and brown rot (*Monilinia* spp.) in the major stone fruit regions in the Western Cape province by sampling from the Unifruco Quality Evaluation Scheme and from 11 stone fruit orchards. Unifruco subjected fruit that were delivered by growers for export to storage conditions simulating overseas shipment. Isolations were made from fruit designated as defective by Unifruco personnel at the end of the storage period. Flowers and fruit from the different orchards were subjected to conditions that facilitated disease expression by surface inoculum and latent infection (unsterile vs. surface-sterilised; untreated vs. paraquat-treated). *Botrytis cinerea* was found to be the most important pathogen causing blossom blight and postharvest decay on stone fruit. The pathogen was most prominent on early- and mid-season cultivars. Brown rot was exclusively caused by *M. laxa* and no evidence was found that *M. fructicola* had been introduced into South Africa. *M. laxa* was most prominent on the later maturing cultivars. In the case of *B. cinerea*, blossom infection did not contribute directly to postharvest decay. Both surface inoculum and latent infection consistently occurred on fruit in each orchard, although at fluctuating levels. Disease expression on developing fruit was not governed by the amount of *B. cinerea* occurring on fruit surfaces, but by the ability of fruit to resist disease expression. This was shown by the finding that paraquat-treated fruit yielded substantially higher levels of decay than non-treated fruit. The amount of *B. cinerea* on fruits was generally higher during spring than summer. *Monilinia laxa* occurred sporadically on the blossoms of late-maturing cultivars. Immature fruit were generally pathogen-free and disease expression only occurred on maturing fruit. Long-term latency therefore does not seem to play a prominent role in *M. laxa* fruit rot. These findings suggest that conidia of *M. laxa* are generally produced in orchards when fruits are approaching maturity and can only penetrate and infect maturing fruit.

INTRODUCTION

A survey of postharvest decay of stone fruit in the Western Cape province of South Africa (Fourie & Holz, 1985a) attributed 73% of decay to *Botrytis cinerea* (grey mould) and 12 % to *Monilinia laxa* (brown rot). This finding is in contrast with reports from other parts of the world where *M. fructicola*, *M. laxa* or *Rhizopus stolonifer* are more important (Smith, 1971; Wells, 1972; Wells & Bennett, 1975; French, 1976; Phillips & Harris, 1979). In America and Australasia *M. fructicola* is considered the main causal agent of brown rot, and *M. laxa* is primarily associated with the blossom and spur blight phase of the *Monilinia* disease syndrome (Hewitt & Leach, 1939; Ogawa *et al.*, 1954; Ogawa & English, 1960; Ogawa *et al.*, 1975; Byrde & Willetts, 1977; Ogawa *et al.*, 1983; Jones & Sutton, 1996). *Monilinia fructicola* is absent in South Africa, and all stages of this disease is caused by *M. laxa* (Fourie & Holz, 1985a; 1985b; 1987a; 1987b; Schlagbauer & Holz, 1989a; 1989b). Inoculation studies on plum and nectarine flowers on shoots in the laboratory and orchard showed that *B. cinerea* infections resembled *Monilinia* blossom blight (Fourie & Holz, 1995). These findings suggest that *B. cinerea*, and not *M. laxa*, may be the principal pathogen responsible for blossom blight in South African stone fruit orchards.

The significance of different inoculum sources of *M. fructicola* to brown rot development is well known. Ascospores produced from mummies on/in the soil (Roberts & Dunegan, 1926; Tate & Corbin, 1978; Hong *et al.*, 1996; Hong & Michailides, 1998) and conidia shed from infected areas remaining on the tree from a previous year (Byrde & Willetts, 1977; Tate & Corbin, 1978; Landgraf & Zehr, 1982; Ogawa *et al.*, 1983; Biggs & Northover, 1985) provide inoculum for infection of flowers, which has been associated with latent infection (Wade, 1956; Jenkins & Reinganum, 1965; Tate & Corbin, 1978). Latent infections appear to be of great significance under humid temperature conditions because many develop into vigorously sporulating lesions that envelop maturing stone fruits (Jenkins & Reinganum, 1965; Wade & Cruickshank, 1992; Northover & Cerkaskas, 1994; Emery *et al.*, 2000). Nonabscised, aborted fruits in the tree and thinned fruits on the ground are other important sources of conidia as fruits are approaching maturity (Landgraf & Zehr, 1982; Biggs & Northover, 1985; Hong *et al.*, 1997). However, little is known of inoculum sources of *M. laxa* and *B. cinerea*. It has been suggested that *B. cinerea* flower infection may

contribute indirectly to grey mould fruit decay (Fourie & Holz, 1995). Locally, different types and cultivars of stone fruit with different blossoming periods are cultivated in adjacent and nearby orchards. The production of secondary inoculum on colonised floral parts may contribute to the total infection pressure, supplying the required inoculum for infection of different stone fruit types as their fruit ripen. Considerable reinfection will therefore be caused by infected floral parts that land on healthy tissue (Ogawa & English, 1960). Data on inoculum behaviour of both *M. laxa* and *B. cinerea* are needed to contribute to a better understanding of the two diseases and will provide a basis for more effective management of grey mould and brown rot. The aim of this study was to determine the relative importance and seasonal occurrence of *B. cinerea* and *M. laxa* in nectarine and plum orchards and to ascertain the ecology of their inocula on stone fruit at various phenological stages in Western Cape orchards.

MATERIALS AND METHODS

General survey. In the past, Unifruco (Pty.) Ltd., an export marketing company, controlled the export and marketing of a large proportion of stone fruit from South Africa. In order to regulate the quality of export fruit, the company enforced a Quality Evaluation Scheme, which requires that each producer's fruit be sampled prior to overseas shipment. Sample sizes were statistically determined according to the number of cartons in each consignment. After sampling, the fruit were subjected to storage conditions simulating overseas shipment (4 wk storage at -0.5°C). Following cold storage, Unifruco personnel determined the different categories of fruit defects. Symptomatic fruit were collected on a regular basis during the 1996/97, 1997/98 and 1998/99 seasons from Unifruco. The symptomatic fruit were placed in separate plastic bags and incubated on a laboratory bench at 22°C to induce sporulation. The causal organism was identified with a dissecting microscope, and in the case of brown rot isolated onto potato dextrose agar (PDA) for cultural identification to species-level (Hewitt & Leach, 1939; Calavan & Keitt, 1948; Ogawa *et al.*, 1954; Jenkins, 1965; Heyns, 1968; Penrose *et al.*, 1976; Sonoda *et al.*, 1982; Corazza *et al.*, 1998; Leeuwen & Kesteren, 1998). Reference cultures from California (R.M. Sonoda), Australia (H.J. Willetts), and the United Kingdom (R.J.W. Byrde) were included. When isolates differed from the reference cultures in appearance on PDA, their identity was verified

by comparing their germ tube morphology (Hewitt & Leach, 1939; Calavan & Keitt, 1948; Ogawa *et al.*, 1954; Jenkins, 1965; Heyns, 1968; Leeuwen & Kesteren, 1998), the extent of hyphal anastomosis between germ tubes (Ogawa & English, 1964; Hoffmann, 1972; 1974) and their interactions with reference cultures on oatmeal agar (Sonoda *et al.*, 1982).

Orchards. The investigation was conducted on flowers and fruit obtained over a period of 3 years (1996-1999) from 11 orchards located in the main stone fruit producing regions of the Western Cape province. Three nectarine and two plum cultivars with differing ripening periods were selected for the study: Mayglo (nectarine), early-season cultivar harvested early November; Sunlite (nectarine) and Santa Rosa (plum), mid-season cultivars harvested early December; and Flamekist (nectarine) and Casselman (plum), late-season cultivars harvested early February. Mayglo and Sunlite orchards were selected in Simondium, Wellington and Klein-Drakenstein, and Flamekist orchards in Prince Alfred Hamlet, Koue Bokkeveld and Vyeboom (Fig. 1). A Santa Rosa and a Casselman orchard were selected in the Blaauwklippen valley, Stellenbosch (Fig. 1). All orchards were well established with tree age of at least 5 years. Orchards were micro-irrigated and orchard practices for the production of first grade export fruit, as prescribed by the Unifruco Producer Guide (Unifruco (Pty.) Ltd., Parc du Cap, Mispel Road, Bellville, 7550), were maintained. Different programmes for the control of *B. cinerea* and *M. laxa* were followed in the orchards. Sprays against *B. cinerea* were applied at blossom, after shuck fall and 1 wk before harvest. Fungicides used were benomyl (Benlate, 500 WP, Du Pont) and iprodione (Rovral, 255 SC, Aventis). Sprays against *M. laxa* were applied at blossom, 10 and 3 days before harvest. Fungicides used were benomyl, iprodione, bitertanol (Baycor, 300 EC, Bayer), triforine (Denarin, 190 EC, Cyanamid) and fenbuconazole (Indar, 50 EC, Algro-Chem).

Disease expression on moist incubated flowers. Sound unblemished flowers (400 per orchard) were sampled at full bloom, placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm) and used for determining the ecology of *B. cinerea* and *M. laxa* inoculum on flowers. For these studies, half the number of flowers had been sterilised (30 s in 70% ethanol and air-dried), whereas the other half was left unsterile. The screens were placed in ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper (45 x 57 cm) with the base resting in deionised water to establish high relative humidity ($\geq 93\%$ RH), and were incubated at 23°C

under a 12 h light schedule to induce the development of *B. cinerea* and *M. laxa*. These treatments provided conditions that facilitated the development of symptom expression by different inocula during the period of moist incubation. On untreated flowers, disease expression was the result of natural infection by surface inoculum and the development of latent infections. Surface sterilisation completely eliminated the pathogens from the flower surface. This treatment prevented natural infection and promoted the development of only latent infection. The flowers were regularly monitored for disease expression and the primary site of pathogen development was recorded. The causal organism was identified using a dissecting microscope. Percentages flowers yielding *B. cinerea* or *M. laxa* were calculated after 14 days. All the *Monilinia* isolates were identified to species-level as described previously.

Disease expression on moist incubated fruit. Sound unblemished fruit were collected at shuck fall (400 fruit per orchard), pit hardening (200 fruit per orchard), 2 wk before harvest (200 fruit per orchard) and at harvest (200 fruit per orchard), placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm) and used for determining the ecology of *B. cinerea* and *M. laxa* inoculum on fruit. For these studies, fruit on the screens were divided in four groups. One group on each screen was sterilised (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried. The second group was sterilised as above, immersed in a 3% paraquat (WPK Paraquat, 200 g/l (bipyridyl), WPK Agricultural) solution for 30 seconds, rinsed in sterile deionised water and air-dried. The third group was left unsterile and received no paraquat, whereas the fourth group was left unsterile but was treated with paraquat. The screens were placed in ethanol-disinfected moist chambers and were incubated under laboratory conditions (23°C under a 12 h light schedule) to induce the development of *B. cinerea* and *M. laxa*. These treatments provided conditions that facilitated disease expression by different inocula during the period of moist incubation. On untreated fruit, decay was the result of natural infection by surface inoculum and the development of latent infection, as influenced by host resistance. Surface sterilisation completely eliminated the pathogens from the fruit surface. This treatment prevented natural infection and promoted the development of latent infections only. Paraquat terminated host resistance in the outer cell layers and consequently promoted the development of surface inoculum and latent infection (Baur *et al.*, 1969; Cerkauskas & Sinclair, 1980; Pscheidt & Pearson, 1989). This treatment therefore enhanced the development of latent infection on surface-sterilised fruit.

On unsterile fruit, both natural infection by surface inoculum and development of latent infection was promoted. The fruits were regularly monitored for disease expression and the primary site of pathogen development was recorded. The causal microorganisms were identified using a dissecting microscope. Percentages fruit yielding *B. cinerea* or *M. laxa* were calculated after 14 days. All the *Monilinia* isolates were identified to species-level as described previously.

Postharvest decay on cold stored fruit. An additional 200 fruit per orchard were sampled at harvest, packed in packing cartons and kept under conditions simulating overseas shipment and marketing (nectarines 4 wk at -0.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH; plums 2 wk at -0.5°C , 2 wk at 10°C followed by 1 wk at 23°C at $\pm 56\%$ RH). The development of brown rot and grey mould was monitored and the percentage fruit yielding *B. cinerea* or *M. laxa* calculated. All the *Monilinia* isolates were identified to species-level as described before.

RESULTS

General survey. A total of 286 plum and 181 symptomatic nectarine and peach fruit were obtained during the investigation period from the Unifruco Quality Evaluation Scheme (Tables 1 and 2). *Botrytis cinerea* was the most frequently isolated decay pathogen. Brown rot decay was exclusively caused by *M. laxa*. *Botrytis cinerea* was generally the major pathogen on the early and mid-season ripening cultivars of both stone fruit types, with *M. laxa* becoming the major pathogen on the later maturing cultivars.

Disease expression on moist incubated flowers. Mean percentages of flowers of each cultivar yielding either *B. cinerea* or *M. laxa* are given in Table 3. The sites on flowers where the organisms were primarily recorded are given in Table 4. On unsterile flowers nearly a third of the flowers of each cultivar yielded *B. cinerea* (Table 3). The pathogen was generally less common on flowers of the early-season nectarine, Mayglo (22.8%), than on the late-season cultivars, Flamekist (37.9%) and Casselman (36.8%). Levels of *B. cinerea* yielding flowers were generally lower in the sterile treatment, ranging between 5.2% for the plum cultivar, Santa Rosa, to 20.1% for the nectarine cultivar, Sunlite. The pathogen developed primarily from the calyx and to a lesser extent from the petals and stamens of the

symptomatic nectarine flowers (Table 4). On plum, calyx and petal infection were predominant. *Monilinia laxa*, on the other hand, was virtually absent from flowers of both the unsterile and sterile regimes and was associated only with the late-season nectarine cultivar, Flamekist, but at a low level. On these flowers the pathogen developed predominantly from the calyx and from petals.

Trends followed by the two pathogens in each of the different orchards are given in Figs. 2 to 9. The occurrence of *B. cinerea* on flowers varied greatly between orchards of a specific cultivar and between seasons. On unsterile flowers, levels of pathogen-yielding flowers were generally relatively low in the early-season nectarine, Mayglo (Fig. 2), and the mid-season plum, Santa Rosa (Fig. 4). Levels were generally higher in flowers of the mid-season nectarine, Sunlite (Fig. 3), the late-season nectarine, Flamekist (Fig. 4), and the late-season plum, Casselman (Fig. 5). Levels of sterile pathogen-yielding flowers in orchards of Mayglo (Fig. 2), Sunlite (Fig. 3) and Casselman (Fig. 5) generally corresponded to those recorded on the unsterile flowers. Therefore, in most of these orchards levels in a specific season were mostly either high in both the unsterile and the sterile sample, or low. This was not the case in Flamekist (Fig. 4) orchards, which in certain seasons yielded flowers showing high levels of *B. cinerea* in the unsterile sample, but low levels in the sterile sample.

Monilinia laxa followed a different pattern compared with *B. cinerea*. Of all the samplings only the one collected in the 1996/97 season from the Flamekist orchard at Koue Bokkeveld yielded *M. laxa* at approximately 40% under both sterility regimes (Fig. 8). Flowers from the other nectarine orchards and both the plum cultivars were free from *M. laxa* in each season or showed less than 1% infection (Figs. 6, 7 and 9).

Disease expression on moist incubated fruit. Mean percentages of fruit of each cultivar yielding *B. cinerea* are given in Table 5. In the case of the non-paraquat treatment, fruit from all the developmental stages were highly resistant to disease expression, with very low levels (0-2.7%) of pathogen-yielding fruit recorded. The level of sporulating fruit was furthermore unaffected by the sterility regime, which indicated that fruits were not infected by surface inoculum during the period of moist incubation. Exceptions were the early- and mid-season nectarine cultivars Mayglo and Sunlite, which were markedly less susceptible to disease expression at harvest and yielded the pathogen at higher levels in the unsterile regime.

Resistance to decay was markedly reduced on fruit of all the cultivars by the paraquat treatment. Paraquat-treated fruit at each phenological stage therefore yielded higher levels of pathogen-yielding fruit than the non-paraquat treated fruit. Frequencies of sporulating fruit were furthermore markedly influenced by the sterility regime, showing consistently higher levels on fruit subjected to the unsterile than the sterile regime. This indicated that surface inoculum occurred on fruit causing new infections during the period of moist incubation. Levels of sporulating fruit under the sterile and unsterile regimes differed drastically for some of the cultivars. On Mayglo and Sunlite high levels of sporulating fruit were recorded for the unsterile treatment at pit hardening, 2 wk before harvest and at harvest, whereas high levels were recorded in the unsterile regime for the pit hardening and 2 wk before harvest samplings on Flamekist. On Santa Rosa decay levels were high on the unsterile fruit sampled at shuck fall.

The primary sites of *B. cinerea* disease expression on fruits (mean values of all cultivars for sterile fruit of both paraquat regimes) are given in Table 6. Decay originated at shuck fall and pit hardening in approximately 50% of the symptomatic fruit from either the cheek or peduncle-end. The proportion of cheek-associated infections increased with ripening, reaching 65% at harvest. Peduncle-associated infections, on the other hand, decreased to 35% at harvest. The proportion of tip-associated infections were between 10 and 20% from shuck fall to 2 wk before harvest and increased to 28% at harvest.

Trends followed by *B. cinerea* in each of the different orchards are given in Figs. 2 to 5 and are summarised below. In each of the seasons, fruit from nearly all the orchards under both sterility regimes in the non-paraquat treatment remained asymptomatic during moist incubation at the shuck fall to 2 wk before harvest samplings. Disease expression only occurred on non-paraquated fruits when sampled at harvest and only on fruits of the early- and mid-season nectarine cultivars, Mayglo and Sunlite. For these cultivars disease expression was recorded on fruit from each orchard, but the levels in an orchard fluctuated between seasons. In the case of Mayglo (Fig. 2), six of the nine samplings yielded fruit of which approximately 20% developed *B. cinerea* decay. On Sunlite (Fig. 3) only one sampling made from the Wellington orchard in 1996/97 yielded fruit with a relatively high level (40%) of disease expression. Decay levels in the other samplings ranged between 1 and 10%. Disease expression on paraquat-treated fruit followed a different trend and was largely

regulated by the cultivar. On both Mayglo and Sunlite, high (60%) to very high (80%) levels of symptomatic fruit were found on samplings made at pit hardening and 2 wk before harvest. High levels were occasionally found at shuck fall and harvest. Levels were furthermore generally markedly higher on fruit under the unsterile than the sterile regime. On Flamekist (Fig. 4) high levels of symptomatic fruit were only recorded in the 1996/1997 season and then predominantly at pit hardening and 2 wk before harvest. Disease expression was mostly at a low level in the two plum cultivars, Santa Rosa and Casselman (Fig. 5).

The mean percentages of fruit of each cultivar yielding *M. laxa* are given in Table 7. The primary site of disease expression on fruits (mean values of all cultivars for sterile fruit of both paraquat regimes) is given in Table 6. The pathogen was recorded only on ripening Sunlite and Flamekist fruit. On these fruits disease expression was not influenced by paraquat treatment or sterility regime. Cheek-associated infections were predominant on ripening fruit, comprising 80% or more of the infections at 2 wk before harvest and at harvest (Table 6). The proportion tip-associated infections decreased with fruit development.

Trends followed by *M. laxa* in each of the different orchards are given in Figs. 6 to 9 and are summarised below. In each season fruit from all the Mayglo, Santa Rosa and Casselman orchards remained mostly asymptomatic, irrespective of sterility or paraquat regime. Disease expression was only found on Sunlite and Flamekist and was dependant on orchard and season for both cultivars. In all these samplings fruit generally remained asymptomatic at the shuck fall and pit hardening stages and developed symptoms when sampled 2 wk before harvest and at harvest. Furthermore, no distinct pattern of disease expression was observed between the different treatments.

Postharvest decay of cold stored fruit. Overall, two distinct patterns of postharvest decay were noted (Table 8). *Botrytis cinerea* decay was pronounced on the early- and mid-season nectarine cultivars Mayglo and Sunlite, but caused a very low level of decay on the late-season nectarine Flamekist. *Monilinia laxa*, on the other hand, was recorded at a very low level on Mayglo, but was pronounced on Sunlite. The level of decay was, however, very high on Flamekist. Both pathogens were of minor importance on the two plum cultivars Santa Rosa and Casselman.

DISCUSSION

By sampling from the Unifruco Quality Evaluation Scheme and from 11 stone fruit orchards in the major stone fruit regions, isolates were obtained over a 3-year period from a large geographical area, comprising many producers and a wide variety of cultivars. Data from this investigation showed that *B. cinerea* is the most important pathogen causing decay on stone fruit in the Western Cape province. This study therefore confirmed the findings of Fourie and Holz (1985a) and found no evidence indicating a shift in the prevalence of *B. cinerea* and *M. laxa* in local stone fruit orchards. Furthermore, no evidence was found that *M. fructicola* had been introduced into the region. This confirmed previous reports, which stated that brown rot in South Africa is caused by *M. laxa* (Fourie & Holz, 1985a; Schlagbauer & Holz, 1987).

In this study flowers and fruit of different cultivars of two stone fruit types were kept under conditions facilitating disease expression by both new and established infections of *B. cinerea* and *M. laxa*. In the case of *B. cinerea*, both infection types consistently occurred on flowers and fruit in each orchard, although at fluctuating levels. By comparing the levels of sporulating flowers in the two sterility regimes, it became apparent that moist incubation of these flowers gave a good indication of the amount of *B. cinerea* occurring on flower surfaces. Disease expression on developing fruit was however not governed by the amount of *B. cinerea* occurring on fruit surfaces, but by the ability of fruit to resist disease expression. This is shown by the finding that paraquat-treated fruit yielded substantially higher levels of decayed fruit than non-treated fruit. Paraquat is known for its terminating effect on natural host resistance of harvested fruit, thereby facilitating development of established infections on sterile fruit and infection by surface inoculum on unsterile fruit (Baur *et al.*, 1969; Cerkaskas & Sinclair, 1980; Pscheidt & Pearson, 1989). The occasional occurrence of high levels of sporulating fruit in both the unsterile and sterile paraquat treatments thus indicated the presence of high amounts of *B. cinerea* on fruit surfaces and high incidences of latent infected fruit, respectively. Generally, conidia of *B. cinerea* are regarded as short-lived propagules, but there is evidence to suggest they possess considerable survival abilities (Blakeman, 1980; Coley-Smith, 1980). In this regard it was recently shown that conidia of this pathogen were able to survive on fruit surfaces of kiwifruit, remaining viable and

infectious throughout the growing season (Walter *et al.*, 1999). The role of predisposing factors, such as wounding, resulting from injuries sustained during or after harvest, may therefore be underestimated in symptom expression and the epidemiology of *B. cinerea* on stone fruit.

Losses from postharvest decay have been ascribed to direct penetration of ripening fruit and not to flower or early fruit infections (Fourie & Holz, 1994; 1995). Trends followed by the pathogen during natural infection in the different orchards substantiate these findings on artificially inoculated material. Decay of sterile fruit developed in a scattered pattern and was style-associated in the minority of cases. The amount of *B. cinerea* on developing fruit and disease expression were not governed by the level of flower infection. This trend is clearly shown when levels of disease expression on paraquat-treated fruit are compared with those on flowers. These comparisons indicated that in each orchard drastic seasonal fluctuations occurred between levels of disease expression on flowers and on fruit at shuck fall and pit hardening stages. In spite of these fluctuations, levels of decayed fruit of early, mid- and late-season nectarine cultivars were generally relatively high during the period pit hardening to the beginning of ripening. Decay levels were furthermore mostly high at harvest on the Mayglo and Sunlite, but low on Flamekist. These differences in the amount of *B. cinerea* occurring on ripening fruit may be partially responsible for the higher levels of postharvest decay recorded on early- and mid-season cultivars.

No evidence was found for the assumption that *B. cinerea*-infected floral parts contribute indirectly to grey mould fruit decay by supplying the required inoculum for infection of different stone fruit types as their fruit ripen (Fourie & Holz, 1994). On the other hand, inoculum for infection was readily available in orchards as infection occurred throughout the growing season under natural orchard conditions. Infection levels on fruit, and therefore the amount of *B. cinerea* in orchards, were however higher during spring than summer. *Botrytis cinerea* is among the fungi of which the conidia are most frequently trapped in air (Pady & Kelly, 1954; Richards, 1956) and it occurs worldwide on a variety of plants (Jarvis, 1980). Kobayashi (1984) observed conidial masses of the organism throughout the year on fallen petals of 28 plant species from in 19 genera of 14 families. The Western Cape province, a winter rainfall area, is well known for its diversity of plant species. During spring (late August to early November), which is traditionally a wet period, *B. cinerea*

was observed sporulating on senescing weed tissue in drainage ditches between the tree rows in the orchard and on weeds beneath the trees that were killed by herbicides (Data not shown). Weeds colonised were mostly *Oxalis pes-caprae* (yellow sorrel), *Anagallis arrenis* (bird's eye weed), *Taraxacum officinale* (common dandelion), *Sonchus oleracens* (milk thistle), and *Conyza bonariensis* (horseweed). These weeds may directly contribute to the high *B. cinerea* inoculum levels occurring during the early season in local stone fruit orchards and consequently to the prominence of grey mould on the early maturing cultivars.

Blossom and spur blight are considered the principal features and the most destructive phase of the *M. laxa* disease syndrome (Hewitt & Leach, 1939; Ogawa *et al.*, 1954; Ogawa & English, 1960; Ogawa *et al.*, 1975; Byrde & Willetts, 1977; Ogawa *et al.*, 1983; Jones & Sutton, 1996). However, in a study on the occurrence of latent infections on different stone fruit types in South Africa, *M. laxa* was never seen on stigmas or styles of plum blossoms, as these parts were usually heavily colonised by *Cladosporium cladosporioides* or *Alternaria alternata* (Schlagbauer & Holz, 1989a). In a subsequent study on *M. laxa* blossom blight, *B. cinerea* was commonly found on attached and abscised Reubennel plum flowers (Schlagbauer & Holz, 1990). Fourie and Holz (1994) artificially inoculated Harry Pickstone plum and Sunlite nectarine flowers with *B. cinerea* on shoots in the laboratory and orchard and showed that the infections resembled *Monilinia* blossom blight. During this 3-year investigation period typical blossom blight was observed only once, namely in the 1996/97 season in the Sunlite orchard at Wellington. Orchard observations and isolations from symptomatic and asymptomatic flowers from this orchard ascribed the disease to *B. cinerea*. These findings, and the fact that asymptomatic flowers from the 11 stone fruit orchards consistently yielded *B. cinerea* and not *M. laxa*, suggest that *B. cinerea* is the principal pathogen responsible for blossom blight in South African stone fruit orchards. *Botrytis cinerea* blossom blight has previously been reported on other stone fruit types (Ogawa & Lyda, 1960; Fourie & Holz, 1994) and pistachio (Michailides, 1991).

Monilinia fructicola brown rot of ripe fruits may develop from different types of infection. Latent infections may be initiated in young fruits (Wade, 1956; Northover & Cerkauskas, 1994), sometimes as early as blossom (Wittig *et al.*, 1997) and shuck fall (Wade & Cruickshank, 1992). Short-term latent infections may be formed as late as a few weeks before harvest (Wade & Cruickshank, 1992). Latent infections can serve as a source of

inoculum for subsequent fruit rot of maturing stone fruits (Jenkins & Reinganum, 1965; Phillips & Harvey, 1975; Wade & Cruickshank, 1992; Northover & Cerkauskas, 1994; Emery *et al.*, 2000). In *M. laxa*, short-term latent infections initiated shortly before fruit are harvest-ripe, rather than long-term latent infections, are considered the main cause of fruit losses (Kable, 1971; Schlagbauer & Holz, 1989a; 1989b). In this study fruit in both the unsterile and sterile paraquat treatments were virtually free of *M. laxa* from shuck fall until 2 wk before harvest. Only ripening fruit yielded the pathogen and decay primarily developed in a scattered pattern on the cheek and in a minority of cases from the style end of fruits. Furthermore, no distinct pattern of symptom expression emerged on ripening fruit subjected to different paraquat regimes. Events of high inoculum loads on fruit surfaces and establishment of latent infection seems therefore not to occur in the orchards during the early stages of fruit development. On the other hand, if such events did occur, conidial viability might decrease rapidly due to bacterial antagonism, effects of UV light or the presence of substances in fruit exudates deleterious to germinating conidia and germlings of *M. laxa*. Latent contamination (Jerome, 1958) of fruit approaching maturity should however be considered of major importance when handling and storing harvested fruit. Disease management strategies should therefore focus on the eradication of inoculum sources that contribute to latent contamination, eradication of latent conidia on highly susceptible mature fruit and disease prediction during the preharvest period.

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Table 1. Percentage plum fruit showing grey mould (*Botrytis cinerea*) or brown rot (*Monilinia laxa*) in samples classified as defective by the Unifruco Quality Evaluation Scheme^a

Sample date	Number of fruit per sample ^b	Incidence (%)	
		<i>B. cinerea</i>	<i>M. laxa</i>
December 1996	40	92.5	7.5
January 1997	100	71.0	29.0
February 1997	79	45.6	54.4
March 1997	3	0	100
Total (1996/97 season)	222	65.0	35.0
November 1997	8	87.5	12.5
December 1997	4	75.0	25.0
January 1998	13	69.2	30.8
February 1998	6	0	100
Total (1997/98 season)	24	62.5	37.5
January 1999	35	97.1	2.9
February 1999	5	40.0	60.0
Total (1998/99 season)	40	90.0	10.0

^a Fruit of various plum cultivars, grown in the main stone fruit regions of the Western Cape province, were sampled for quality control by Unifruco. Sample sizes were statistically determined in accordance with the number of cartons in each consignment.

^b Isolations were made from fruit classified as defective due to grey mould or brown rot by personnel of Unifruco after a cold storage period simulating overseas shipment.

Table 2. Percentage nectarine and peach fruit showing grey mould (*Botrytis cinerea*) or brown rot (*Monilinia laxa*) in samples classified as defective by the Unifruco Quality Evaluation Scheme^a

Sample date	Number of fruit per sample ^b	Incidence (%)	
		<i>B. cinerea</i>	<i>M. laxa</i>
November 1996	55	96.4	3.6
December 1996	43	67.4	32.6
January 1997	1	0	100
February 1997	19	10.5	89.5
March 1997	1	0	100
Total (1996/97 season)	119	70.6	29.4
October 1997	5	100	0
November 1997	23	95.6	4.4
December 1997	1	100	0
Total (1997/98 season)	29	96.6	3.4
December 1998	22	95.5	4.5
January 1999	8	62.5	37.5
February 1999	3	0	100
Total (1998/99 season)	33	78.8	21.2

^aFruit of various peach and nectarine cultivars, grown in the main stone fruit regions of the Western Cape province, were sampled for quality control by Unifruco. Sample sizes were statistically determined in accordance with the number of cartons in each consignment.

^bIsolations were made from fruit classified as defective due to grey mould or brown rot by personnel of Unifruco after a cold storage period simulating overseas shipment.

Table 3. Mean percentage flowers^a of different plum and nectarine cultivars yielding *Botrytis cinerea* or *Monilinia laxa* after 14 days incubation at high relative humidity ($\geq 93\%$ RH)

Cultivars ^b	Symptomatic flowers (%)			
	<i>B. cinerea</i>		<i>M. laxa</i>	
	Unsterile	Sterile	Unsterile	Sterile
Early-season				
Mayglo	22.8	8.8	0.1	0.1
Mid-season				
Sunlite	30.3	20.1	0.1	0
Santa Rosa	24.2	5.2	0	0
Late-season				
Casselman	36.8	18.8	0	0
Flamekist	37.9	8.3	5.4	5.0

^a Based on the total number of flowers obtained for three consecutive seasons.

^b Nectarine cultivars: Mayglo and Sunlite orchards located in Simondium, Klein-Drakenstein and Wellington; Flamekist orchards located in Koue Bokkeveld, Prince Alfred Hamlet and Vyeboom.

Plum cultivars: Santa Rosa and Casselman orchards located in the Blaauwklippen valley, Stellenbosch.

Table 4. Development of *Botrytis cinerea* and *Monilinia laxa* in parts of surface-sterilised nectarine^a and plum^b flowers after incubation at high relative humidity ($\geq 93\%$ RH)

Fruit type	Flowers (%) yielding <i>B. cinerea</i> from:			Flowers (%) yielding <i>M. laxa</i> from:		
	Calyx	Petal	Stamen	Calyx	Petal	Stamen
Nectarine	67.1	44.8	48.3	58.2	55.1	16.3
Plum	92.4	79.2	53.5	0	0	0

^a Based on 681 and 98 nectarine flowers infected with *B. cinerea* and *M. laxa*, respectively.

^b Based on 144 and 0 plum flowers infected with *B. cinerea* and *M. laxa*, respectively.

Table 5. Mean percentage plum and nectarine fruit^a at different growth stages yielding *Botrytis cinerea* after being subjected to a differential set of treatments^b and 14 days incubation at high humidity ($\geq 93\%$ RH)

Cultivars ^c	Untreated								Paraquat-treated							
	Shuck fall		Pit hardening		2 wk before harvest		Harvest		Shuck fall		Pit hardening		2 wk before harvest		Harvest	
	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
Early-season																
Mayglo	0	0.1	0	0.2	1.1	0.9	9.6	22.0	5.9	10.0	14.7	31.1	20.2	44.9	10.9	27.1
Mid-season																
Sunlite	0	0.2	0	0	1.8	2.7	4.7	8.4	14.8	12.7	6.9	24.0	29.6	54.2	17.6	35.3
Santa Rosa	0.7	0.7	1.3	1.3	0.7	0.7	2.7	0	10.7	25.7	0.7	11.3	3.3	8.7	3.3	7.3
Late-season																
Casselman	0	0	0	0	0	0	0	0	2.3	8.3	0.7	5.3	5.3	5.3	0.7	2.7
Flamekist	0.4	0	0	0	0	0.2	0.9	0.9	7.6	5.2	8.4	14.7	22.9	13.6	5.1	7.1

^a Based on the total number of fruit sampled at different growth stages for three consecutive seasons.

^b Fruit were divided in four groups. One group was sterilised (S) (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried. The second group was sterilised, immersed in a 3% paraquat solution for 30 seconds, rinsed in sterile deionised water and air-dried. The third group was left unsterile (NS) and received no paraquat, whereas the fourth group was left unsterile but was treated with paraquat. The screens were placed in ethanol-disinfected moist chambers and were incubated at 23°C under a 12 h light schedule to induce the development of *B. cinerea*.

^c Nectarine cultivars: Mayglo and Sunlite orchards located in Simondium, Klein-Drakenstein and Wellington; Flamekist orchards located in Koue Bokkeveld, Prince Alfred Hamlet and Vyeboom.

Plum cultivars: Santa Rosa and Casselman orchards located in the Blaauwklippen valley, Stellenbosch.

Table 6. Development of *Botrytis cinerea* and *Monilinia laxa* from sites on surface-sterilised nectarine and plum fruit at different growth stages after incubation at high relative humidity ($\geq 93\%$ RH)

Growth stage	Fruit (%) yielding <i>B. cinerea</i> from: ^a			Fruit (%) yielding <i>M. laxa</i> from: ^b		
	Peduncle	Tip	Cheek	Peduncle	Tip	Cheek
Shuck fall	47.0	11.4	51.9	42.9	28.6	42.9
Pit hardening	44.5	19.0	51.1	60.0	10.0	50.0
2 wk before harvest	40.2	12.5	62.6	20.6	9.5	86.5
Harvest	35.6	28.4	65.3	24.9	14.2	79.1

^aBased on the infection of 264, 137, 353 and 225 nectarine and plum fruits from the shuck fall, pit hardening, 2 wk before harvest and harvest stages, respectively.

^bBased on the infection of 7, 10, 126 and 225 nectarine and plum fruits from the shuck fall, pit hardening, 2 wk before harvest and harvest stages, respectively.

Table 7. Mean percentage plum and nectarine fruit^a at different growth stages yielding *Monilinia laxa* after being subjected to a differential set of treatments^b and 14 days incubation at high humidity ($\geq 93\%$ RH)

Cultivars ^c	Untreated								Paraquat-treated							
	Shuck fall		Pit hardening		2 wk before harvest		Harvest		Shuck fall		Pit hardening		2 wk before harvest		Harvest	
	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
Early-season																
Mayglo	0	0	0	0	0	0	0.2	0	0.2	0	0	0.2	0.2	0.2	0	0.2
Mid-season																
Sunlite	0.2	0.1	0	0	4.4	7.3	7.6	12.4	0.3	0.6	0	0	9.1	8.7	5.6	11.1
Santa Rosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Late-season																
Casselman	0	0	0	0	0	0	0	0	0	0	1.3	1.3	1.3	2.0	1.2	0
Flamekist	0	0	0.9	0.2	1.1	4.9	18.2	19.8	0	0.1	0.7	0.2	10.4	13.6	14.5	24.0

^a Based on the total number of fruit sampled at different growth stages for three consecutive seasons.

^b Fruit were divided in four groups. One group was sterilised (S) (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried. The second group was sterilised, immersed in a 3% paraquat solution for 30 seconds, rinsed in sterile deionised water and air-dried. The third group was left unsterile (NS) and received no paraquat, whereas the fourth group was left unsterile but was treated with paraquat. The screens were placed in ethanol-disinfected moist chambers and were incubated at 23°C under a 12 h light schedule to induce the development of *M. laxa*.

^c Nectarine cultivars: Mayglo and Sunlite orchards located in Simondium, Klein-Drakenstein and Wellington; Flamekist orchards located in Koue Bokkeveld, Prince Alfred Hamlet and Vyeboom.

Plum cultivars: Santa Rosa and Casselman orchards located in the Blaauwklippen valley, Stellenbosch.

Table 8. Mean percentage fruit^a of different plum and nectarine cultivars developing postharvest^b grey mould (*Botrytis cinerea*) or brown rot (*Monilinia laxa*)

Cultivars ^c	Symptomatic fruit (%)	
	<i>B. cinerea</i>	<i>M. laxa</i>
Early-season		
Mayglo	3.1	0.1
Mid-season		
Sunlite	4.2	3.7
Santa Rosa	0.7	0
Late-season		
Casselman	0	0
Flamekist	0.4	8.9

^a Based on the total number of fruit examined in three consecutive seasons.

^b Fruit were sampled at harvest (200 per orchard per season for three consecutive seasons [1996-1999]), packed in packing cartons and kept at conditions simulating overseas shipment and marketing (nectarines 4 wk at -0.5°C followed by 1 wk at 23°C at ±56% RH; plums 2 wk at -0.5°C, 2 wk at 10°C followed by 1 wk at 23°C at ±56% RH).

^c Nectarine cultivars: Mayglo and Sunlite orchards located in Simondium, Klein-Drakenstein and Wellington; Flamekist orchards located in Koue Bokkeveld, Prince Alfred Hamlet and Vyeboom.

Plum cultivars: Santa Rosa and Casselman orchards located in the Blaauwklippen valley, Stellenbosch.

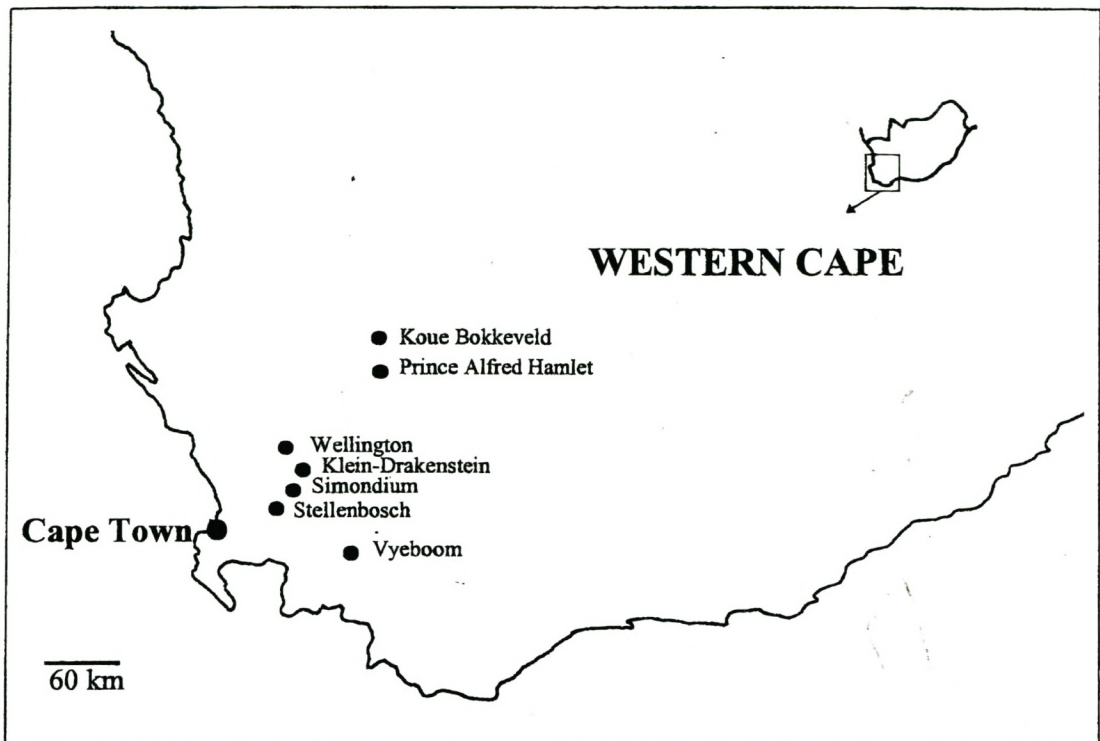


Figure 1. Localities in the Western Cape province where flowers and fruit were collected from different nectarine and plum orchards.

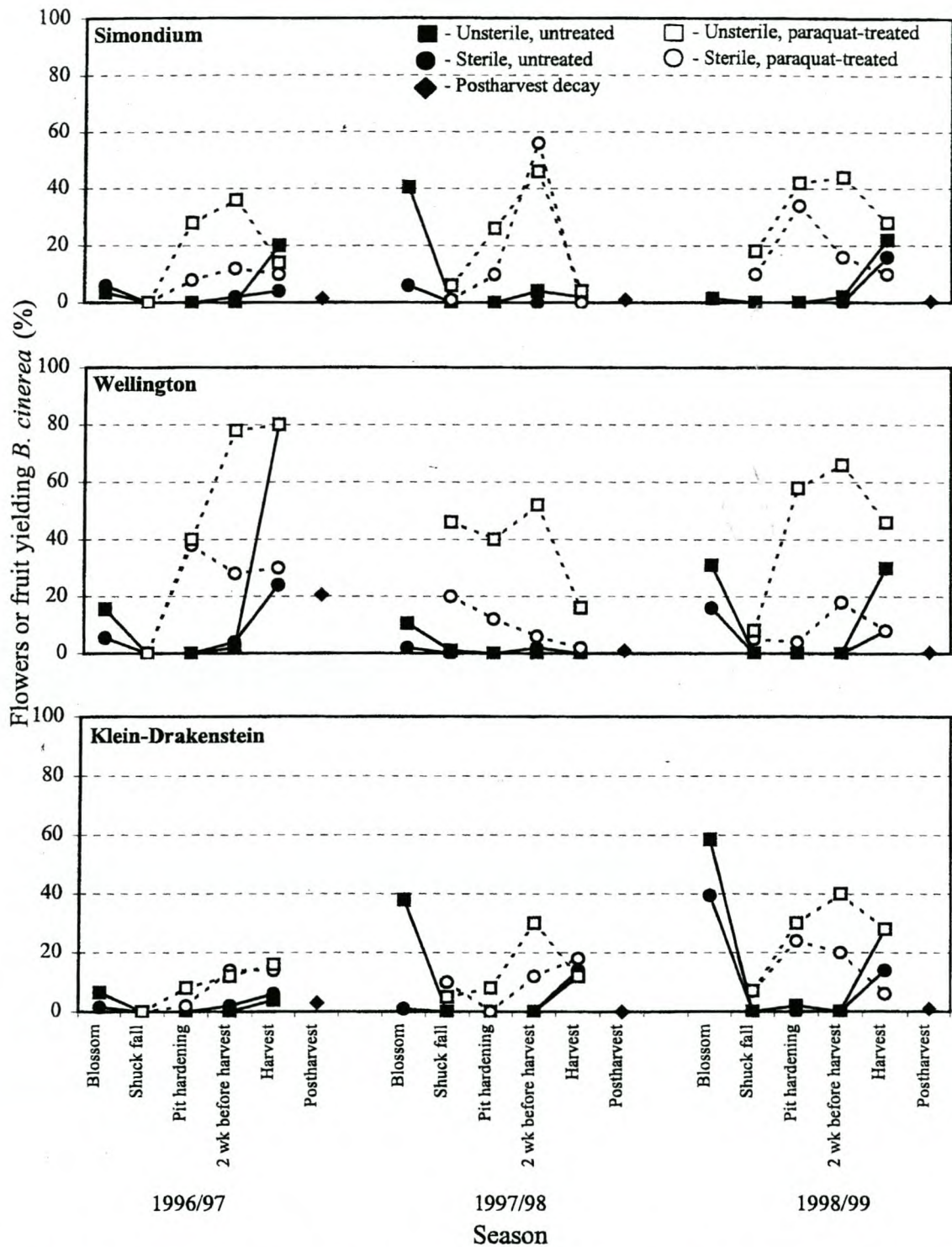


Figure 2. Percentage flowers or fruit obtained at different growth stages from three nectarine (cultivar Mayglo) orchards yielding *Botrytis cinerea* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

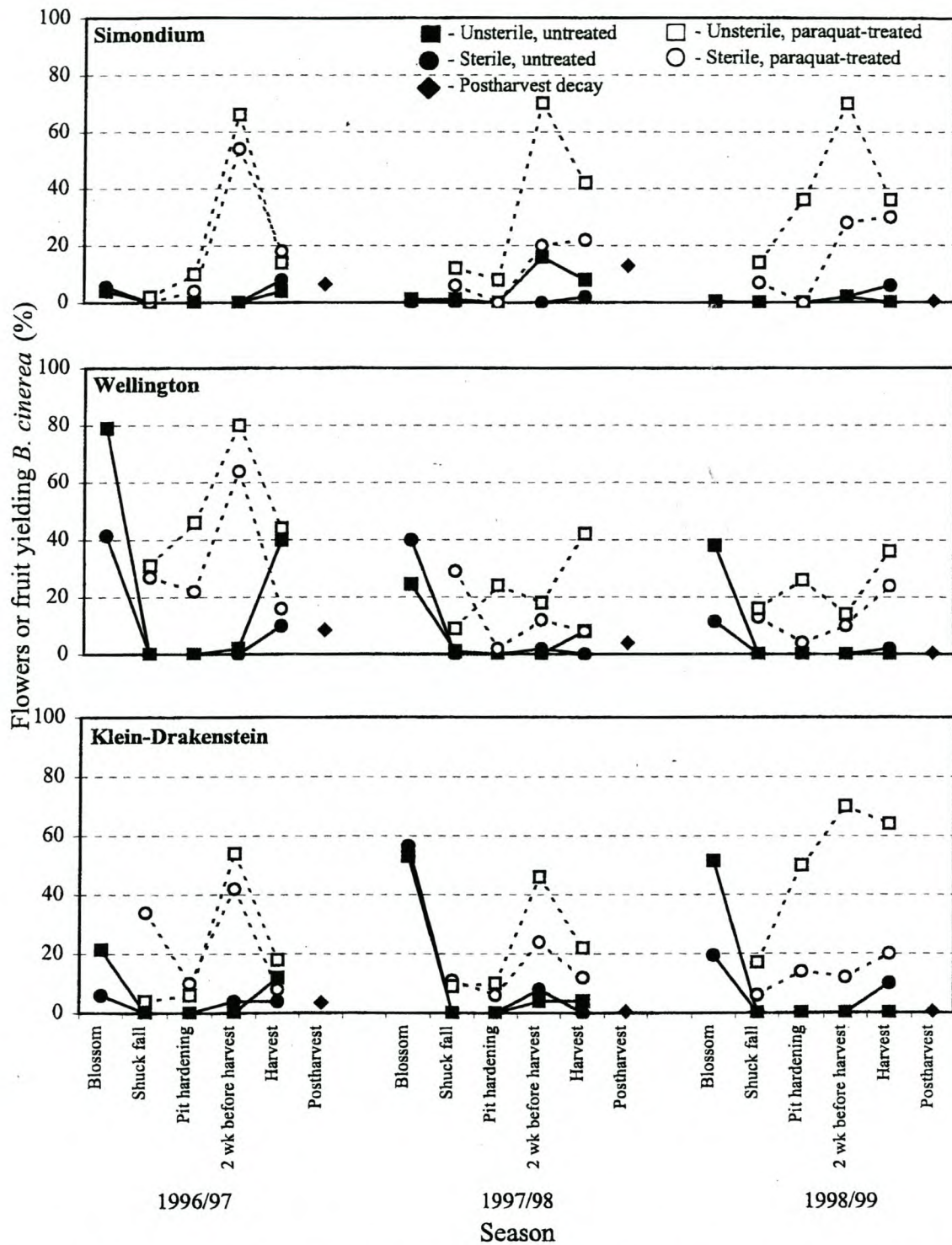


Figure 3. Percentage flowers or fruit obtained at different growth stages from three nectarine (cultivar Sunlite) orchards yielding *Botrytis cinerea* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

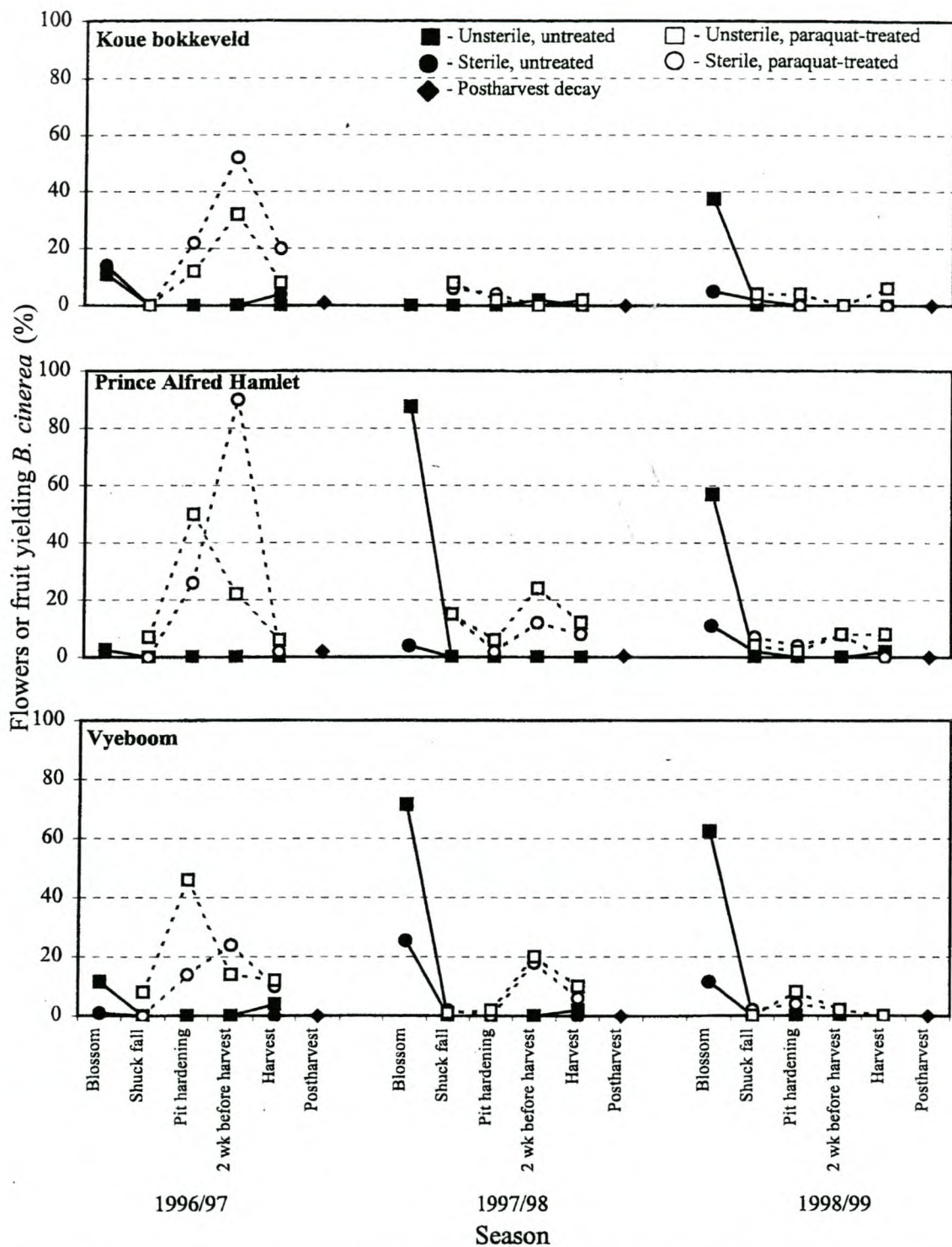


Figure 4. Percentage flowers or fruit obtained at different growth stages from three nectarine (cultivar Flamekist) orchards yielding *Botrytis cinerea* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

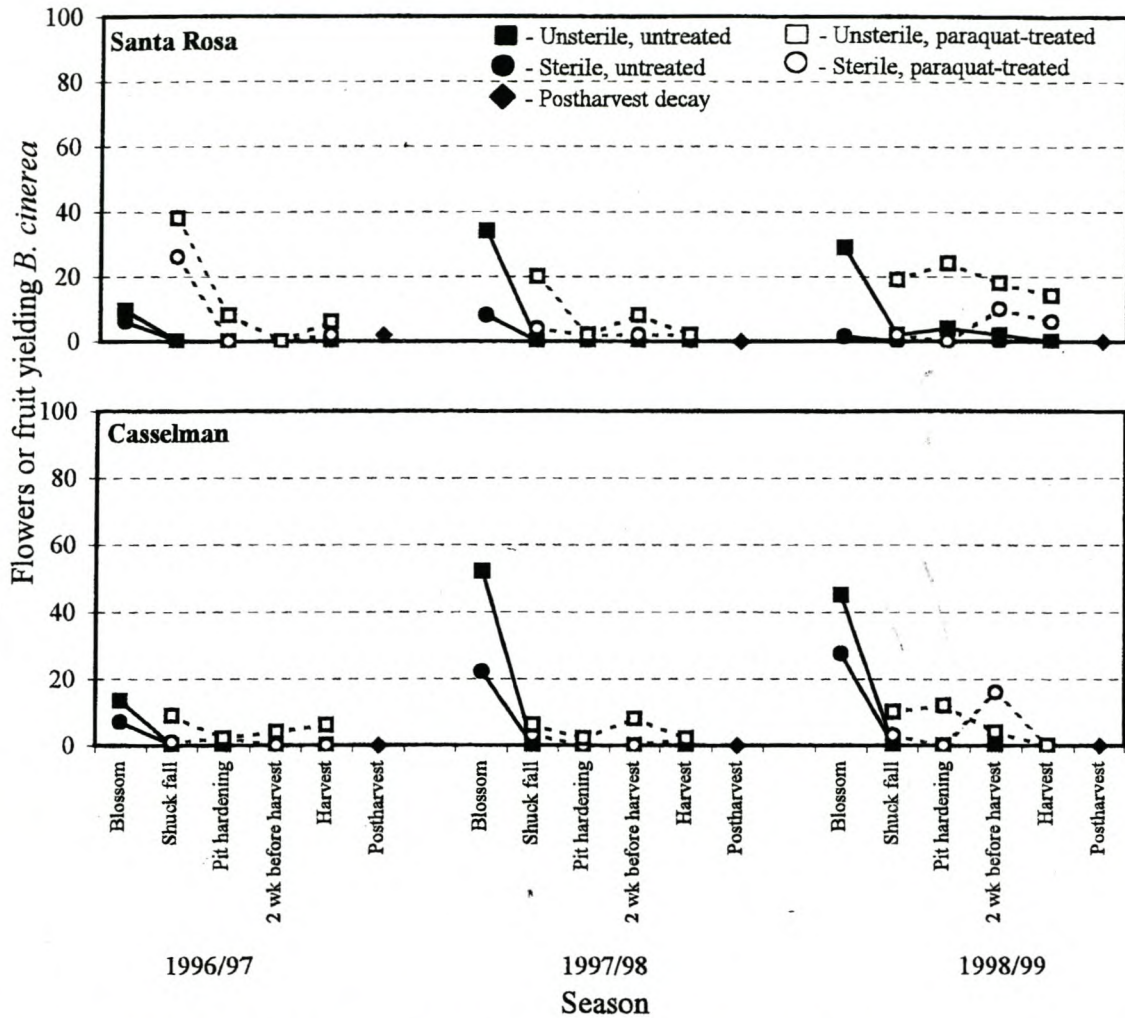


Figure 5. Percentage flowers or fruit obtained at different growth stages from two plum (cultivars Santa Rosa and Casselman) orchards yielding *Botrytis cinerea* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

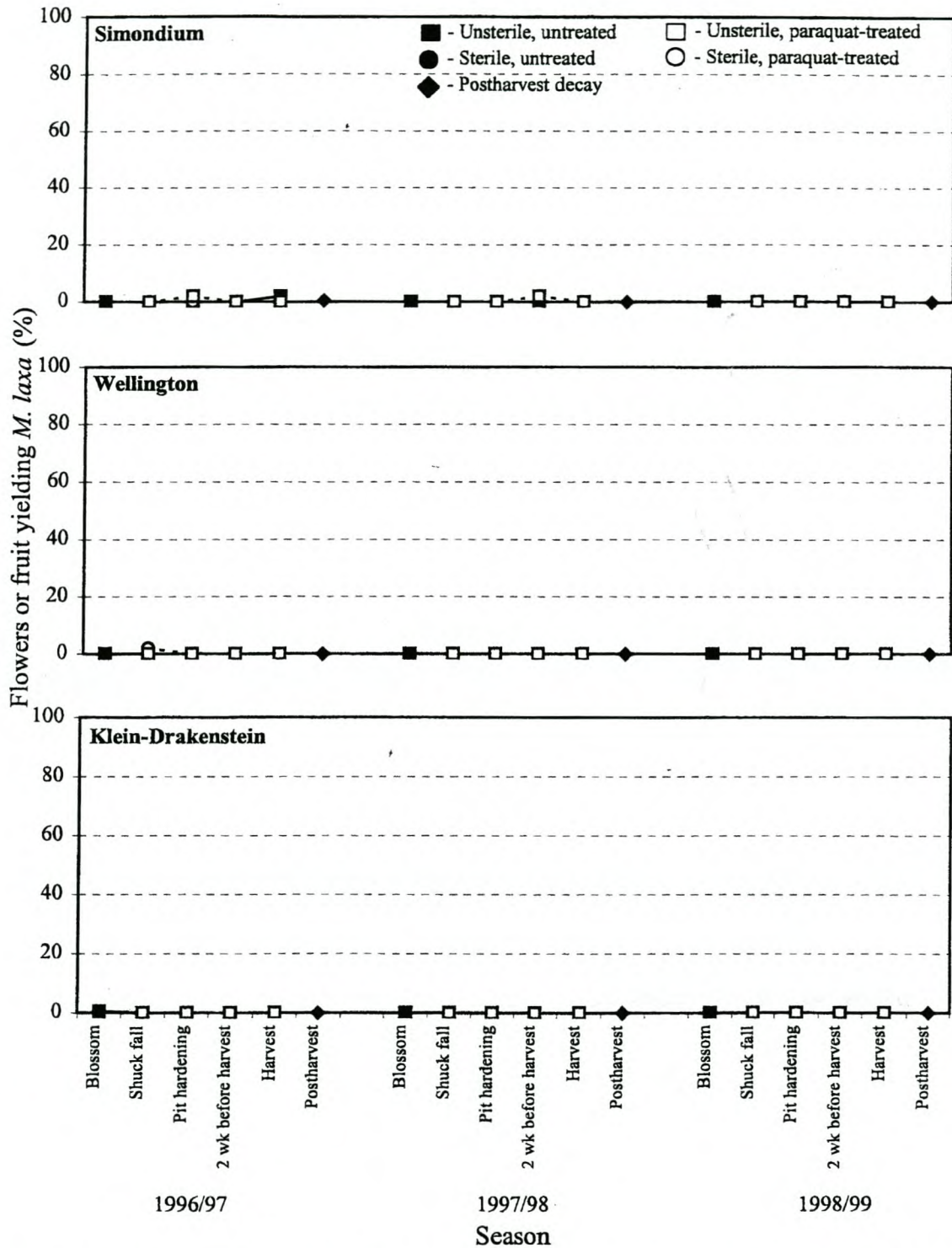


Figure 6. Percentage flowers or fruit obtained at different growth stages from three nectarine (cultivar Mayglo) orchards yielding *Monilinia laxa* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

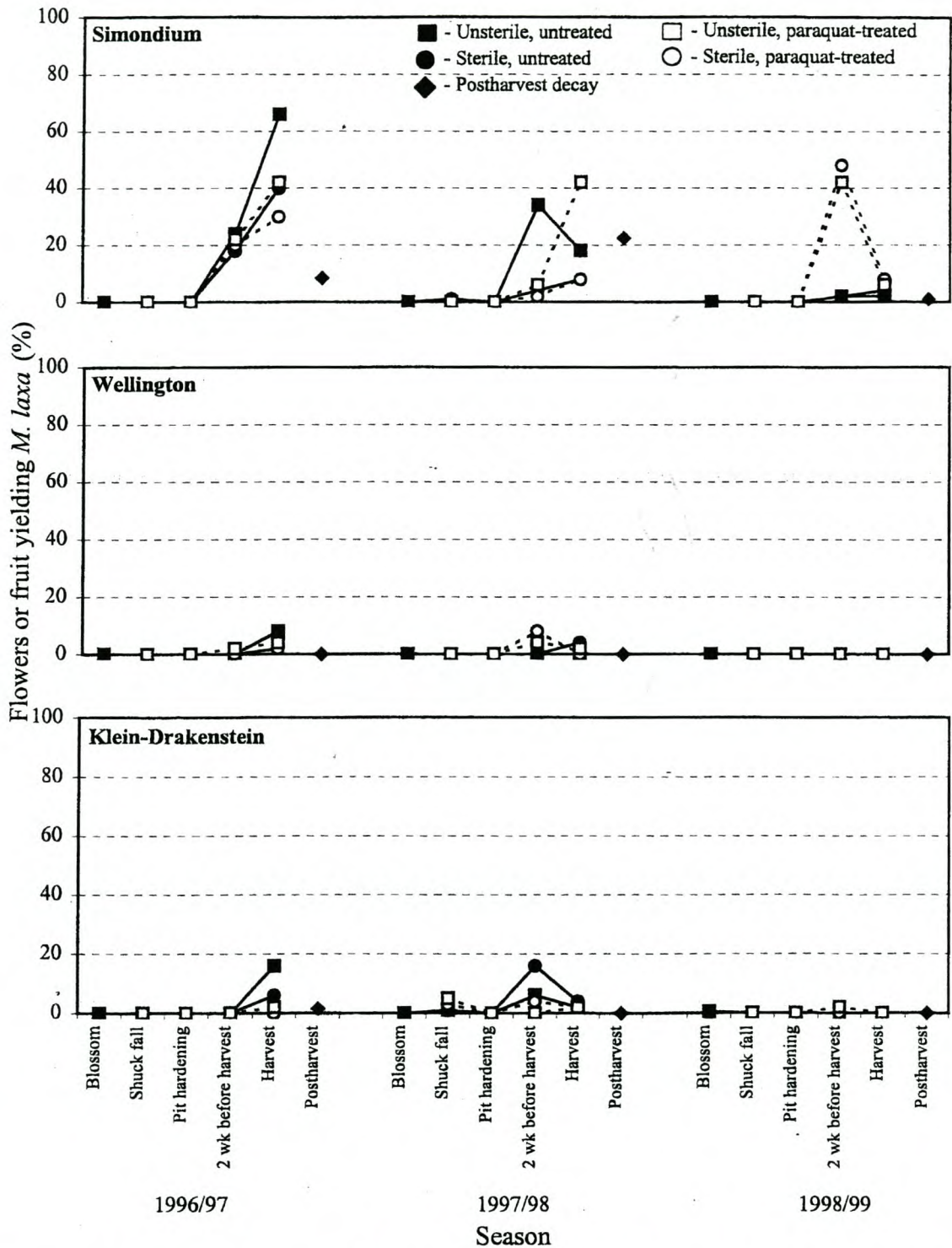


Figure 7. Percentage flowers or fruit obtained at different growth stages from three nectarine (cultivar Sunlite) orchards yielding *Monilinia laxa* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

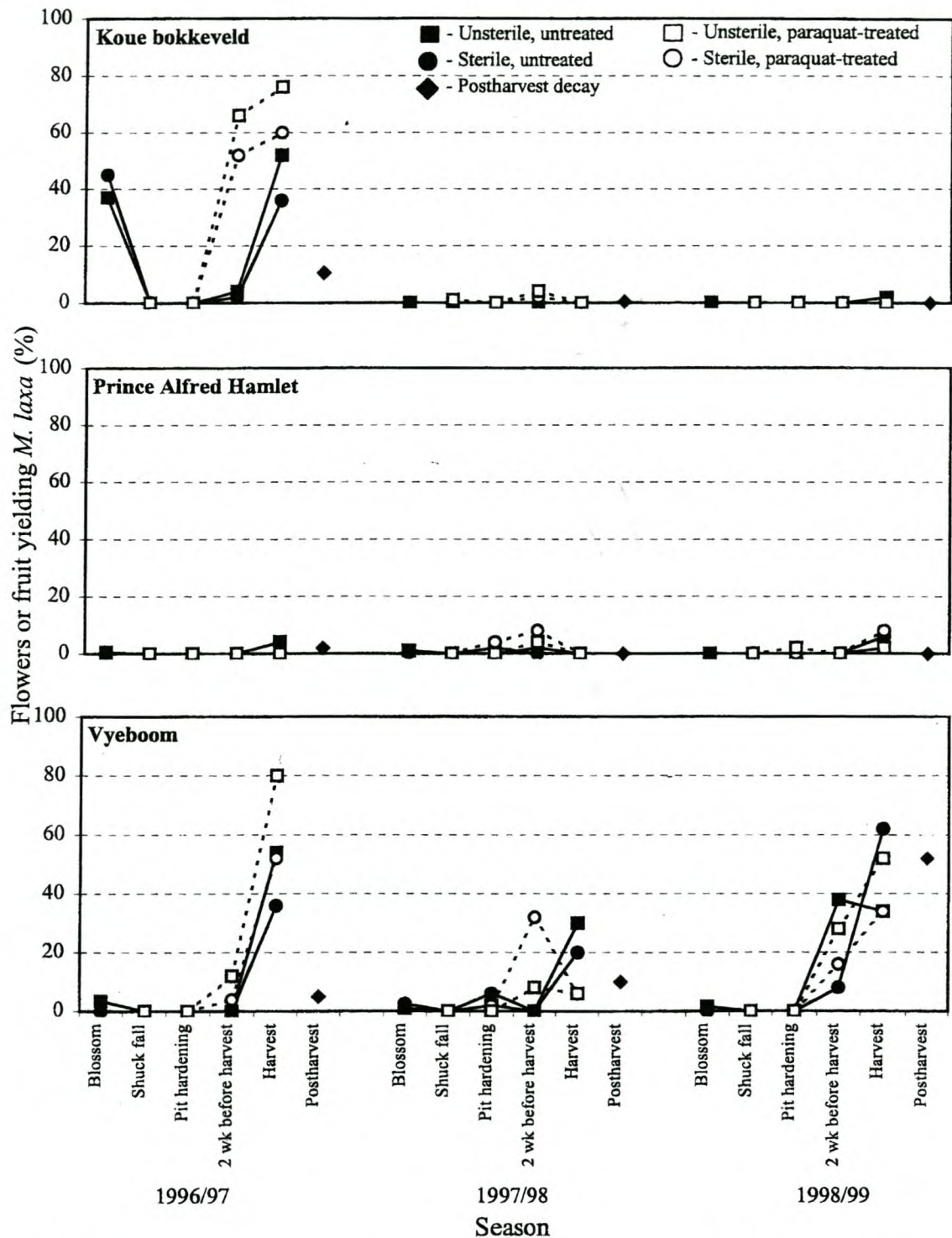


Figure 8. Percentage flowers or fruit obtained at different growth stages from three nectarine (cultivar Flamekist) orchards yielding *Monilinia laxa* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

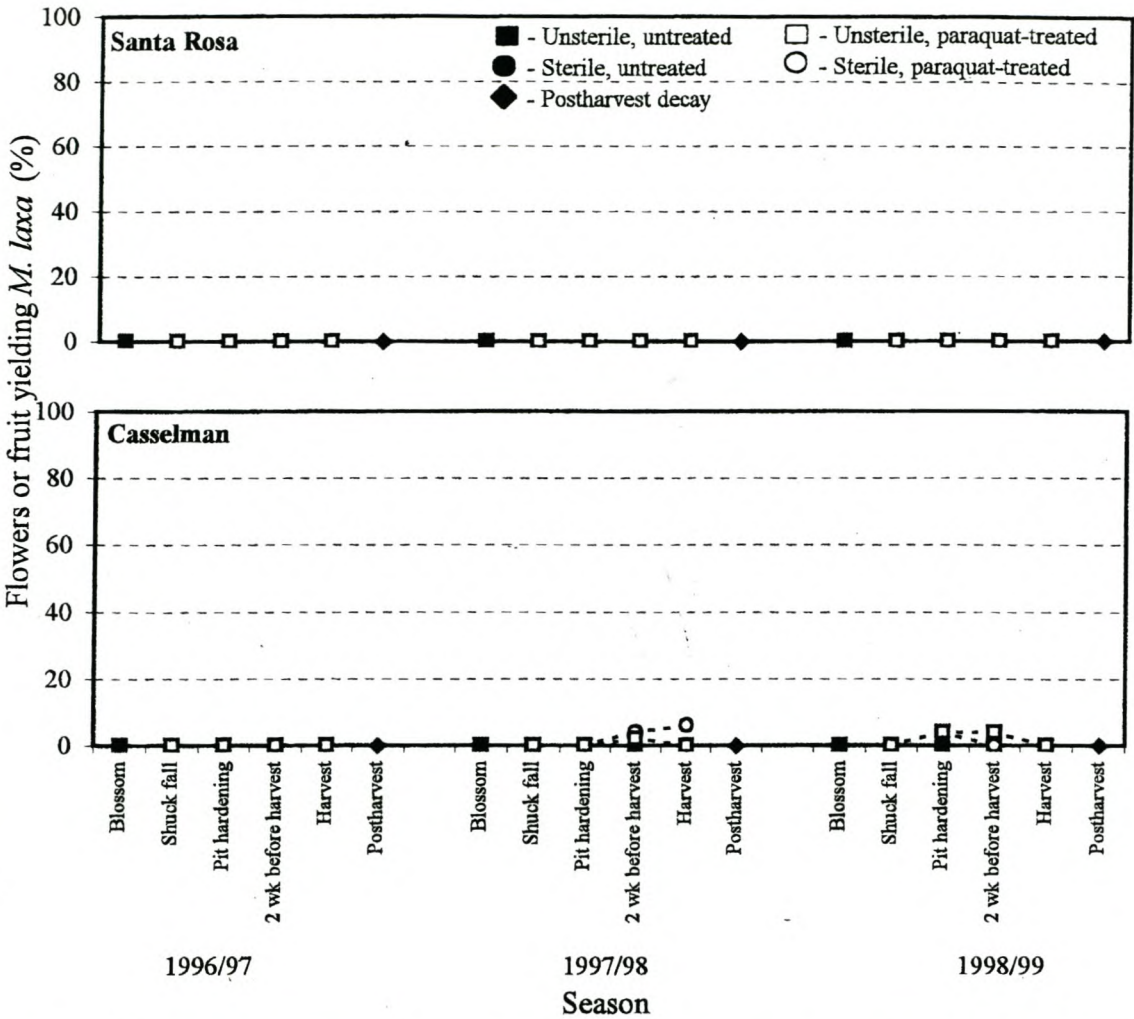


Figure 9. Percentage flowers or fruit obtained at different growth stages from two plum (cultivars Santa Rosa and Casselman) orchards yielding *Monilinia laxa* after being subjected to a differential set of treatments (see legend) and 14 days incubation at high relative humidity (>93% RH).

3. BEHAVIOUR OF SOLITARY CONIDIA OF *MONILINIA LAXA* AND DISEASE EXPRESSION ON NECTARINE FRUIT

ABSTRACT

Nectarine fruit (cultivar Flamekist) at pit hardening, 2 wk before harvest, harvest stage and after cold storage (4 wk at -0.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH) were dusted with dry conidia of *Monilinia laxa* in a settling tower. The fruits were incubated for periods ranging from 3 to 48 h at high relative humidity ($\geq 93\%$, humid fruit) or covered with a film of water (wet fruit). Behaviour of the solitary conidia was examined with an epifluorescence microscope on skin segments stained in a differential stain containing fluorescein diacetate, aniline blue and blankophor. The ability of solitary conidia to colonise the fruit surface, penetrate fruit skins and to induce disease expression was determined by using a differential set of tests. For these tests, fruit were surface-sterilised (30 s in 70% ethanol) or left unsterile. From each group, fruit were selected for isolation (skin segment test), immersed in a 3% paraquat solution (paraquat fruit test) or left untreated (sound fruit test). The tests showed that at the pit hardening stage, fruit skins were not penetrated under both wetness regimes, latent infections were not established and fruits reacted resistant to disease expression. However, the barrier capacity of fruit skins decreased as fruits ripened. The disease reaction on ripening fruit was furthermore influenced by wetness. The change in fruit susceptibility on maturing fruit could be ascribed to changes of components of the fruit skin. The behaviour of inoculum on fruit surfaces complemented these findings. On humid fruit germ tube growth was invariably restricted on all fruits, but germ tubes grew slightly longer on mature than on immature fruit. Germination rates were higher on wet fruit, but were not affected by host phenology. However, germ tubes grew more restricted on immature fruit, compared with the extensive growth on mature fruit. Appressorium formation and direct penetration were not observed on any of the fruits. Germ tubes penetrated fruit predominantly through stomata, lenticels and microfissures in the fruit skin. The tendency to grow towards a specific site and to penetrate was also influenced by fruit phenology. On fruit at pit hardening and 2 wk before harvest, germlings were inclined to grow primarily towards

stomata, and predominantly entered these sites. This tendency changed when fruit were inoculated at harvest. On these fruits germings were attracted in nearly equal proportions by stomata, lenticels and microfissures and had entered them in nearly equal proportions. The findings indicate that short-term latency and latent contamination are important factors contributing to *M. laxa* decay of nectarine fruit.

INTRODUCTION

Blossom blight and brown rot of stone fruit can be caused by *Monilinia fructicola* (Wint.) Honey or *M. laxa* (Aderh. & Ruhl.) Honey. In America and Australasia *M. fructicola* is mostly associated with the fruit rot phase, and *M. laxa* with the blossom and spur blight phase of the *Monilinia* disease syndrome (Hewitt & Leach, 1939; Ogawa *et al.*, 1954; Ogawa & English, 1960; Ogawa *et al.*, 1975; Byrde & Willetts, 1977; Ogawa *et al.*, 1983; Jones & Sutton, 1996). *Monilinia fructicola*, considered to be the more virulent of the brown rot fungi (Hewitt & Leach, 1939; Ogawa & English, 1960; Penrose *et al.*, 1976) is absent in Europe (Byrde & Willetts, 1977; Willetts & Bullock, 1993) and South Africa (Fourie & Holz, 1985a; Schlagbauer & Holz, 1987; Part 2), and in these countries all stages of this disease are caused by *M. laxa* (Byrde & Willetts, 1977; Fourie & Holz, 1985a; 1985b; 1987a; 1987b; Schlagbauer & Holz, 1989a; 1989b; Tamm & Flückiger, 1993; Willetts & Bullock, 1993; Tamm, 1994; Tamm *et al.*, 1995; Part 2).

With *M. fructicola*, ascospores are considered as an important part of the primary inoculum (Roberts & Dunegan, 1926; Byrde & Willetts, 1977; Tate & Corbin, 1978; Hong *et al.*, 1996; Hong & Michailides, 1998). Ascospores produced from mummies on/in the soil and conidia shed from infected areas remaining on the tree from a previous year, provide inoculum for infection of flowers, which has been associated with latent infection (Wade, 1956a; Jenkins & Reinganum, 1965; Tate & Corbin, 1978). Latent infections appear to be of great significance under humid temperature conditions because many develop into vigorously sporulating lesions that envelop maturing stone fruits (Jenkins & Reinganum, 1965; Wade & Cruickshank, 1992b; Northover & Cerkaskas, 1994; Emery *et al.*, 2000). Nonabscised, aborted fruits in the tree and thinned fruits on the ground are other important sources of conidia as fruits approach maturity (Landgraf & Zehr, 1982; Biggs & Northover, 1985; Hong *et al.*, 1997). Fruits that rot before harvest and are covered by sporodochia, serve as a source

of inoculum for the potential infection of the very susceptible mature fruits. Propagules of *M. fructicola* were found covering sound fruit only in orchards where sporulation had occurred on nearby infected fruit (Phillips & Harvey, 1975). Infection of blossoms and fruits by this fungus is well recorded (Wormald, 1919; Curtis, 1928; Smith, 1936; Hewitt & Leach, 1939; Weaver, 1950; Wade, 1956a; Wade, 1956b; Ogawa & English, 1960; Corbin, 1963; Jenkins & Reinganum, 1965; Corbin *et al.*, 1968; Kable, 1969a; Kable, 1969b; Hall, 1971; Kable, 1971; Byrde & Willetts, 1977; Tate & Corbin, 1978; Ritchie, 1983a; Biggs & Northover, 1988a; Biggs & Northover, 1988b; Biggs & Northover, 1989; Brown & Wilcox, 1989; Northover & Biggs, 1990; Adaskaveg *et al.*, 1991; Cruickshank & Wade, 1992a; Wade & Cruickshank, 1992a; Cruickshank & Wade, 1992b; Wade & Cruickshank, 1992b; Willetts & Bullock, 1993; Elmer & Gaunt, 1994; Northover & Cerkaskas, 1994; Northover & Biggs, 1995; Biggs *et al.*, 1997; Hong *et al.*, 1998; Bostock *et al.*, 1999).

Monilinia laxa, on the other hand, rarely forms apothecia (Willetts & Harada, 1984). The fungus overwinters on mummified fruits, fruit stalks, scars, buds, as well as in cankerous lesions (Batra, 1985). *Monilinia laxa* infects by means of conidia that may be produced throughout the year. There is however only limited quantitative information on the importance of the different inoculum sources and infection of blossoms and fruit by this pathogen. In stone fruit orchards of the Western Cape province of South Africa, brown rot, and not blossom and spur blight, is the most destructive phase of the *M. laxa* disease syndrome. A recent study (Part 2), which investigated symptom expression by both new and established *M. laxa* infections in different stone fruit orchards over a three-year period, showed that the pathogen was virtually absent from flowers and occurred only sporadically. Immature fruit were generally pathogen free and symptom expression only occurred on maturing fruit. Contrary to *M. fructicola*, long-term latency does not seem to play a prominent role in *M. laxa* fruit rot (Kable, 1971; Schlagbauer & Holz, 1989a; 1989b; Part 2). These findings suggest that conidia of *M. laxa* are generally produced in orchards when fruits are approaching maturity and can only penetrate and infect maturing fruit.

Conidia of *Monilinia* spp. are primarily dispersed by air currents, splashing water droplets (Byrde & Willetts, 1977; Ogawa *et al.*, 1983) and by insects (Ogawa, 1957; Kable, 1969a; Tate & Ogawa, 1975). Conidia are therefore carried either in groups, or as single cells. Studies with *Botrytis cinerea* showed that of those conidia dispersed by rain drops,

very few become wet enough to enter the droplets and that the majority are carried on the droplet surface as a dry coating. Raindrops may therefore deposit conidia carried on their surfaces as single units onto fruit during runoff (G. Holz, pers. comm.). These findings imply that in the field, infection may not always be caused by clusters of conidia, but more often by solitary conidia. Relatively little information has been published on the interactions between solitary conidia, temperature, and wetting duration for *M. laxa* on stone fruit. The aims of this study were to use an inoculation method that simulates natural infection by airborne conidia, to study germination and germ tube behaviour on the surface of humid or wet nectarine fruit at different phenological stages, and to record infection and the host response. Preliminary reports of this study have been published (Fourie & Holz, 1999a; 1999b).

MATERIALS AND METHODS

Fruit. A nectarine orchard (cultivar Flamekist) with a history of low levels of brown rot incidence was selected in the Witzenberg valley (Koue Bokkeveld). Four weeks prior to the pit hardening stage, a section of the orchard was demarcated and no fungicides were applied. Sound, unblemished fruit were selected at pit hardening, 2 wk before harvest, and at the harvest stage from the latter trees. Fruit obtained at harvest stage were either used, or kept under conditions simulating overseas shipment and marketing before being used (4 wk at -0.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH). Before usage, fruits were surface sterilised (30 s in 70% ethanol, 2 min in 2% sodium hypochlorite, 30 s in 70% ethanol), packed on sterile, epoxy-coated steel mesh screens (53 x 28 x 2 cm) and allowed to air-dry. Picking wounds at or near the peduncle-end were covered with petroleum jelly. In order to recognize the inoculated cheek of the fruit at a later stage, a 0.5 cm mark was made near the peduncle-end with a soft-tipped koki pen. Preliminary studies showed no phytotoxic effect. Before inoculation, surface sterilised fruit were kept for 24 h in ethanol-disinfected perspex (Cape Plastics) chambers (60 x 30 x 60 cm) at 22°C and $\pm 56\%$ RH to allow re-establishment of surface nutrients.

Inoculation. A virulent *M. laxa* isolate, sensitive to iprodione and benomyl and obtained from a naturally-infected nectarine fruit, was maintained in the laboratory at 22°C on a synthetic agar medium amended with sugars, minerals and malic acid at concentrations occurring in grape berry exudates (1.85 g glucose; 1.95 g fructose; 0.25 g sucrose; 0.15 g

malic acid; 5 g peptone; 5 g sodium chloride; 15 g agar; and 2 g yeast extract per litre deionised water), or was kept on malt extract agar (MEA) slopes at 5°C in the dark. Inoculum was prepared by inoculating ripe surface-sterilised nectarines with mycelium discs, or conidia, obtained from fresh cultures growing on potato dextrose agar (PDA). Inoculated fruit were incubated for 10 to 14 days at 22°C on screens in moist perspex chambers (see below) to allow infection, colonisation and profuse sporulation by *M. laxa*. The mummified fruit were then kept in dry chambers at $\pm 56\%$ RH. For inoculation, a mummified fruit was placed on a shelf 10 cm below the ceiling of a spore settling tower (1.5 x 1.0 x 1.5 m [length x width x height]). Conidia were blown for 1 s from the mummy with a pressure pump (Rietchle VTE 3 [3.5-4.2 m³/h]) and the lid in the ceiling closed. The conidia were allowed 10 min to settle onto the fruit that were positioned on three screens on the floor of the tower. Petri dishes with PDA were placed among the fruit 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 6 ethanol-disinfected perspex chambers lined with a sheet of chromatography paper (45 x 57 cm), with the base resting in deionised water to establish high relative humidity ($\geq 93\%$ RH). Each chamber contained five screens carrying 31 fruits, and each screen in a chamber was randomly assigned one of five incubation periods (first incubation cycle). These were 3, 6, 12, 24 and 48 h post inoculation (hpi). Each chamber was considered as a block and the screens were randomised within each chamber. In nature, frequent runoff of raindrops and a half-day or more of sunny weather may lead to different durations of continuous fruit wetness, or of high humidity on the fruit surface. Therefore, in three of the chambers, fruits were overlaid with sterile paper towels wetted with sterile deionised water. Fruits in the other three chambers were left dry. These conditions provided two different wetness regimes with different durations for the pathogen; dry conidia on dry fruit under high relative humidity (humid fruit), and conidia exposed to a film of water on the fruit surface (wet fruit). The chambers containing the fruit were incubated at 22°C with a 12 h photoperiod daily. After a set incubation period, the appropriate screens with fruit were removed from the chambers, the paper towels removed and the fruits air-dried before they were used for histological studies, and for the determination of surface colonisation, skin penetration and disease expression.

Histology. One fruit (i.e. 3 fruit per wetness regime per incubation period) was randomly selected from each screen per chamber. Thin hand-sectioned pieces (5 x 5 mm) of skin consisting of the cuticle, epidermis, and a few cell layers, were cut with a razor blade from the inoculated cheek of the fruit. The sections were stained for 5 min in a differential stain containing fluorescein diacetate ([FDA] Sigma Chemical Co., St. Louis, MO), aniline blue ([AB] B.D.H. Laboratory Chemicals Division, Poole, England) and blankophor ([BP] Bayer), 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/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 prepared by mixing 25 μl of FDA stock solution with 1 ml of AB/BP stock solution in a 1.5 ml polypropylene Eppendorf tube, which was then kept on crushed ice. Conidial germination, germ tube and hyphal growth, apressorium formation, penetration sites, host responses and viability of fungal structures were examined with the aid of 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 filter No. 02, 06 and 18. Protoplasts of dead cells were blue-black (filter No. 06, 18), whereas cells without protoplasts fluoresced white (filter No. 02) or yellow (filter No. 18) (O'Brien & McCully, 1981). Formation of phenolic substances became visible by irradiation with ultra-violet light (filter No. 02) resulting in a bright bluish fluorescence (Langcake, 1981). Suberised cell walls showed a light blue or bright yellow fluorescence (Hill, 1985). Microfissures and -cracks in the skin became visible due to accumulation of phenolic substances, lignification and suberisation of surrounding cell walls. Lenticels were brown with yellow fluorescence of suberised cells.

Surface colonisation, skin penetration and disease expression. The ability of the solitary conidia to colonise the fruit surface, to penetrate the fruit skins, and to induce disease expression during each incubation period was determined by using a differential set of tests. For these tests, fruit on the screens from each chamber were divided into two groups. One group on each screen was surface-sterilised (30 s in 70% ethanol), while the second group was left unsterile. Five fruit from each group on each screen were selected for isolation (skin segment test). Nine epidermal tissue segments (5 x 7 mm) (45 segments per treatment) were

cut from the inoculated cheek of each fruit, placed with the cuticle upward on PDA amended with 40 mg/l streptomycin sulfate (PDAS) and incubated at 23°C (second incubation cycle). Another 15 fruit from each group on each screen were left untreated (sound fruit test). The remainder of the fruit on the screens were immersed in a 3.1% paraquat (WPK Paraquat, WPK Agricultural) solution for 30 seconds, rinsed in sterile deionised water and air-dried (paraquat fruit test). Fruit were replaced on the screens, and the screens were transferred to dry perspex chambers ($\leq 56\%$ RH) and kept under laboratory conditions (23°C under a 12 h light schedule) (second incubation cycle). These treatments provided conditions that facilitated disease expression during the second incubation cycle by different inocula on the test material. In the non-sterilised treatment, disease expression on segments, or fruit, was the result of penetration by germings that had penetrated the cuticle under natural host resistance during the two incubation periods. Surface sterilisation after the first incubation cycle completely eliminated the pathogen from the fruit surface, and allowed the development of only germings that had penetrated the cuticle during this incubation period. Paraquat terminated host resistance in the outer cell layers and consequently promoted the development of epiphytic inoculum, and endophytic inoculum (Baur *et al.*, 1969; Cerkaskas & Sinclair, 1980; Pscheidt & Pearson, 1989). This treatment therefore enhanced the development of latent infection on surface-sterilised fruit. The segments and fruits were regularly monitored for the development of *M. laxa*, and numbers yielding the pathogen were recorded after 14 days. The number of sporulating segments or fruit recorded in each experiment were used to quantify surface colonisation, skin penetration and disease expression.

Statistical analyses. Experimental design of experiments, each of which was repeated twice, was a completely randomised split-plot design and analyses of variance were done using SAS. Regression analyses were performed to investigate possible significant trends in interactions or main effects. Slopes and intercepts of regression lines were compared using Student's *t*-LSD ($P < 0.05$) (Snedecor & Cochran, 1980). Significance values of the regression line slopes were calculated, with $P < 0.05$ providing strong evidence against the H_0 -hypothesis that no change occurred over time. Analysis of variance of the percentage germination and germ tube length were done using SAS. A non-linear natural growth function [$y = A \times \exp(B / x)$] (Hoerl, 1954) was fitted to the data and trends (coefficients) compared using Student's *t*-LSD ($P < 0.05$).

RESULTS

Conidial behaviour. Analyses of variance were done on the germination percentages and germ tube lengths measured during the histological study of the fruit subjected to the various treatments (Table 1 and 2). Non-linear growth curves were fitted to the data and the various trends for percentage germination and germ tube length over incubation time plotted in Fig. 1A-F. Based on fluorescence microscopy of the stained segments, it was obvious that the airborne inoculum consisted of conidia only, since no hyphal fragments were observed. Conidia were consistently deposited on fruit surfaces as single cells, and not in pairs or groups. The average number of conidia recorded per segment for successive inoculations ranged from 50 to 249, with an average conidial density of 5.3 (SD = 2.47) conidia per mm² fruit surface. Although depositions were regulated by counts on PDA plates, it varied markedly between successive inoculations. Conidia used at each inoculation were highly viable and germinated freely on PDA. Germination on PDA usually reached 98 to 100% at 6 hpi. Germination proceeded at a lower rate on fruit than on PDA and followed distinct trends, which were regulated by fruit phenology and wetness regime. Germ tubes protruded at 3 hpi on fruit under both wetness regimes (Fig. 1A-C), but germination rate was significantly ($P < 0.05$) slower on humid than wet fruit. On humid fruit, germination rates tended to be low during the 3 to 6 hpi period, but then increased to approximately 60% at 24 hpi, after which it levelled off. An exception was found at the 2 wk before harvest (Fig. 1B) stage, when rates were exceptionally low during the 3-12 hpi period and tended to remain low for the duration of the incubation period. Germination generally proceeded rapidly on wet fruit. Germination however peaked earlier (12 hpi) on ripening fruit (Fig. 1B,C) than on fruit at pit hardening (Fig. 1A).

Wetness had no influence on the number of germ tubes formed and germ tube branching (Table 3). Conidia germinated forming predominantly one unbranched germ tube. However, on ripening fruit a tendency for the formation of more than one germ tube and branches was noted. Germ tube growth, on the other hand, was markedly affected by wetness regime and fruit phenology. Growth was consistently more restricted on humid than on wet fruit (Fig. 1A-C). This phenomenon was most pronounced at pit hardening stage where germ tube lengths on humid fruit did not exceed 15 μ m. Germ tubes however grew slightly longer on humid, ripening fruit, especially on those inoculated at harvest, where predicted lengths

were 49.5 μm at 24 hpi. Growth proceeded fast on wet fruit and followed a similar trend on the different fruits during the first 6 hpi. Thereafter it slowed down on fruit of the pit hardening stage, but was rapid on ripening fruit, showing extensive growth on harvest ripe fruit (178.7 μm predicted at 24 hpi).

Appressorium formation and direct penetration were not observed on any of the nectarine fruits. Germ tubes penetrated fruit predominantly through stomata, lenticels and microfissures in the fruit skin, although conidia seldom landed on these structures. The tendency to grow towards these sites, and to enter them, was influenced by host phenology and wetness regime (Table 4). Firstly, as the fruit reached maturity, proportionally more germplings grew in close proximity of these sites. The proportion of germ tubes that grew towards them, and those that had entered them, also increased with maturity. The tendency to grow towards a specific site and to penetrate was also influenced by fruit phenology. On fruit at pit hardening and the 2 wk before harvest stages, germplings were inclined to grow primarily towards stomata, and predominantly entered these sites. This tendency changed when fruit were inoculated at harvest. On these fruits, germplings were attracted in nearly equal proportions by stomata, lenticels and microfissures and had entered them in nearly equal proportions. Secondly, at each phenological stage, proportionally more germplings grew in close proximity of these sites and more entered them on wet than on humid fruit.

Different patterns of conidium and germpling dieback were observed amongst individuals on a given fruit. On humid fruit some conidia or germplings died or only certain sections of the germ tube died. A similar pattern of germpling dieback was observed on wet fruit. Trends followed in dieback were regulated by fruit phenology and wetness regime. Conidium and germpling dieback were more pronounced on immature than on mature fruit, and occurred at a considerably higher rate on wet fruit. Data on dieback were however inconclusive and were not analysed.

Bright bluish fluorescence zones, indicative of phenol accumulation, or yellow fluorescence, indicative of suberisation, were not observed in host cells immediately surrounding infection sites. Fluorescence was however observed in cells surrounding ageing stomata, microfissures, old wounds and lenticels.

Surface colonisation. Analyses of variance were done on the data obtained from the skin segment, paraquat fruit and sound fruit tests (Tables 5-8). Significant ($P < 0.05$) treatment vs. incubation period interaction was observed at all stages and the main effects could therefore not be compared. According to the different tests conducted on fruit in the unsterile treatment, surface colonisation during incubation was differentially influenced by wetness at each developmental stage. Trends followed at each stage are summarised below.

Pit hardening stage. The majority of segments removed from humid fruit during the 3 to 24 hpi period supported growth of the pathogen, indicating high levels of germling viability on fruit surfaces during this period (Fig. 2A). The proportion of segments supporting growth then declined, indicating gradual germling dieback during the 24 to 48 hpi period ($P = 0.0005$). Free water, on the other hand, had a constant negative effect on colonisation and germling viability ($P = 0.0269$). Therefore, at each sampling, significantly less segments removed from wet than humid fruit were colonised by *M. laxa*. The drastic decline in the frequency of colonised segments obtained from wet fruit furthermore indicated rapid death of germlings. Fruits in the sound fruit test (Fig. 3A) and the paraquat fruit test (Fig. 4A) remained asymptomatic during dry incubation, notwithstanding the duration of the initial incubation period or wetness regime. Germlings were therefore unable to penetrate and infect the sound fruit or fruit of which active host defence was terminated by paraquat.

Two weeks before harvest stage. The skin segment test (Fig. 2B) showed a gradual decline in the frequency of *M. laxa*-yielding segments removed during the 3 to 24 hpi period from both humid and wet fruit ($P = 0.0796$ and 0.0043 , respectively). Germlings therefore gradually succumbed during the 24 hpi incubation period, but dieback proceeded at a significantly faster rate on wet than humid fruit. The minority of humid fruit in the sound fruit test developed lesions during dry incubation (Fig. 3B). Predicted values on fruit removed 24 and 48 hpi were 5.2% and 10.3%, respectively. Nearly similar values were predicted for the 3 to 24 hpi period on wet fruit. The proportion symptomatic fruit, however, drastically increased to 92.9% on fruit kept wet for 48 hpi before dry incubation. Disease expression occurred on a relative large proportion of fruits where active host responses were terminated by paraquat 3 hpi (Fig. 4B). The frequency then gradually declined on fruits that were initially kept humid ($P = 0.0003$), but gradually increased on fruits kept wet ($P =$

0.0265). Germlings were therefore, notwithstanding their viability levels on humid fruit, less successful in penetrating these fruits.

Harvest stage. In the skin segment test (Fig. 2C), segments constantly yielded the pathogen irrespective of wetness regime or period. In the sound fruit test (Fig. 3C) symptom expression was enhanced by longer incubation, but was significantly higher on wet than humid fruit ($P < 0.05$). Corresponding trends were observed on fruit in the paraquat fruit test (Fig. 4C). According to trends showed by the different tests, germlings remained viable on fruit during the 48 hpi period, but were more successful in penetrating wet than humid fruit.

Cold stored fruit. Data from the three tests (Figs. 2D, 3D, 4D) clearly indicated high germling viability and penetration of fruit inoculated after cold storage. Proportions *M. laxa*-yielding segments or symptomatic fruit were not significantly affected by wetness regime.

Skin penetration. Tests conducted on fruit in the sterile treatment showed that skin penetration was influenced by both fruit phenology and wetness. Trends followed at each developmental stage are summarised below.

Pit hardening stage. Trends displayed by segments yielding *M. laxa* (Fig. 2A) showed that, irrespective of wetness regime or period, less than 5% of segments were penetrated by *M. laxa*. The high level of host resistance to penetration shown by the skin segment test was confirmed by the lack of symptom expression on fruit in the sound fruit test (Fig. 3A) and paraquat fruit test (Fig. 4A). This showed that although fruit surfaces were differentially colonised by germlings during the 48 hpi period, germlings were unable to establish infection.

Two weeks before harvest stage. Data of the skin segment test (Fig 2B) showed that penetration was unrelated to surface colonisation. Segments removed from humid fruit yielded *M. laxa* at a nearly constant low level ($P = 0.5329$). However, the proportion increased at a significant rate when segments were obtained from wet fruit ($P = 0.0043$). A similar trend was found on fruit in the two other tests. On fruit in the sound fruit test (Fig. 3B), levels of disease expression remained low on fruit removed during the 3-24 hpi. Levels on fruit kept wet for 48 hpi increased drastically to 79.7% ($P = 0.0253$), but levels increased to only 11.3% on fruit kept humid ($P = 0.3599$). On fruit in the paraquat fruit test (Fig. 4B),

disease expression levels on humid fruit were constantly low and did not exceed 25% ($P = 0.3747$). Wet fruit, however, already showed high levels of disease expression during the second incubation cycle when they were incubated for 3 hpi in the first cycle. The proportion symptomatic fruit drastically increased when the duration of the first incubation cycle was increased ($P = 0.0008$).

Harvest stage. The three tests showed nearly similar trends in disease expression. In the case of humid fruit, expression levels started from a low base (0-2%) in both the skin segment test (Fig 2C) and the sound fruit test (Fig. 3C) on fruit kept for 3-6 hpi in the first incubation cycle. Levels then gradually increased to reach approximately 40% for the 48 hpi sampling. Disease expression was slightly more pronounced on fruit in the paraquat fruit test, with 10.1% and 57.8% fruit showing symptoms for the 3 and 48 hpi sampling, respectively. In the case of wet fruit, on the other hand, the level of disease expression increased dramatically with incubation ($P = 0.0001$). In both the skin segment and paraquat fruit test, nearly all the segments or fruit expressed symptoms for the 24 hpi sampling. In the sound fruit test, 100% disease expression was recorded in the 48 hpi sampling.

Cold stored fruit. Data from the three tests showed that resistance to infection and disease expression was markedly reduced by cold storage. Although segments (Fig. 2D) only yielded the pathogen when removed at the 6 hpi sampling, levels increased drastically at later samplings. These increases were furthermore significantly higher on wet than humid fruit ($P < 0.05$). Trends displayed in the two fruit tests were however similar. For both tests, high levels of disease expression were already shown by the 3 hpi sampling, with rapid increases at later samplings. Disease expression was furthermore not significantly affected by wetness regime ($P > 0.05$).

DISCUSSION

The mode of penetration of fruits by the brown rot fungi, *M. fructicola*, *M. laxa* and *M. fructigena*, and subsequent disease expression, have not been well documented. Infection by these pathogens is mainly associated with wounds on fruit (Byrde & Willetts, 1977; Fourie & Holz, 1985b; Xu & Robinson, 2000). Entry of fruits inoculated with conidial suspensions has however been observed through undamaged surfaces, including direct

penetration of the cuticle by appressoria of *M. fructicola* (Cruickshank & Wade, 1992b) and structures such as hair sockets, lenticels and stomata (Curtis, 1928; Smith, 1936; Hall, 1971; Byrde & Willetts, 1977; Willetts & Bullock, 1993). The inoculation technique used in this study simulated natural infection of nectarine fruit at different phenological stages under humid and wet conditions and facilitated studies on the behaviour of airborne conidia on the fruit surface, penetration and of disease expression. The findings of the skin segment, paraquat-treated fruit and sound fruit tests clearly showed that nectarine fruits reacted resistant to disease expression at the pit hardening stage. Wetness had no effect on disease expression at the pit hardening stage, but the disease reaction was markedly influenced by wetness on ripening fruit. The findings furthermore suggest that the change in fruit susceptibility on maturing fruit could be ascribed to changes of components of the fruit skin.

The behaviour of the airborne inoculum, based on fluorescence microscopy of the stained segments, complemented these findings. On humid fruit, both germination and germ tube growth were markedly affected by fruit phenology. Germination was poor on fruit at the 2 wk before harvest stage, but was not meaningfully influenced at pit hardening or the harvest stage. Germ tube growth was invariably restricted on all fruits, but germ tubes grew slightly longer on mature than on immature fruit. Germination was not affected by host phenology on wet fruit. However, germ tube elongation was markedly influenced by fruit phenology. Germ tubes grew more restricted on immature fruit, but extensively on mature fruit. Appressorium formation and direct penetration was not observed on any of the fruits. Germ tubes penetrated fruit predominantly through stomata, lenticels and microfissures in the fruit skin. This confirmed earlier findings made with conidial suspensions of *M. laxa*, which described microfissures in the fruit skin (Nguyen-The *et al.*, 1989; Schlagbauer & Holz, 1989b), stomata and lenticels (Den Breeÿen, 1993) as primary sites for penetration. Fluorescence microscopy further revealed that the tendency to grow towards these sites, and to enter them, was regulated by host phenology and wetness regime. Firstly, it was found that the airborne conidia seldom landed on these structures. Secondly, as the fruit reach maturity, proportionally more germlings grew in close proximity of these sites. The proportion that grew towards and penetrated them increased with maturity. The tendency to grow towards a specific site and to penetrate was also influenced by fruit phenology. On fruit at pit hardening and the 2 wk before harvest stages, germlings were inclined to grow primarily towards stomata, and predominantly entered these sites. This tendency changed when fruit

were inoculated at harvest. On these fruits, germlings were attracted in nearly equal proportions by stomata, lenticels and microfissures and entered them in nearly equal proportions. Secondly, at each phenological stage proportionally more germlings grew in close proximity of these sites, and entered more of them on wet than on humid fruit. Counts (data not included) furthermore showed that the number of stomata and lenticels on fruits increased from 1.89 per mm² (SD = 0.356) at the 2 wk before harvest stage to 2.37 per mm² (SD = 0.594) at the harvest stage, thereby offering more sites for penetration on the latter fruits.

It has been shown that temperature and nutrients can affect volume, nuclear number, germination and aggressiveness of conidia of *Monilinia* spp. (Phillips, 1982; Phillips, 1984; Margosan & Phillips, 1985; Phillips & Margosan, 1985; Margosan & Phillips, 1989; Phillips *et al.*, 1989; Tamm & Flückiger, 1993). The conidia used in this study were produced on fresh, harvest-ripe nectarine fruit kept at a constant temperature of 22°C, therefore optimising the viability and virulence of the conidia. Conidial production was furthermore optimised by incubating infected fruits at relatively low humidity, which allowed fragmentation of conidial chains (Byrde & Willetts, 1977) and the even dispersal of conidia on fruit surfaces. The viability of the conidia was at a constant high level, as proved by the fact that nearly all the conidia germinated within 6 hpi on PDA. It can thus be assumed that the conidia dispersed onto the fruit surface at each inoculation were all viable, and that lack of germination or vitality could be ascribed to the effects of substances in fruit exudates, wax layers or other skin components.

The marked effect of fruit phenology and wetness regime on germination, morphogenesis of germ tubes and germling viability can partially be attributed to the influence of fruit exudates on solitary conidia. Phenols, in particular chlorogenic and caffeic acids, are high in resistant immature peach genotypes, and decline with fruit maturity (Bostock *et al.*, 1999). Bostock *et al.* (1999) associated the suppressive action of surface phenolics on the production of cutinase with host resistance of peach fruit to *M. fructicola*. Working with *Botrytis cinerea*, Fourie and Holz (1998b) showed that prior to the period of rapid cell enlargement, growth of this fungus on raised slides was inhibited by nectarine exudates. Germlings of *M. laxa* need an external supply of nutrients for germ tube differentiation and penetration of host surfaces since conidia of the brown rot fungi contain

insufficient reserves (Willetts & Bullock, 1993). Sugar concentrations in the exudates of nectarine fruit are low prior to pit hardening (Fourie & Holz, 1998b), but increase rapidly in the last 2 wk prior to harvest. It was shown with *B. cinerea* that at concentrations corresponding to those found in exudates, glucose, fructose and sucrose did not influence fungal growth in a mineral medium. Fungal growth was only enhanced when either of the reducing sugars or sucrose was supplied in excess of 0.27 and 0.14 mM, respectively. During the last two weeks prior to harvest, total sugar in nectarine exudates was far in excess of these values. Substances in fruit exudates inhibitory or stimulatory to growth will not be readily available to the fungus when an individual conidium germinates under high humidity on the dry fruit surface. On the other hand, exudates will easily dissolve in the film of water on wet fruit and rapidly be taken up by the solitary conidium growing on the fruit. Furthermore, ordinary diffusion should ensure a constant supply of these substances to the germ tube and hyphae of individual conidia. These effects were shown by the solitary conidia grown on humid and wet fruit.

Inoculum dose is of great importance for successful infection and several studies have shown increased infection with an increase in inoculum dose (Roberts & Dunegan, 1926; Corbin, 1963; Hall, 1971; Fourie & Holz, 1985b; Biggs & Northover, 1988a; Brown & Wilcox, 1989; Wilcox, 1989; Northover & Biggs, 1990; Northover & Biggs, 1995; Hong *et al.*, 1998). An increase in the inoculum dose would increase the likelihood of the increased number of germ tubes encountering sites susceptible to penetration. Conidia act independently however, and synergism may only occur at high doses (Hall, 1971). Northover and Biggs (1990) found in a study on sweet and sour cherries that host resistance against *M. fructicola* was overcome when inoculum doses were increased. In a similar study, Northover and Biggs (1995) found that by increasing the inoculum dose of *M. fructicola*, the initial lesion appearance was advanced and infection incidence and the percentage of fruit with sporodochia were increased. Fluorescence microscopy revealed that conidia of *M. laxa* behaved differently when they grew solitary on fruit surfaces, compared with growth in a cluster. Histological studies with *M. laxa* on stone fruit (Schlagbauer & Holz, 1989b; Den Breeÿen, 1993) showed that conidia suspended in droplets were inclined to settle in the centre of the droplet, which caused an agglomeration of conidia. This action forced conidia to settle around or on stomata, and to enter there. Germ tubes grew extensively and hyphal mats formed on the fruit surface in most droplets. It was also noted that microfissures, which acted

as avenues for penetration by hyphal mats, developed with time in the cuticle under the droplet. In these studies synergistic effects of numerous conidia growing in a cluster on a single site on the host surface possibly masked the effects of fruit exudates and skin components described here. By inoculating with conidia in spore suspensions, the synergistic effects of numerous conidia germinating at a single site on the fruit surface masks the effects of host resistance and the behaviour of single germ tubes on the surface is difficult to study.

Resistance to *M. laxa* of stone fruit prior to the pit hardening stage has been reported (Kable, 1971; Fourie & Holz, 1987a; Schlagbauer & Holz, 1989a; 1989b). Several workers concluded that short-term latent infections initiated shortly before fruit are harvest-ripe, rather than long-term latent infections, are the main cause of *M. laxa* fruit losses (Kable, 1971; Schlagbauer & Holz, 1989a; 1989b). A recent study (Part 2) on natural infection, conducted over a 3-year period on plum and nectarine fruit from 11 stone fruit orchards, indicated that fruit generally remained free of latent infection at the shuck fall and pit hardening stages and only developed symptoms when sampled 2 wk before harvest and at harvest. Data obtained in this study on the surface growth and survival of germlings, skin segment penetration and disease expression on sound and paraquat treated fruit provide additional evidence for the unimportance of long-term latency of *M. laxa* on nectarine. Collectively the findings indicate that *M. laxa* fruit rot epidemics on stone fruit are driven by inoculum levels on fruit approaching maturity, and humid temperature conditions prevailing during the preharvest and harvest period. The role of short-term latency and latent contamination (Jerome, 1958) may thus be underestimated in the epidemiology of *M. laxa* on stone fruit. Disease management strategies should therefore focus on the eradication of inoculum sources that contribute to latent contamination, eradication of latent conidia on the very susceptible mature fruits, and disease prediction during the preharvest period. However, information describing inoculum sources and fruit rot epidemics of stone fruit by *M. laxa* is lacking. More information is therefore needed on these aspects of *M. laxa* to fully understand its biology, epidemiology and control on stone fruit.

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Table 1. Analysis of variance for effects of growth stage (G), wetness regime (humid[H]/wet[W]) and incubation period (T) on germination (%) of airborne conidia of *Monilinia laxa* on surfaces of Flamekist nectarine fruit

Source of variation	Df	SS	MS	SL
Model	29	92501	3190	0.0001
G	2	1193	596	0.0697
W/H	1	23120	23120	0.0001
T	4	45335	11334	0.0001
G x W/H	2	8401	4201	0.0001
G x T	8	4564	571	0.0143
W/H x T	4	6636	1659	0.0001
G x W/H x T	8	3252	406	0.0769
Error	60	12850	214	
Corrected total	89	105351		

Table 2. Analysis of variance for effects of growth stage (G), wetness regime (humid[H]/wet[W]) and incubation period (T) on the growth (average length in μm) of *Monilinia laxa* germ tubes on surfaces of Flamekist nectarine fruit

Source of variation	Df	SS	MS	SL
Model	29	250751	8647	0.0001
G	2	37239	18619	0.0001
W/H	1	49731	49731	0.0001
T	4	73711	18428	0.0001
G x W/H	2	12501	6251	0.0001
G x T	8	31776	3972	0.0001
W/H x T	4	28110	7028	0.0001
G x W/H x T	8	17683	2210	0.0001
Error	60	19341	322	
Corrected total	89	270091		

Table 3. Germination pattern of airborne *Monilinia laxa* conidia on surfaces of humid^a or wet^b Flamekist nectarine fruit

Growth stages	Germ tubes observed	Germination pattern					
		One germ tube formed (%)			Two germ tubes formed (%)		
		Total	Unbranched	Branched	Total	Unbranched	Branched
Humid fruit							
Pit hardening	688	98.3	92.6	5.7	1.6	1.2	0.4
2 wk before harvest	375	99.2	96.3	2.9	0.8	0.8	0
Harvest	446	97.5	88.5	9.0	2.5	2.5	0
Wet fruit							
Pit hardening	529	98.8	96.1	2.7	1.3	1.1	0.2
2 wk before harvest	587	93.8	88.4	5.4	6.2	5.2	1.0
Harvest	389	97.5	88.0	9.5	2.6	2.3	0.3

^aFruit incubated at high relative humidity ($\geq 93\%$ RH).^bFruit overlaid with wet paper towels.**Table 4.** Behaviour of germ tubes of solitary *Monilinia laxa* germlings growing in proximity of natural openings^a on humid^b or wet^c Flamekist nectarine fruit

Growth stages	Germ tubes observed	Germ tubes (%) in proximity of natural openings			
		Total	Missing	Towards	Penetrating
Humid fruit					
Pit hardening	688	4.0	1.9	1.6	0.5
2 wk before harvest	375	13.1	4.8	5.1	3.2
Harvest	446	18.7	5.4	8.4	4.9
Wet fruit					
Pit hardening	529	19.9	4.5	6.8	8.6
2 wk before harvest	587	27.4	4.8	9.9	12.7
Harvest	389	32.0	8.2	7.4	16.4

^aStomata, lenticels or microfissures.^bFruit incubated at high relative humidity ($\geq 93\%$ RH).^cFruit overlaid with wet paper towels.

Table 5. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germings growing on Flamekist nectarine fruit at the pit hardening stage

Source of variation	Df	SS	MS	SL
Model	59	155325	2633	0.0001
M	2	55478	27739	0.0001
W/H	1	1567	1567	0.0001
S/NS	1	23729	23729	0.0001
T	4	2648	662	0.0001
M x W/H	2	3134	1567	0.0001
M x S/NS	2	47458	23729	0.0001
M x T	8	5296	662	0.0001
W/H x S/NS	1	1459	1459	0.0001
W/H x T	4	702	176	0.0103
S/NS x T	4	2613	653	0.0001
M x W/H x S/NS	2	2918	1459	0.0001
M x W/H x T	8	140	176	0.0013
M x S/NS x T	8	5226	653	0.0001
W/H x S/NS x T	4	563	141	0.0302
M x W/H x S/NS x T	8	1127	141	0.0075
Error	120	6095	51	
Corrected total	179	161420		

Table 6. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germings growing on Flamekist nectarine fruit at the 2 wk before harvest stage

Source of variation	Df	SS	MS	SL
Model	59	222085	3764	0.0001
M	2	49898	24949	0.0001
W/H	1	8431	8431	0.0005
S/NS	1	38647	38647	0.0001
T	4	27203	6801	0.0001
M x W/H	2	5741	2870	0.0001
M x S/NS	2	37233	18617	0.0001
M x T	8	12447	1556	0.0001
W/H x S/NS	1	4521	4521	0.0001
W/H x T	4	13197	3299	0.0001
S/NS x T	4	4423	1106	0.0028
M x W/H x S/NS	2	3070	1535	0.0034
M x W/H x T	8	8311	1039	0.0003
M x S/NS x T	8	4319	540	0.0417
W/H x S/NS x T	4	1354	339	0.2695
M x W/H x S/NS x T	8	3290	411	0.1338
Error	120	30974	258	
Corrected total	179	253059		

Table 7. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germplings growing on Flamekist nectarine fruit at the harvest stage

Source of variation	Df	SS	MS	SL
Model	59	248974	4220	0.0001
M	2	14629	7314	0.0001
W/H	1	65696	65696	0.0001
S/NS	1	34274	34274	0.0001
T	4	64918	16229	0.0001
M x W/H	2	9254	4627	0.0001
M x S/NS	2	19742	9871	0.0001
M x T	8	2908	363	0.1975
W/H x S/NS	1	1021	1021	0.0485
W/H x T	4	11887	2972	0.0001
S/NS x T	4	4648	1162	0.0019
M x W/H x S/NS	2	3806	1903	0.0009
M x W/H x T	8	5505	688	0.0097
M x S/NS x T	8	7042	880	0.0014
W/H x S/NS x T	4	2663	666	0.0401
M x W/H x S/NS x T	8	981	123	0.8703
Error	120	30850	257	
Corrected total	179	279823		

Table 8. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germplings growing on cold stored Flamekist nectarine fruit

Source of variation	Df	SS	MS	SL
Model	59	190733	3233	0.0001
M	2	21115	10555	0.0001
W/H	1	2712	2712	0.0003
S/NS	1	18461	18461	0.0001
T	4	105882	26471	0.0001
M x W/H	2	221	110	0.5616
M x S/NS	2	11563	5781	0.0001
M x T	8	8808	1101	0.0001
W/H x S/NS	1	519	519	0.1017
W/H x T	4	452	113	0.6679
S/NS x T	4	8046	2012	0.0001
M x W/H x S/NS	2	2956	1478	0.0007
M x W/H x T	8	5585	698	0.0007
M x S/NS x T	8	2655	332	0.0956
W/H x S/NS x T	4	1030	258	0.2550
M x W/H x S/NS x T	8	727	91	0.8706
Error	120	22867	191	
Corrected total	179	213600		

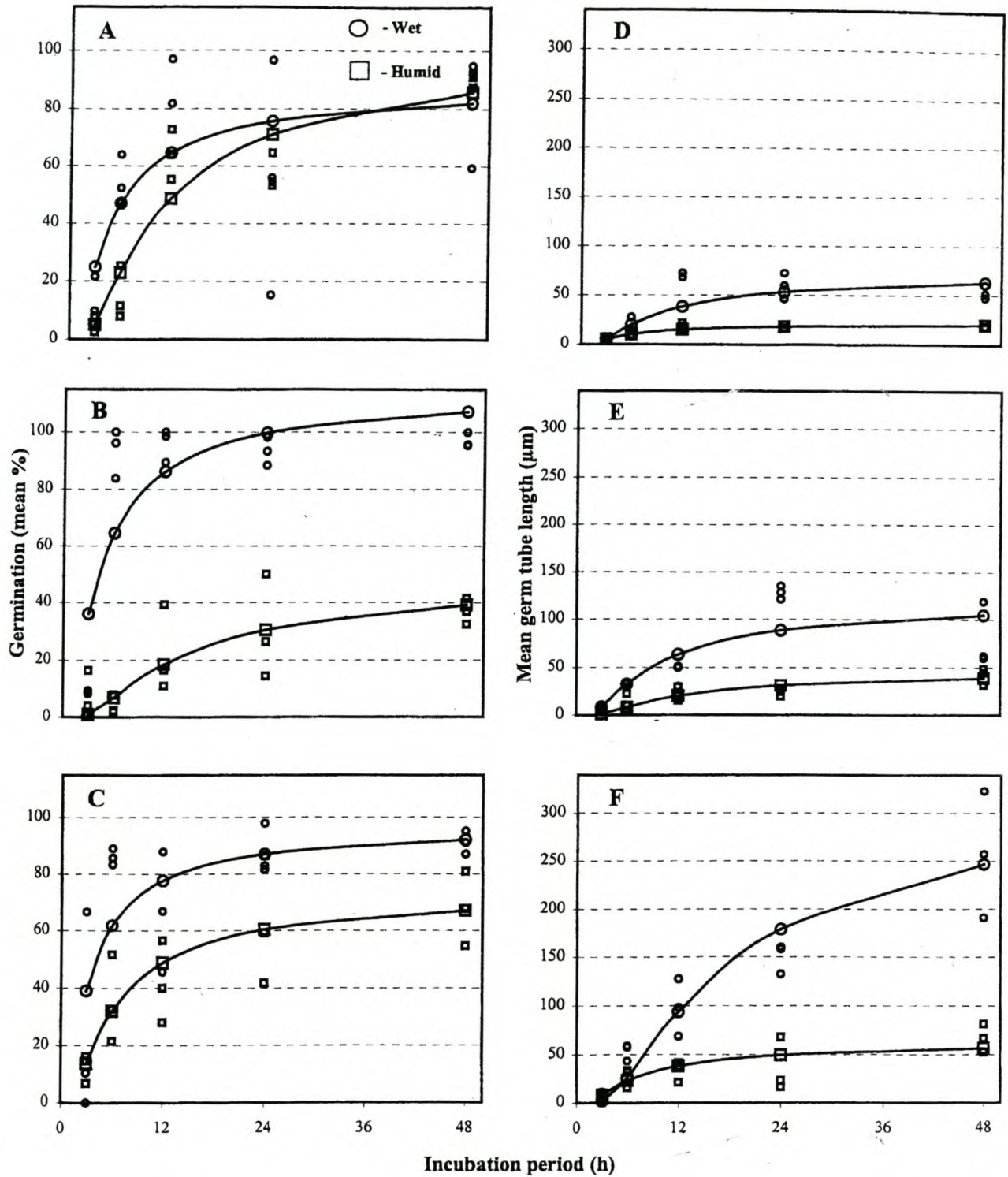


Figure 1. Effect of wetness regime and incubation period on germination rate and germ tube growth of airborne conidia of *Monilinia laxa* on Flamekist nectarine fruit at pit hardening (A,D), 2 wk before harvest (B,E) and harvest (C,F) stage. Points represent actual means of germination percentages or germ tube lengths (μm) of three experiments, whereas lines represent predicted values based on a non-linear growth curve $[y=A\exp(B/x)]$ fitted to the point data to observe and compare trends.

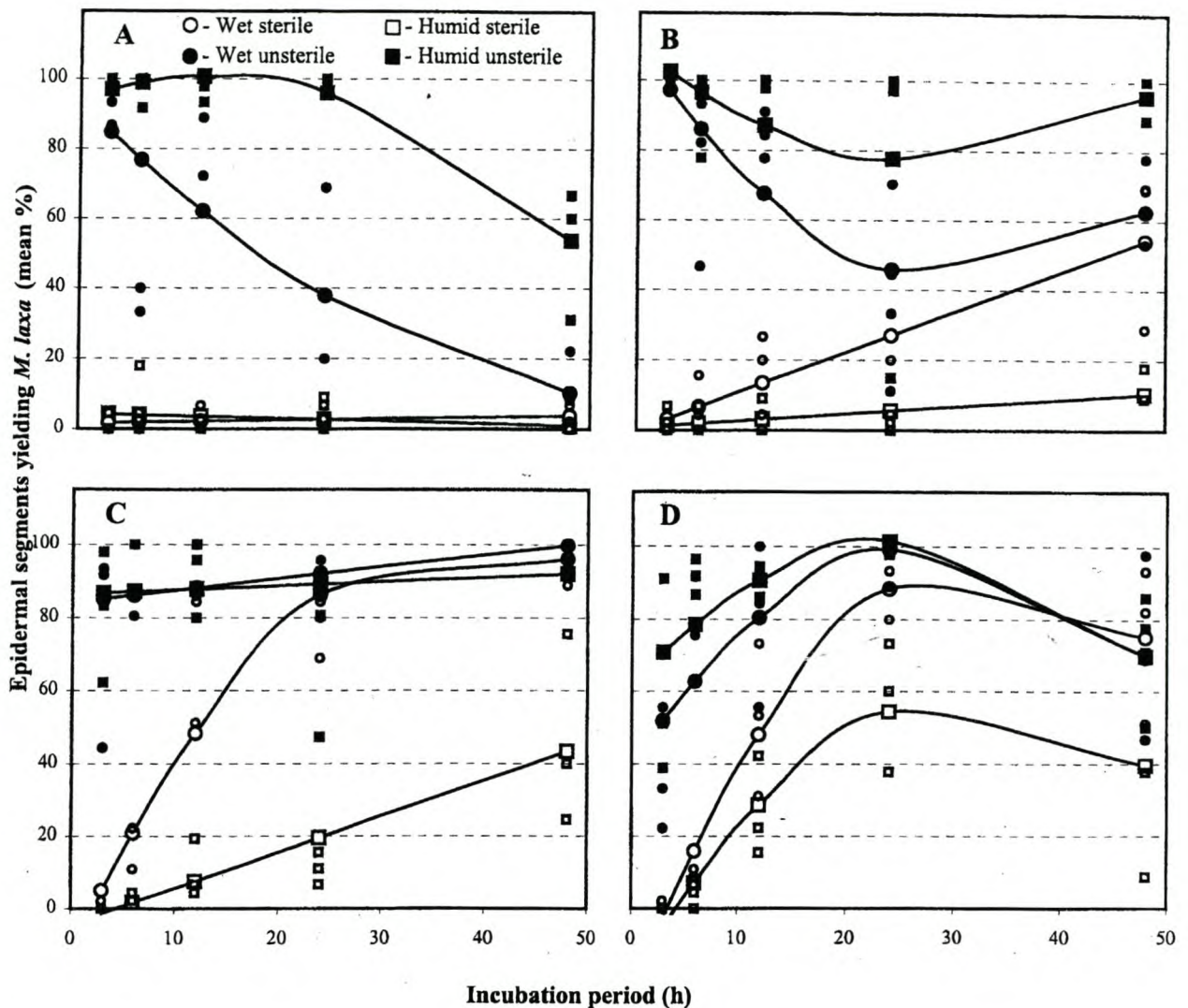


Figure 2. Effect of wetness regime and incubation period on the surface colonising ability and infectivity of solitary *Monilinia laxa* germlings on Flamekist nectarine fruit at pit hardening (A), 2 wk before harvest (B), harvest stage (C) and after cold storage (D). Surface colonising ability is represented by the percentage segments removed from unsterile fruit at each incubation period that yielded the pathogen after 14 days incubation. Infectivity is represented by the percentage segments removed from surface-sterilised fruit that yielded the pathogen. Points represent actual means from three experiments, whereas lines represent predicted values based on linear or quadratic regression equations derived from the point data.

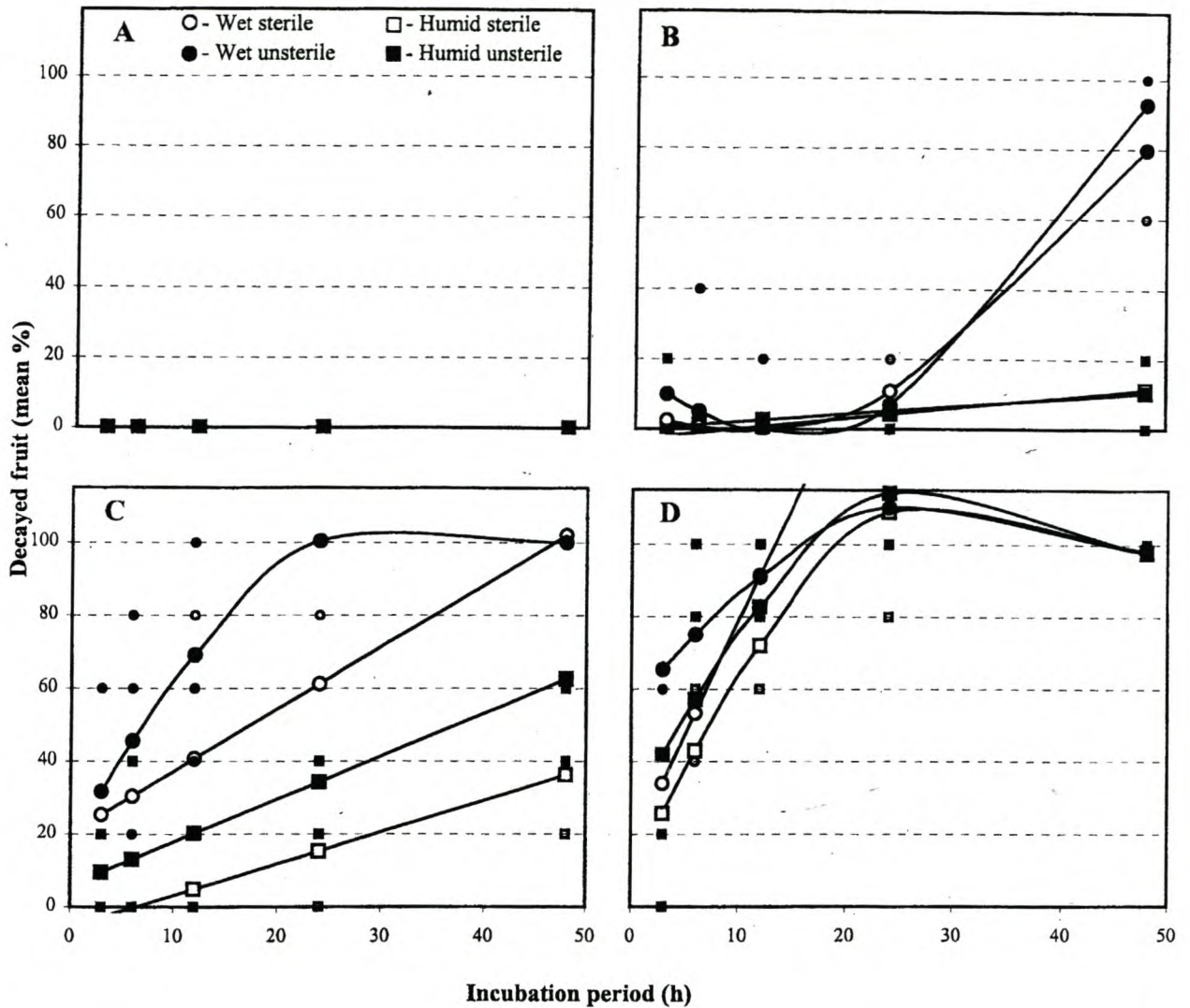


Figure 3. Effect of wetness regime and incubation period on disease expression by solitary *Monilinia laxa* germlings on Flamekist nectarine fruit at pit hardening (A), 2 wk before harvest (B), harvest stage (C) and after cold storage (D). Fruits were incubated at a specific wetness regime, then kept dry. Points represent mean percentages decayed fruit recorded in three experiments, whereas lines represent predicted values based on linear or quadratic regression equations derived from the point data.

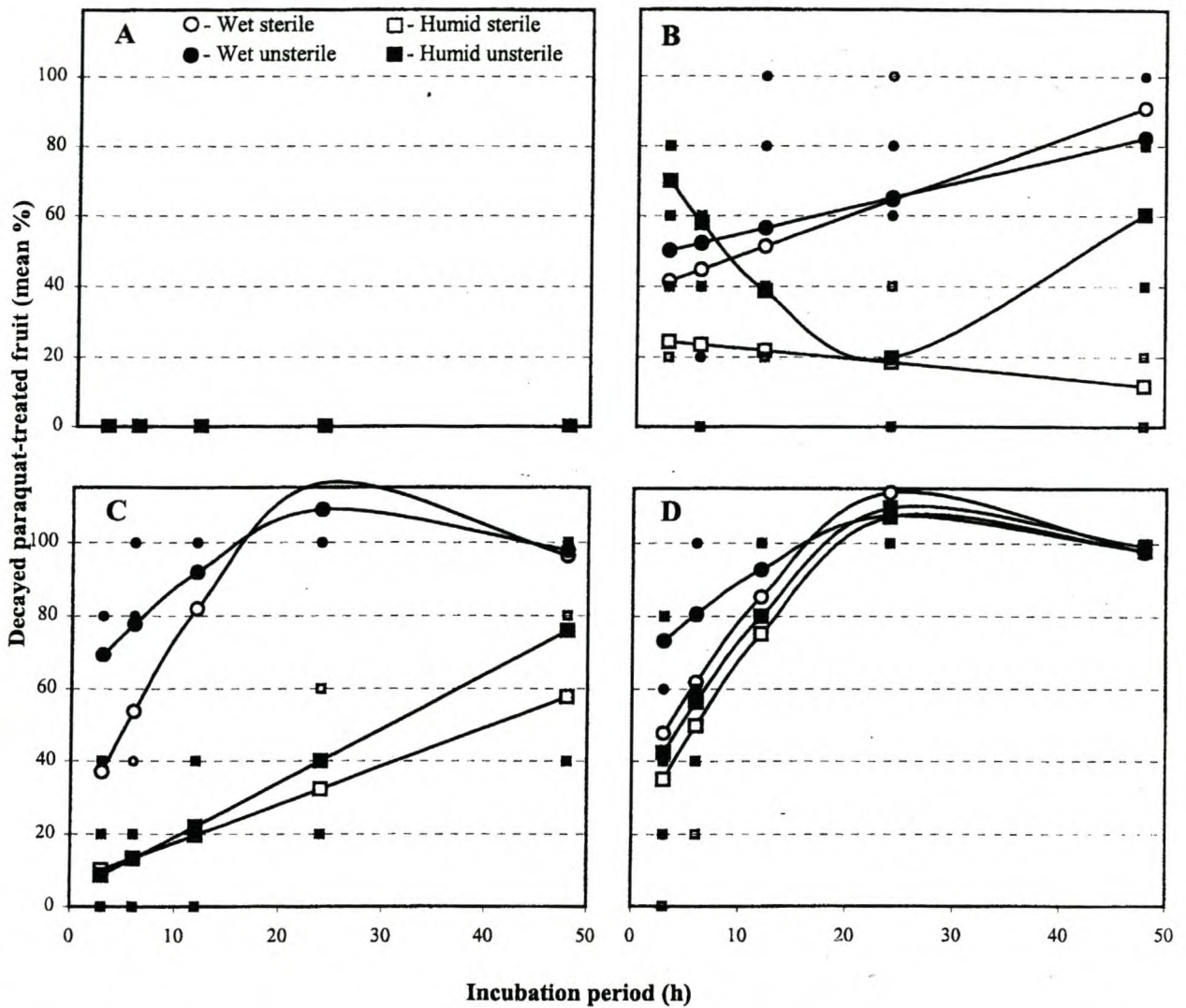


Figure 4. Effect of wetness regime and incubation period on disease expression by solitary *Monilinia laxa* germlings on Flamekist nectarine fruit at pit hardening (A), 2 wk before harvest (B), harvest stage (C) and after cold storage (D). Fruits were removed after each incubation period at a given wetness regime, immersed in 3% paraquat and rinsed in water, then kept dry. Points represent mean percentages decayed fruit recorded in three experiments, whereas lines represent predicted values based on linear or quadratic regression equations derived from the point data.

4. BEHAVIOUR OF SOLITARY CONIDIA OF *MONILINIA LAXA* AND DISEASE EXPRESSION ON PLUM FRUIT

ABSTRACT

Plum fruit (cultivar Laetitia) at pit hardening, 2 wk before harvest, harvest stage and after cold storage were dusted with dry conidia of *Monilinia laxa* in a settling tower. The fruits were incubated for periods ranging from 3 to 48 h at high relative humidity ($\geq 93\%$, humid fruit), or were covered with a film of water (wet fruit). Behaviour of the solitary conidia was examined with an epifluorescence microscope on skin segments stained in a differential stain containing fluorescein diacetate, aniline blue and blankophor. The ability of the solitary conidia to colonise the fruit surface, to penetrate the fruit skins and to induce disease expression was determined by using a differential set of tests. For these tests, fruit were surface-sterilised or left unsterile. From each group, fruit were selected for isolation (skin segment test), immersed in a 3% paraquat solution (paraquat fruit test) or left untreated (sound fruit test). The tests showed that at the pit hardening stage, fruit skins were not penetrated under both wetness regimes, latent infections were not established and fruits reacted resistant to disease expression. Humid fruit at the 2 wk before harvest stage, harvest stage and after cold storage remained asymptomatic in the sound fruit test. Fruit at these stages only developed disease after a prolonged period (≥ 12 h) of wet incubation. The paraquat fruit test revealed that these fruits became more susceptible to latent infection as the fruit ripened. However, maturing fruit did not display a drastic change in the barrier capacity of fruit skins. The behaviour of the inoculum on fruit surfaces complemented these findings. Germination was not affected by fruit phenology, but was markedly affected by wetness. The number of conidia that germinated after 48 h on wet and humid fruit was similar, but most germination on wet fruit occurred during the first 12 h, whereas a lag phase of 12 to 24 h was observed on humid fruit. Germ tube elongation on humid fruit was unaffected by fruit phenology and was slightly more restricted than on wet fruit. However, on wet harvest-ripe fruit, germ tube growth was markedly more extensive. Appressorium formation and direct penetration was not observed on any of the fruits. Germ tubes penetrated fruit predominantly

through stomata, lenticels and microfissures in the fruit skin. Conidium and germling survival was drastically reduced by prolonged wet incubation of fruits. These findings indicate that on plum, *M. laxa* fruit infection is influenced by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period.

INTRODUCTION

Two *Monilinia* species, namely *M. fructicola* (Wint.) Honey and *M. laxa* (Aderh. & Ruhl.) Honey, are associated with blossom blight and brown rot of stone fruit. *Monilinia fructicola* is absent in Europe (Byrde & Willetts, 1977; Willetts & Bullock, 1993) and South Africa (Fourie & Holz, 1985; Schlagbauer & Holz, 1987; Part 2) and in these countries all stages of this disease are caused by *M. laxa* (Byrde & Willetts, 1977; Fourie & Holz, 1985; Schlagbauer & Holz, 1987; Willetts & Bullock, 1993; Part 2). A recent study (Part 2), which investigated disease expression by both new and established *M. laxa* infections in different nectarine and plum orchards over a three-year period, showed that the pathogen was virtually absent from flowers and occurred only sporadically. Immature fruit were generally pathogen-free and disease expression only occurred on fruit approaching maturity. Contrary to *M. fructicola*, long-term latency does not seem to play a prominent role in *M. laxa* fruit rot of plum and nectarine (Kable, 1971; Schlagbauer & Holz, 1989a; Schlagbauer & Holz, 1989b; Part 2; Part 3). A recent study (Part 3) with airborne conidia simulating natural infection of fruit at different phenological stages under humid and wet conditions, confirmed this conclusion on nectarine. The findings clearly showed that nectarine fruits reacted resistant to disease expression at the pit hardening stage. Wetness had no effect on disease expression at the pit hardening stage, but the disease reaction was markedly influenced by wetness on ripening fruit. The findings furthermore indicated that the change in fruit susceptibility on maturing fruit could be ascribed to changes in the components of the skin. Collectively, the findings indicate that *M. laxa* fruit rot epidemics on nectarine fruit are driven by inoculum levels on fruit approaching maturity and climatic conditions prevailing during the preharvest and harvest period. The role of short-term latent infection and latent contamination (Jerome, 1958) may thus be underestimated in the epidemiology of *M. laxa* on stone fruit. However, information describing inoculum sources and fruit rot epidemics of stone fruit by *M. laxa* is

lacking and more information is needed on these aspects of *M. laxa* to fully understand its biology, epidemiology and control on stone fruit.

The mode of penetration of plum fruits by *M. laxa*, and subsequent disease expression, have not been well documented. Studies made with conidial suspensions of *M. laxa* described microfissures in the fruit skin (Nguyen-The *et al.*, 1989; Schlagbauer & Holz, 1989b) stomata and lenticels (Den Breeÿen, 1993) as primary sites for penetration. The aims of this investigation were to study the behaviour of airborne conidia and to record penetration and disease expression under conditions simulating natural infection. To compare the behaviour of the pathogen on different stone fruit types, similar experimental procedures were used as those reported for a previous study with airborne conidia of *M. laxa* on nectarine (Part 3).

MATERIALS AND METHODS

Fruit. A plum orchard (cultivar Laetitia) with a history of low levels of brown rot incidence was selected in the Blaauwklippen valley, Stellenbosch. Four weeks prior to the pit hardening stage, a section of this orchard was demarcated and no fungicides were applied. Sound, unblemished fruit were selected at pit hardening, 2 wk before harvest, and at the harvest stage from these trees. Fruit obtained at harvest stage were either used, or kept under conditions simulating overseas shipment and marketing before being used (10 days at -0.5°C , 18 days at 7.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH). Before usage, fruits were surface sterilised (30 s in 70% ethanol, 2 min in 2% sodium hypochlorite, 30 s in 70% ethanol), packed on sterile, epoxy-coated steel mesh screens (53 x 28 x 2 cm) and allowed to air-dry. Picking wounds at or near the peduncle-end were covered with petroleum jelly. In order to recognize the inoculated cheek of the fruit at a later stage, a 0.5 cm mark was made near the peduncle-end with a soft-tipped koki pen. Preliminary studies showed no phytotoxic effect. Before inoculation, surface sterilised fruit were kept for at least 24 h in ethanol-disinfected perspex (Cape Plastics) chambers (60 x 30 x 60 cm) at 22°C at $\pm 56\%$ RH to allow re-establishment of surface nutrients.

Inoculation. A virulent *M. laxa* isolate, sensitive to iprodione and benomyl and obtained from a naturally-infected nectarine fruit, was maintained in the laboratory at 22°C

on a synthetic agar medium amended with sugars, minerals and malic acid at concentrations occurring in grape berry exudates (1.85 g glucose; 1.95 g fructose; 0.25 g sucrose; 0.15 g malic acid; 5 g peptone; 5 g sodium chloride; 15 g agar; and 2 g yeast extract per liter deionised water), or was kept on malt extract agar (MEA) slopes at 5°C in the dark. Inoculum was prepared by inoculating ripe surface-sterilised nectarines with mycelium discs, or conidia, obtained from fresh cultures growing on potato dextrose agar (PDA). Inoculated fruit were incubated for 10 to 14 days at 22°C on screens in moist perspex chambers (see below) to allow infection, colonisation and profuse sporulation by *M. laxa*. The mummified fruit were then kept in dry chambers at $\pm 56\%$ RH. For inoculation, a mummified fruit was placed on a shelf 10 cm below the ceiling of a spore settling tower (1.5 x 1.0 x 1.5 m [length x width x height]). Conidia were blown for 1 s from the mummy with a pressure pump (Rietzle VTE 3 [3.5–4.2 m³/h]) and the lid in the ceiling closed. The conidia were allowed 10 min to settle onto the fruit that were positioned on three screens on the floor of the tower. Petri dishes with PDA were placed among the fruit 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 6 ethanol-disinfected perspex chambers lined with a sheet of chromatography paper (45 x 57 cm) with the base resting in deionised water to establish high relative humidity ($\geq 93\%$ RH). Each chamber contained five screens carrying 31 fruits, and each screen in a chamber was randomly assigned one of five incubation periods (first incubation cycle). These were 3, 6, 12, 24 and 48 h post inoculation (hpi). Each chamber was considered as a block and the screens were randomised within each chamber. In nature, frequent runoff of raindrops and a half-day or more of sunny weather may lead to different durations of continuous fruit wetness, or of high humidity on the fruit surface. Therefore, in three of the chambers, fruits were overlaid with sterile paper towels wetted with sterile deionised water. Fruits in the other three chambers were left dry. These conditions provided two different wetness regimes with different durations for the pathogen; dry conidia on dry fruit under high relative humidity (humid fruit), and conidia exposed to a film of water on the fruit surface (wet fruit). The chambers containing the fruit were incubated at 22°C with a 12 h photoperiod daily. After a set incubation period, the appropriate screens with fruit were removed from the chambers, the paper towels were removed and the fruits air-dried before

they were used for histological studies and for the determination of surface colonisation, skin penetration and disease expression.

Histology. One fruit (i.e. 3 fruit per wetness regime per incubation period) was randomly selected from each screen per chamber. Thin hand-sectioned pieces (5 x 5 mm) of skin consisting of the cuticle, epidermis, and a few cell layers, were cut with a razor blade from the inoculated cheek of the fruit. The sections were stained for 5 min in a differential stain containing fluorescein diacetate ([FDA] Sigma Chemical Co., St. Louis, MO), aniline blue ([AB] B.D.H. Laboratory Chemicals Division, Poole, England) and blankophor ([BP] Bayer), 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/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 prepared by mixing 25 μl of FDA stock solution with 1 ml of AB/BP stock solution in a 1.5 ml polypropylene Eppendorf tube, which was then kept on crushed ice. Conidial germination, germ tube and hyphal growth, apressorium formation, penetration sites, host responses and viability of fungal structures were examined with the aid of 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 filter No. 02, 06 and 18. Protoplasts of dead cells were blue-black (filter No. 06, 18), whereas cells without protoplasts fluoresced white (filter No. 02) or yellow (filter No. 18) (O'Brien & McCully, 1981). Formation of phenolic substances became visible by irradiation with ultra-violet light (filter No. 02) resulting in a bright bluish fluorescence (Langcake, 1981). Suberised cell walls showed a light blue or bright yellow fluorescence (Hill, 1985). Microfissures and –cracks in the skin became visible due to accumulation of phenolic substances, lignification and suberisation of surrounding cell walls. Lenticels were brown with yellow fluorescence of suberised cells.

Surface colonisation, skin penetration and disease expression. The ability of the solitary conidia to colonise the fruit surface, to penetrate the fruit skins and to induce disease expression during each incubation period was determined by using a differential set of tests. For these tests, fruit on the screens from each chamber were divided into two groups. One

group on each screen was surface-sterilised (30 s in 70% ethanol), while the second group was left unsterile. Five fruit from each group on each screen were selected for isolation (skin segment test). Nine epidermal tissue segments (5 x 7 mm) (45 segments per treatment) were cut from the inoculated cheek of each fruit, placed with the cuticle upward on PDA amended with 40 mg/l streptomycin sulfate (PDAS) and incubated at 23°C (second incubation cycle). Another 15 fruit from each group on each screen were left untreated (sound fruit test). The remainder of the fruit on the screens were immersed in a 3.1% paraquat (WPK Paraquat, WPK Agricultural) solution for 30 seconds, rinsed in sterile deionised water and air-dried (paraquat fruit test). Fruit were replaced on the screens and the screens were transferred to dry perspex chambers ($\leq 56\%$ RH) and kept under laboratory conditions (23°C under a 12 h light schedule) (second incubation cycle). These treatments provided conditions that facilitated disease expression during the second incubation cycle by different inocula on the test material. In the non-sterilised treatment disease expression on segments or fruit was the result of penetration by germlings that had penetrated the cuticle under natural host resistance during the two incubation periods. Surface sterilisation after the first incubation cycle completely eliminated the pathogen from the fruit surface and allowed the development of only germlings that had penetrated the cuticle during this incubation period. Paraquat terminated host resistance in the outer cell layers and consequently promoted the development of epiphytic inoculum, and endophytic inoculum (Baur *et al.*, 1969; Cerkaukas & Sinclair, 1980; Pscheidt & Pearson, 1989). This treatment therefore enhanced the development of latent infection on surface-sterilised fruit. The segments and fruits were regularly monitored for the development of *M. laxa* and numbers yielding the pathogen were recorded after 14 days. The number of sporulating segments or fruit recorded in each experiment was used to quantify surface colonisation, skin penetration and disease expression.

Statistical analyses. Experimental design of experiments, each of which was repeated twice, was a completely randomised split-plot design and analyses of variance were done using SAS. Regression analyses were performed to investigate possible significant trends in interactions or main effects. Slopes and intercepts of regression lines were compared using Student's *t*-LSD ($P < 0.05$) (Snedecor & Cochran, 1980). Significance values of the regression line slopes were calculated, with $P < 0.05$ providing strong evidence against the H_0 -hypothesis that no change occurred over time. Analysis of variance of the

percentage germination and germ tube length were done using SAS. A non-linear natural growth function [$y = A \times \exp(B / x)$] (Hoerl, 1954) was fitted to the data and trends (coefficients) compared using Student's *t*-LSD ($P < 0.05$).

RESULTS

Conidial behaviour. Analyses of variance were done on the germination percentages and germ tube lengths measured during the histological study of the fruit subjected to the various treatments (Table 1 and 2). Non-linear growth curves were fitted to the data and the various trends for percentage germination and germ tube length over incubation time plotted in Figure 1A-F. Based on fluorescence microscopy of the stained segments, it was obvious that the airborne inoculum consisted of conidia only, since no hyphal fragments were observed. Conidia were consistently deposited on fruit surfaces as single cells and not in pairs or groups. The average number of conidia recorded per segment for successive inoculations ranged from 117 to 429, with an average conidial density of 8.97 (SD = 3.284) conidia per mm² fruit surface. Although depositions were regulated by counts on PDA plates, it varied markedly between successive inoculations. Conidia used at each inoculation were highly viable and germinated freely on PDA. Germination on PDA usually reached 98 to 100% at 6 hpi. Germination rate on fruit was markedly lower than on PDA and was significantly ($P < 0.05$) lower on humid than on wet fruit (Fig. 1A-C). On humid fruit rates tended to be very low during the 3 to 6 hpi period (0-4%), but then increased reaching approximately 63-72% at 48 hpi. Germination generally proceeded rapidly on wet fruit, but peaked at 24 hpi (51-60%) when it levelled off to 56-67% at 48 hpi.

Wetness did not markedly influence the number of germ tubes formed (Table 3), but a higher proportion of germ tube branching was observed on humid fruit, with the proportion increasing with fruit ripeness (4 to 12% compared with 1 to 3%). Germ tube growth, on the other hand, was markedly affected by wetness regime and fruit phenology. Growth was more restricted on humid than on wet fruit (Fig. 2D-F). At the pit hardening stage germ tube lengths on humid fruit did not exceed 50 μ m compared with germ tubes longer than 100 μ m measured at 48 hpi on wet fruit. Despite slower germ tube growth observed in the initial incubation phases on humid fruit at 2 wk before harvest, little difference was observed in the germ tube lengths on humid or wet fruit at 24 and 48 hpi. On harvest stage fruit, however,

germ tube growth was proportionally longer on wet fruit compared with humid fruit (197 μm and 52 μm predicted at 48 hpi, respectively). Growth proceeded fast on wet fruit, but progressively slowed down from 12 to 48 hpi.

Appressorium formation and direct penetration were not observed on any of the plum fruits. Germ tubes penetrated fruit predominantly through stomata, lenticels and microfissures in the fruit skin (Fig. 2A-C). Conidia however seldom landed on these structures. The tendency to grow towards these sites, and to enter them, was influenced by host phenology and wetness regime (Table 4). Firstly, the highest proportion of germ tubes growing in the vicinity of these sites was recorded on fruit from the pit hardening stage. The highest density of stomata was also recorded at this stage (0.8 [SD=0.399] stomata/ mm^2), which was markedly more than on fruit from the 2 wk before harvest (0.19 [SD=0.154] stomata/ mm^2) and harvest stage (0.16 [SD=0.081] stomata/ mm^2). Consequently a smaller proportion of germlings grew in the vicinity of these sites on ripening fruit. The highest proportion of stoma and lenticel penetration was nonetheless recorded on wet harvest ripe fruit, corresponding with the more extensive surface colonisation observed on these fruit. Similar trends were observed on humid fruit, but a smaller proportion of germ tubes grew in the vicinity of penetration sites. On the humid plum fruit, only one successful penetration was observed at 2 wk before harvest, and none at pit hardening and harvest.

Different patterns of conidium and germling dieback were observed amongst individuals on a given fruit. On humid fruit some conidia or germlings died or only certain sections of the germ tube died. A similar pattern of germling dieback was observed on wet fruit. Trends followed in dieback were regulated by fruit phenology and wetness regime. Conidium and germling dieback were more pronounced on immature than on mature fruit and occurred at a considerably higher rate on wet fruit. Data on dieback were however inconclusive and were not analysed.

Bright bluish fluorescence zones, indicative of phenol accumulation, or yellow fluorescence, indicative of suberisation, were not observed in host cells immediately surrounding infection sites. Fluorescence was however observed in cells surrounding ageing stomata, microfissures, old wounds and lenticels.

Surface colonisation. Analyses of variance were done on the data obtained from the skin segment, paraquat fruit and sound fruit tests (Tables 5 to 8). Significant ($P < 0.05$) treatment vs. incubation period interaction was observed at all stages and the main effects could therefore not be compared. According to the different tests conducted on fruit in the unsterile treatment, surface colonisation during incubation was differentially influenced by wetness at each developmental stage. Trends followed at each stage are summarised below.

Pit hardening stage. Segments removed from humid fruit during the 3 to 48 hpi period consistently supported growth of the pathogen, indicating high levels of conidium and germling viability on fruit surfaces during this period (Fig. 3A). Free water, on the other hand, had a constant negative effect on colonisation and germling viability ($P=0.0001$). Therefore, at each sampling, significantly less segments removed from wet than humid fruit were colonised by *M. laxa*. The drastic decline in the frequency of colonised segments obtained from wet fruit furthermore indicated rapid death of germlings. Fruits in the sound fruit test (Fig. 4A) and the paraquat fruit test (Fig. 5A) remained asymptomatic during dry incubation notwithstanding the initial wetness duration period or wetness regime. Germlings were therefore unable to penetrate and to infect the sound fruit or fruit on which active host defence was terminated by paraquat.

Two weeks before harvest stage. According to the skin segment test (Fig. 3B) colonisation on humid fruit surfaces was constantly high during incubation ($P=0.9684$). The decline in percentage segments from wet fruit yielding *M. laxa* was however significant ($P=0.0064$), indicating that conidia and germlings gradually succumbed during the 24 hpi incubation period. Higher percentages of segments yielded *M. laxa* when isolated from wet fruit at 48 hpi compared with 24 hpi and a quadratic regression line was fitted to the data. Almost no humid fruit in the sound fruit test developed lesions during dry incubation (Fig. 4B). The predicted decay values on humid fruit removed 3 to 48 hpi were below 2%. Slightly higher decay values were observed on wet fruit with 5% and 12% predicted after 24 and 48 hpi, respectively. Symptom expression occurred more frequently on fruit where active host responses were terminated by paraquat at 3 hpi (Fig. 5B). The predicted decay levels on humid fruit increased slowly with 10% fruit decay predicted at 48 hpi ($P=0.378$). Significantly higher decay levels were observed on wet fruit ($P<0.05$) and decay incidence increased linearly from 12% at 3 hpi to 98% at 48 hpi ($P=0.0001$). Germlings were therefore,

notwithstanding their viability levels on humid fruit, markedly less successful in penetrating these fruits.

Harvest stage. Results from the skin segment test (Fig. 3C) indicated no decline in the viability of conidia or germlings on humid fruit ($P=0.8808$). A significant decline was however observed in the 3-24 hpi period on wet fruit ($P=0.0081$). Similar to the 2 wk before harvest fruit, higher percentages of segments yielded the pathogen at 48 hpi, allowing a quadratic line to be fitted to the data. Predicted decay levels on humid sound fruit (Fig. 4C) remained below 2% with only one fruit each from the 12, 24 and 48 hpi expressing brown rot symptoms. Symptom expression on wet fruit however increased from 5% at 6 hpi to 44% at 24 hpi, and then declined slightly to 34% at 48 hpi ($P=0.001$). Corresponding trends were observed on wet fruit in the paraquat fruit test (Fig. 5C), but decay levels increased faster and higher levels of decay were recorded (86% and 68% at 24 and 48 hpi, respectively) ($P=0.0001$). Low levels of decay on humid fruit were also observed with maximum decay predicted at 24 hpi (25.7%) and less at 48 hpi (14.1%) ($P=0.0247$). According to trends showed by the different tests, germlings remained viable on fruit during the 48 hpi period, but were more successful in penetrating wet than humid fruit. A decline in disease expression from 24 to 48 hpi on wet sound fruit and paraquat-treated fruit was furthermore observed, whereas the skin segment test showed more segments yielded *M. laxa* at 48 hpi on wet fruit (Figs. 3C, 4C, 5C).

Cold stored fruit. Data of the skin segment test (Fig. 3D) clearly indicated high conidium and germling viability on humid fruit ($P=0.931$), but a similar decline in viability to that observed at 2 wk before harvest was observed on wet fruit ($P=0.0001$). Disease expression on humid sound fruit increased slightly to 6% at 48 hpi ($P=0.5108$), whereas decay levels increased linearly to 73% at 48 hpi on wet fruit ($P=0.0001$) (Fig. 4D). Similar observations were made in the paraquat fruit test (Fig. 5D). Data from the three tests clearly showed that wetness regime significantly affected conidium and germling viability on cold stored plum fruit.

Skin penetration. Tests conducted on fruit in the sterile treatment showed that skin penetration was influenced by both fruit phenology and wetness. Trends followed at each developmental stage are summarised below.

Pit hardening stage. Trends displayed by segments yielding *M. laxa* (Fig. 3A) showed that irrespective of wetness regime, or period, less than 4% of segments were penetrated by *M. laxa*. The high level of host resistance to penetration shown by the skin segment test was confirmed by the lack of disease expression on fruit in the sound fruit test (Fig. 4A) and paraquat fruit test (Fig. 5A). This showed that although fruit surfaces were differentially colonised by germings during the 48 hpi period, germings were unable to establish infection.

Two weeks before harvest stage. Data from the skin segment test (Fig 3B) showed that wetness significantly influenced penetration. Very few segments (<3%) from humid fruit were penetrated by *M. laxa*, whereas successful penetration of wet fruit increased linearly to 40% at 48 hpi. Similar trends were observed in the sound fruit test (Fig. 4B). Data from the paraquat fruit test (Fig. 5B) indicated however that successful penetration of humid fruit did occur with decay levels at a near-constant level ranging from 23% to 19% ($P=0.704$). Markedly more wet fruit were infected and decay levels were constant from 3 to 12 hpi (24.0 to 22.9%) and increased to 34% and 98% at 24 hpi and 48 hpi, respectively ($P=0.0001$). The proportion symptomatic fruit drastically increased when the duration of the first wet incubation cycle was increased.

Harvest stage. Data from the skin segment test (Fig. 3C) followed similar trends to that observed at 2 wk before harvest. Data from the sound fruit test (Fig. 4C), on the other hand, followed similar trends to that observed on the unsterile harvest fruit, indicating that fruit were not penetrated during the second incubation period. The same conclusion could be drawn from the trends observed in the paraquat fruit test (Fig. 5C).

Cold stored fruit. Surprisingly more successful penetrations of humid fruit compared with that observed on wet fruit during the 12-24 hpi period was observed in the skin segment test (Fig. 3D). Decay levels were however similar at 48 hpi. None of the humid fruit in the sound fruit test (Fig. 4D) showed brown rot symptoms, whereas 59% decay was predicted on wet fruit at 48 hpi after a lag phase during the 3-24 hpi period (<5% decay) ($P=0.0053$). Decay levels were however markedly lower than that observed on the unsterile fruit, indicating additional infection during the second incubation period. Despite the successful penetration of humid fruit predicted by the skin segment test, low levels of decay (>5%) was

observed in the paraquat fruit test ($P=0.7574$). Penetration of wet fruit however increased linearly to 66% at 48 hpi ($P=0.0001$). Disease expression was therefore significantly affected by wetness regime.

DISCUSSION

This study, which simulates natural infection of plum fruit under humid and wet conditions, demonstrated that solitary conidia of *M. laxa* behaved consistently on plum and nectarine (Part 3) fruit surfaces. Appressorium formation and direct penetration was not observed on any of the fruits. Germ tubes penetrated fruit predominantly through stomata, lenticels and microfissures in the fruit skin. This confirmed earlier findings made with conidial suspensions of *M. laxa*, which described microfissures in the fruit skin (Nguyen-The *et al.*, 1989; Schlagbauer & Holz, 1989b), stomata and lenticels (Den Breeÿen, 1993) as primary sites for penetration. As was found on nectarine (Part 3), the monitoring of airborne conidia revealed subtle effects of plum fruit on the behaviour of solitary germlings, which could not be seen when using conidial suspensions. On both fruit types, no deleterious effect was seen on conidial and germling survival when fruit were kept humid at pit hardening, 2 wk before harvest and harvest. However, conidial and germling survival were drastically reduced by prolonged wet incubation of fruits. The findings on disease expression in the skin segment, paraquat-treated fruit and sound fruit tests clearly showed that the skin of both nectarine and plum fruits were not penetrated at the pit hardening stage, latent infections were not established and fruits reacted resistant to disease expression. These facets on both fruit types were furthermore unaffected by wetness. The barrier capacity of the fruit skin of the two stone fruit types however differed drastically later in the season. On nectarine, fruit skins were more readily penetrated and disease expression became more pronounced when fruit approached maturity (Part 3). Penetration and disease expression on ripening nectarine fruit were furthermore greatly influenced by wetness. The segment and paraquat-treated fruit tests showed that at the 2 wk before harvest stage, more skins were penetrated and more latent infections were established on wet than humid fruit. The levels of skin penetration and latent infection increased on fruit inoculated at harvest stage and were even higher on fruit inoculated after cold storage. Levels were furthermore consistently higher on wet than on humid fruit. Maturing plum fruit, on the other hand, did not display the drastic change in the

barrier capacity of fruit skins as observed on nectarine. The influence of wetness on infection and disease expression was also less pronounced than on nectarine. In fact, fruit remained asymptomatic in the sound fruit test after inoculation and humid incubation at the 2 wk before harvest stage, harvest stage and after cold storage. Fruit at these stages only developed disease after a prolonged period (≥ 12 h) of wet incubation. The paraquat fruit test revealed that these fruits became more susceptible to latent infection, but they were not as susceptible as nectarine.

The behaviour of airborne inoculum, based on fluorescence microscopy of the stained segments, complemented these findings. Germination generally proceeded at a much lower rate on plum than on nectarine fruit. This effect was most pronounced on humid fruit, where germ tubes were usually first recorded at 12 hpi, contrary to 3 hpi on nectarine. Unlike the behaviour on nectarine fruit, where it was found that as the fruit reached maturity, proportionally more germlings grew in close proximity of penetration sites, and penetrated them (Part 3), proportionally less germlings grew in the vicinity of these sites on ripening plum fruit. This was partly ascribed to the fact that four to five times less stomata or lenticels occurred on ripening *Laetitia* plums compared with the 0.8 stomata/mm² counted at pit hardening. The tendency to grow towards a specific site, and to enter, was also influenced by fruit wetness. Markedly less germlings grew in the vicinity of penetration sites on humid fruit, and penetration was observed only once. Fruit phenology also influenced germling behaviour. On plum fruit at pit hardening stage germlings were inclined to grow primarily towards stomata, and predominantly entered stomata. This tendency changed when fruit were inoculated at 2 wk before harvest and at harvest. On these fruits, germlings were attracted predominantly by lenticels and entered proportionally more of these structures than stomata or microfissures.

The marked effect of fruit phenology and wetness regime on germination, morphogenesis of germ tubes and germling viability on nectarine fruit was partially attributed to the effects of substances in fruit exudates, wax layers or other skin components on the solitary conidia and germlings (Part 3). Phenolic compounds in the epidermal cell layer, which are associated with host resistance, decline with fruit maturity (Bostock *et al.*, 1999), whereas sugar concentrations in the exudates of plum fruit are low prior to pit hardening, but increase rapidly in the 2 weeks prior to harvest (Fourie & Holz, 1998). Substances in fruit

exudates inhibitory or stimulatory to growth will not be readily available to the fungus when an individual conidium germinates under high humidity on the dry fruit surface. On the other hand, exudates will easily dissolve in the film of water on wet fruit and rapidly be taken up by solitary conidia on the fruit surface. Diffusion would furthermore ensure a constant supply of these substances to the germ tube and hyphae of individual germings. Since the viability of the inoculum used in this study was proven on PDA, the lack of germination and decline in viability on plum fruit must also be ascribed to the effects of substances in fruit exudates, wax layers or other skin components. Increased wax buildup during cold storage, and therewith the increased accumulation of substances inhibitory to the fungus, might also be partially responsible for the more pronounced dieback and reduced symptom expression on wet cold stored fruit compared with the harvest stage fruit. Epicuticular waxes have been shown to protect apple leaves against *Sphaerotheca pannosa* (Martin *et al.*, 1957), *Ginkgo biloba* against *M. fructicola* and other fungi (Major *et al.*, 1960; Adams *et al.*, 1962), cherry leaves against *Stemphylium sarcinaeforme* (Johnston & Sproston, 1965) and beetroot leaves against *Botrytis cinerea* (Blakeman & Szejnberg, 1973).

Schlagbauer and Holz (1989b) studied the penetration of plum by *M. laxa* and the histology of small pinpoint necrotic lesions observed on immature fruit approximately 5 wk after inoculation with spore suspensions. The authors described plum fruit as highly resistant throughout the growing season. Light and scanning electron microscopy of latent infections on immature plum fruit revealed extensive periderm formation in the cortex beneath the necrotic tissue, with evidence of gum deposits and the presence of suberin or lignin. The inability of these lesions to yield *M. laxa* indicated that necrotic lesions on plums were due to host defence reactions leading to loss of pathogen viability. Consequently Schlagbauer and Holz (1989b) concluded that long-term latent infections of plum fruit by *M. laxa* were unimportant. Resistance of stone fruit to *M. laxa* prior to the pit hardening stage has also been reported by other workers (Kable, 1971; Fourie & Holz, 1987). A recent study (Part 2) on natural infection, conducted over a 3-year period on plum and nectarine fruit from 11 stone fruit orchards, indicated that fruit generally remained free of latent infection at the shuck fall and pit hardening stages and only developed symptoms when sampled 2 wk before harvest and at harvest. Data obtained with solitary *M. laxa* conidia on nectarine (Part 3) provide additional evidence for the unimportance of long-term latency, but stress the importance of short-term latency in disease outbreaks on nectarine. Collectively, these

findings indicate that *M. laxa* fruit rot epidemics on plum and nectarine are driven by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period. However, the barrier capacity of plum skins is considerably more effective than that of nectarine fruit. Unlike nectarine fruit, infection of intact plum fruit during humid conditions is negligible and relatively low levels of successful penetration would almost exclusively occur following precipitation during the ripening stages. No decline in the viability of surface inocula was however observed on humid fruit, emphasising the importance of latent contamination (Jerome, 1958) on this fruit type. Wounds would therefore play an important role in the epidemiology of *M. laxa* on plum fruit. Disease management strategies should therefore focus on the eradication of inoculum sources that contribute to latent contamination, eradication of latent conidia on the more susceptible mature fruits and disease prediction during the preharvest period. Furthermore, as the skin of plum acts as an effective barrier to *M. laxa*, more emphasis should be placed on the careful handling of fruit and the prevention of injury during harvest and handling practices. However, information describing inoculum sources, wounding and fruit rot epidemics of stone fruit by *M. laxa* is lacking. More information is therefore needed on these aspects of *M. laxa* to fully understand its biology, epidemiology and control on stone fruit.

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Table 1. Analysis of variance for effects of growth stage (G), wetness regime (humid[H]/wet[W]) and incubation period (T) on germination (%) of airborne conidia of *Monilinia laxa* on surfaces of Laetitia plum fruit

Source of variation	Df	SS	MS	SL
Model	29	52946	1826	0.0001
G	2	380	190	0.4460
W/H	1	16268	16268	0.0001
T	4	22609	5652	0.0001
G x W/H	2	678	339	0.2407
G x T	8	3504	438	0.0791
W/H x T	4	8244	2061	0.0001
G x W/H x T	8	1264	158	0.7072
Error	60	13940	232	
Corrected total	89	66886		

Table 2. Analysis of variance for effects of growth stage (G), wetness regime (humid[H]/wet[W]) and incubation period (T) on the growth (average length in μm) of *Monilinia laxa* germ tubes on surfaces of Laetitia plum fruit

Source of variation	Df	SS	MS	SL
Model	29	141093	4865	0.0001
G	2	21691	10846	0.0012
W/H	1	18523	18523	0.0007
T	4	41179	10295	0.0001
G x W/H	2	18279	9140	0.0032
G x T	8	15535	1942	0.2410
W/H x T	4	9627	2407	0.1704
G x W/H x T	8	16259	2032	0.2133
Error	60	86830	1447	
Corrected total	89	227923		

Table 3. Germination pattern of airborne *Monilinia laxa* conidia on surfaces of humid^a or wet^b Laetitia plum fruit

Growth stages	Germ tubes observed	Germination pattern					
		One germ tube formed (%)			Two germ tubes formed (%)		
		Total	Unbranched	Branched	Total	Unbranched	Branched
Humid fruit							
Pit hardening	397	98.0	93.5	4.5	2.0	2.0	0
2 wk before harvest	186	100	95.2	4.8	0	0	0
Harvest	154	100	88.2	11.8	0	0	0
Wet fruit							
Pit hardening	508	98.6	97.6	1.0	1.4	1.4	0
2 wk before harvest	498	99.6	99.0	0.6	0.4	0.4	0
Harvest	367	97.3	94.3	3.0	2.7	2.4	0.3

^a Fruit incubated at high relative humidity ($\geq 93\%$ RH).^b Fruit overlaid with wet paper towels.**Table 4.** Behaviour of germ tubes of solitary *Monilinia laxa* germlings growing in proximity of natural openings^a on humid^b or wet^c Laetitia plum fruit

Growth stages	Germ tubes observed	Germ tubes (%) in proximity of natural openings			
		Total	Missing	Towards	Penetrating
Humid fruit					
Pit hardening	397	7.5	3.5	4.0	0
2 wk before harvest	186	2.0	1.0	0.5	0.5
Harvest	154	2.5	0.6	1.9	0
Wet fruit					
Pit hardening	508	11.5	1.4	6.9	3.2
2 wk before harvest	498	5.4	0.4	3.8	1.2
Harvest	367	7.0	0.8	1.6	4.6

^a Stomata, lenticels or microfissures.^b Fruit incubated at high relative humidity ($\geq 93\%$ RH).^c Fruit overlaid with wet paper towels.

Table 5. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germlings growing on Laetitia plum fruit at the pit hardening stage

Source of variation	Df	SS	MS	SL
Model	59	163678	2774	0.0001
M	2	56203	28102	0.0001
W/H	1	2687	2687	0.0001
S/NS	1	26728	26728	0.0001
T	4	1661	415	0.0001
M x W/H	2	4499	2250	0.0001
M x S/NS	2	53456	26728	0.0001
M x T	8	2437	305	0.0001
W/H x S/NS	1	2046	2046	0.0001
W/H x T	4	697	174	0.0012
S/NS x T	4	1162	290	0.0001
M x W/H x S/NS	2	4091	2046	0.0001
M x W/H x T	8	2180	272	0.0001
M x S/NS x T	8	2323	290	0.0001
W/H x S/NS x T	4	1170	292	0.0001
M x W/H x S/NS x T	8	2339	292	0.0001
Error	120	4332	36	
Corrected total	179	168010		

Table 6. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germlings growing on Laetitia plum fruit at the 2 wk before harvest stage

Source of variation	Df	SS	MS	SL
Model	59	1987838	3368	0.0001
M	2	50274	25137	0.0001
W/H	1	2036	2036	0.0005
S/NS	1	19043	19043	0.0001
T	4	15534	3884	0.0001
M x W/H	2	8555	4277	0.0001
M x S/NS	2	67624	33812	0.0001
M x T	8	7393	924	0.0001
W/H x S/NS	1	1703	1703	0.0015
W/H x T	4	9905	2476	0.0001
S/NS x T	4	1419	355	0.0731
M x W/H x S/NS	2	5522	2761	0.0001
M x W/H x T	8	3231	404	0.0150
M x S/NS x T	8	2205	276	0.1032
W/H x S/NS x T	4	2873	718	0.0022
M x W/H x S/NS x T	8	1422	178	0.3668
Error	120	19365	161	
Corrected total	179	218103		

Table 7. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germlings growing on Laetitia plum fruit at the harvest stage

Source of variation	Df	SS	MS	SL
Model	59	219433	3719	0.0001
M	2	37361	18681	0.0001
W/H	1	7363	7363	0.0001
S/NS	1	34661	34661	0.0001
T	4	26174	6544	0.0001
M x W/H	2	8286	4143	0.0001
M x S/NS	2	66045	33023	0.0001
M x T	8	18815	2352	0.0001
W/H x S/NS	1	991	991	0.0177
W/H x T	4	8528	2132	0.0001
S/NS x T	4	1111	278	0.1737
M x W/H x S/NS	2	3482	1741	0.0001
M x W/H x T	8	3202	400	0.0229
M x S/NS x T	8	930	116	0.7103
W/H x S/NS x T	4	472	118	0.6020
M x W/H x S/NS x T	8	2011	251	0.1766
Error	120	20578	171	
Corrected total	179	240011		

Table 8. Analysis of variance for effects of test method (M), wetness regime (humid[H]/wet[W]), sterility regime (sterile[S]/unsterile[NS]) and incubation period (T) on disease expression by solitary *Monilinia laxa* germlings growing on cold stored Laetitia plum fruit

Source of variation	Df	SS	MS	SL
Model	59	202631	3434	0.0001
M	2	63357	31679	0.0001
W/H	1	3319	3319	0.0001
S/NS	1	23295	23295	0.0001
T	4	16857	4214	0.0001
M x W/H	2	14269	7135	0.0001
M x S/NS	2	45985	22992	0.0001
M x T	8	3963	495	0.0008
W/H x S/NS	1	590	590	0.0396
W/H x T	4	10440	2610	0.0001
S/NS x T	4	703	176	0.2776
M x W/H x S/NS	2	1536	768	0.0046
M x W/H x T	8	8623	1078	0.0001
M x S/NS x T	8	8046	1006	0.0001
W/H x S/NS x T	4	904	226	0.1640
M x W/H x S/NS x T	8	743	93	0.7071
Error	120	16350	136	
Corrected total	179	218981		

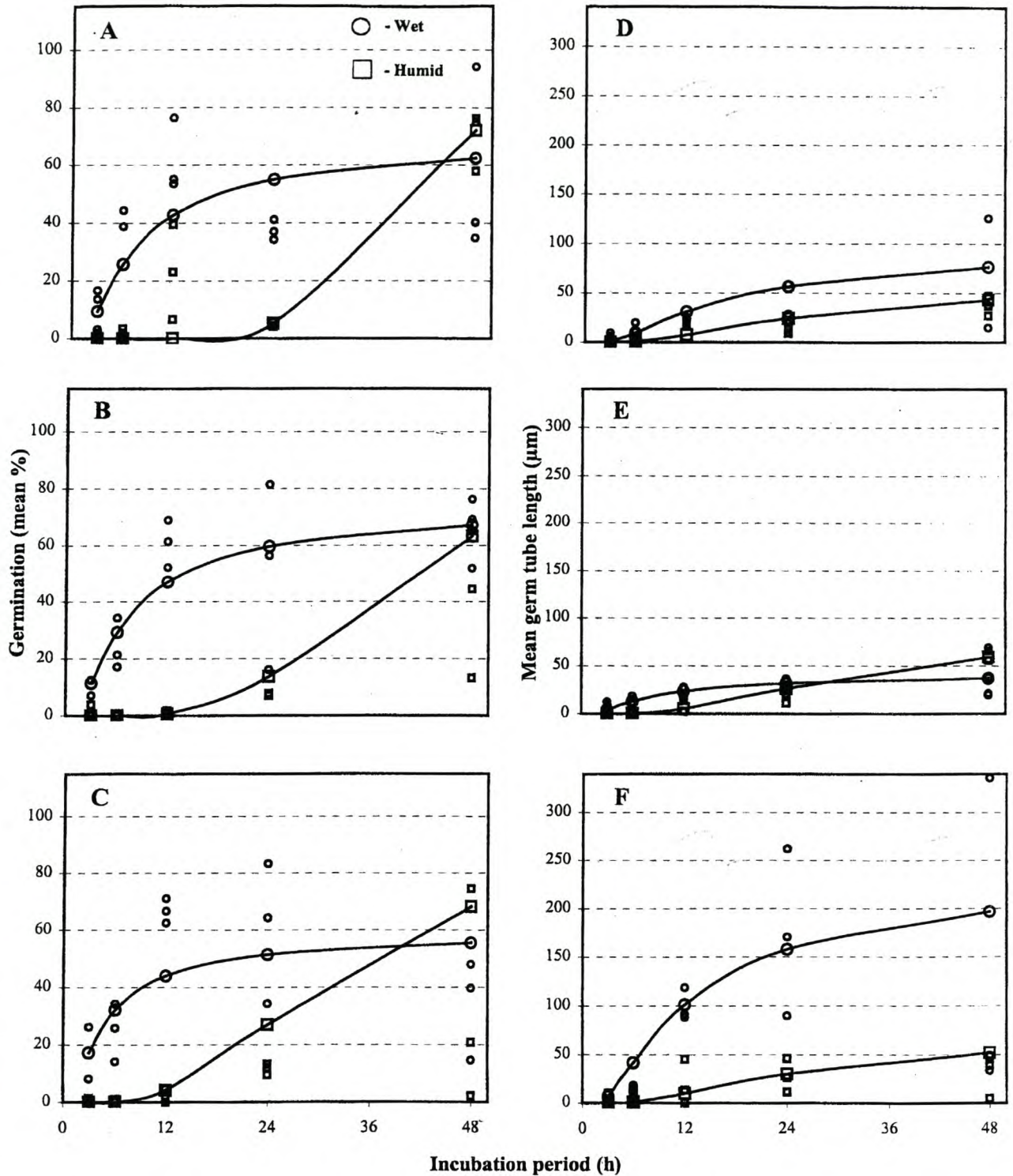


Figure 1. Effect of wetness regime and incubation period on germination rate and germ tube growth of airborne conidia of *Monilinia laxa* on Laetitia plum fruit at pit hardening (A,D), 2 wk before harvest (B,E) and harvest (C,F) stage. Points represent actual means of germination percentages or germ tube lengths (µm) of three experiments, whereas lines represent predicted values based on a non-linear growth curve [$y=A\exp(B/x)$] fitted to the point data to observe and compare trends.

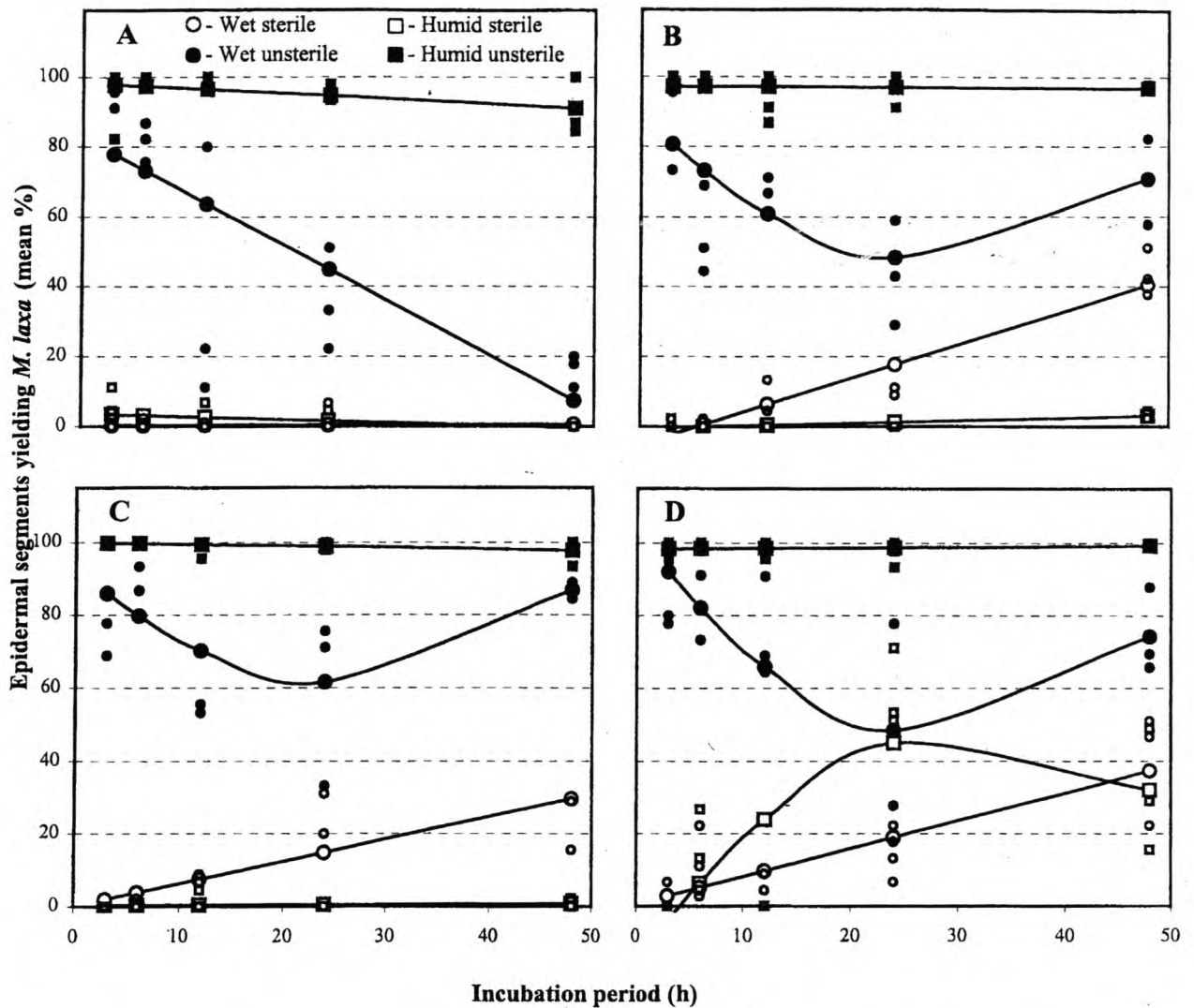


Figure 2. Effect of wetness regime and incubation period on the surface colonising ability and infectivity of solitary *Monilinia laxa* germlings on Laetitia plum fruit at pit hardening (A), 2 wk before harvest (B), harvest stage (C) and after cold storage (D). Surface colonising ability is represented by the percentage segments removed from unsterile fruit at each incubation period that yielded the pathogen after 14 days incubation. Infectivity is represented by the percentage segments removed from surface-sterilised fruit that yielded the pathogen. Points represent actual means from three experiments, whereas lines represent predicted values based on linear or quadratic regression equations derived from the point data.

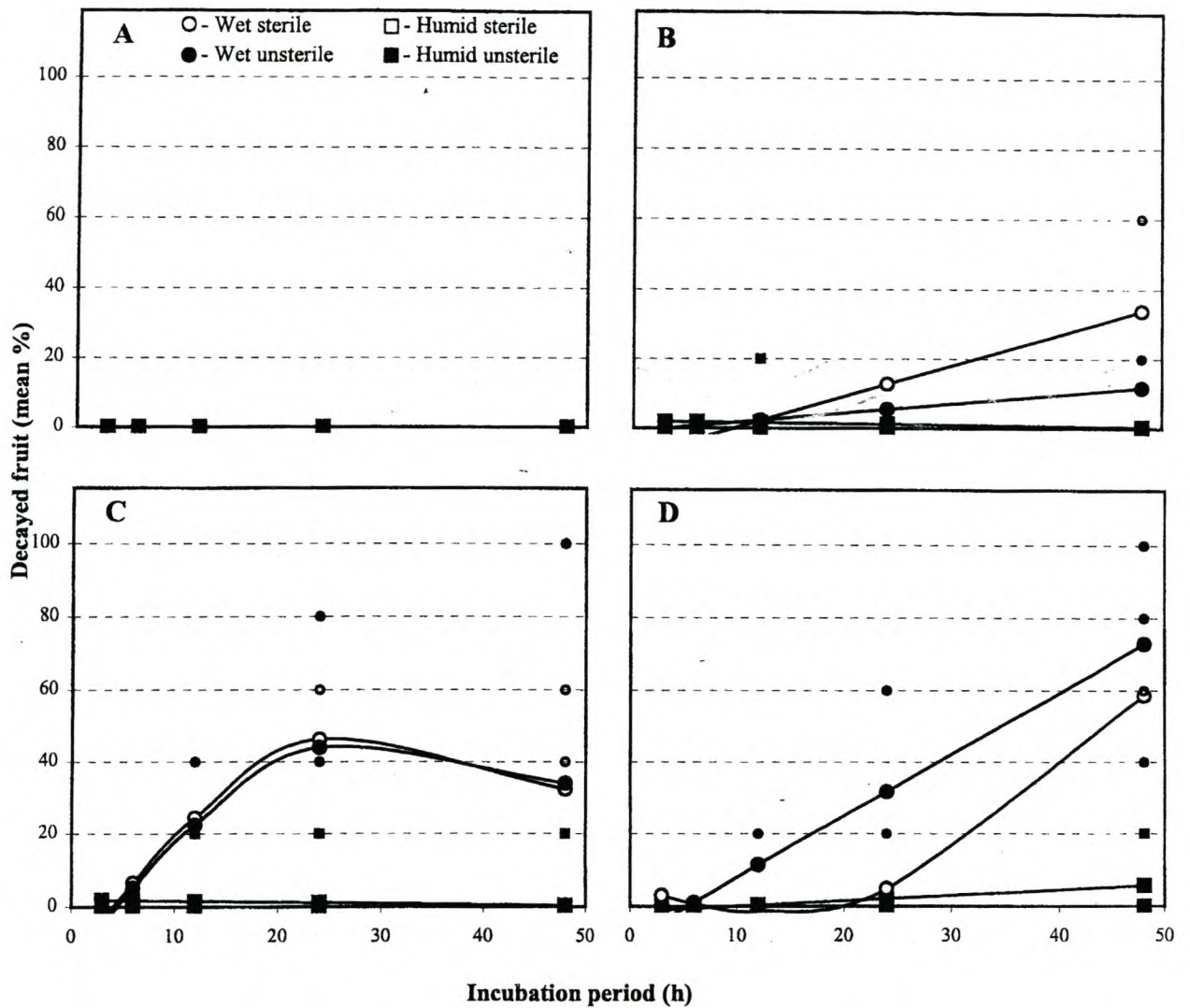


Figure 3. Effect of wetness regime and incubation period on disease expression by solitary *Monilinia laxa* germlings on Laetitia plum fruit at pit hardening (A), 2 wk before harvest (B), harvest stage (C) and after cold storage (D). Fruits were incubated at a specific wetness regime, then kept dry. Points represent mean percentages decayed fruit recorded in three experiments, whereas lines represent predicted values based on linear or quadratic regression equations derived from the point data.

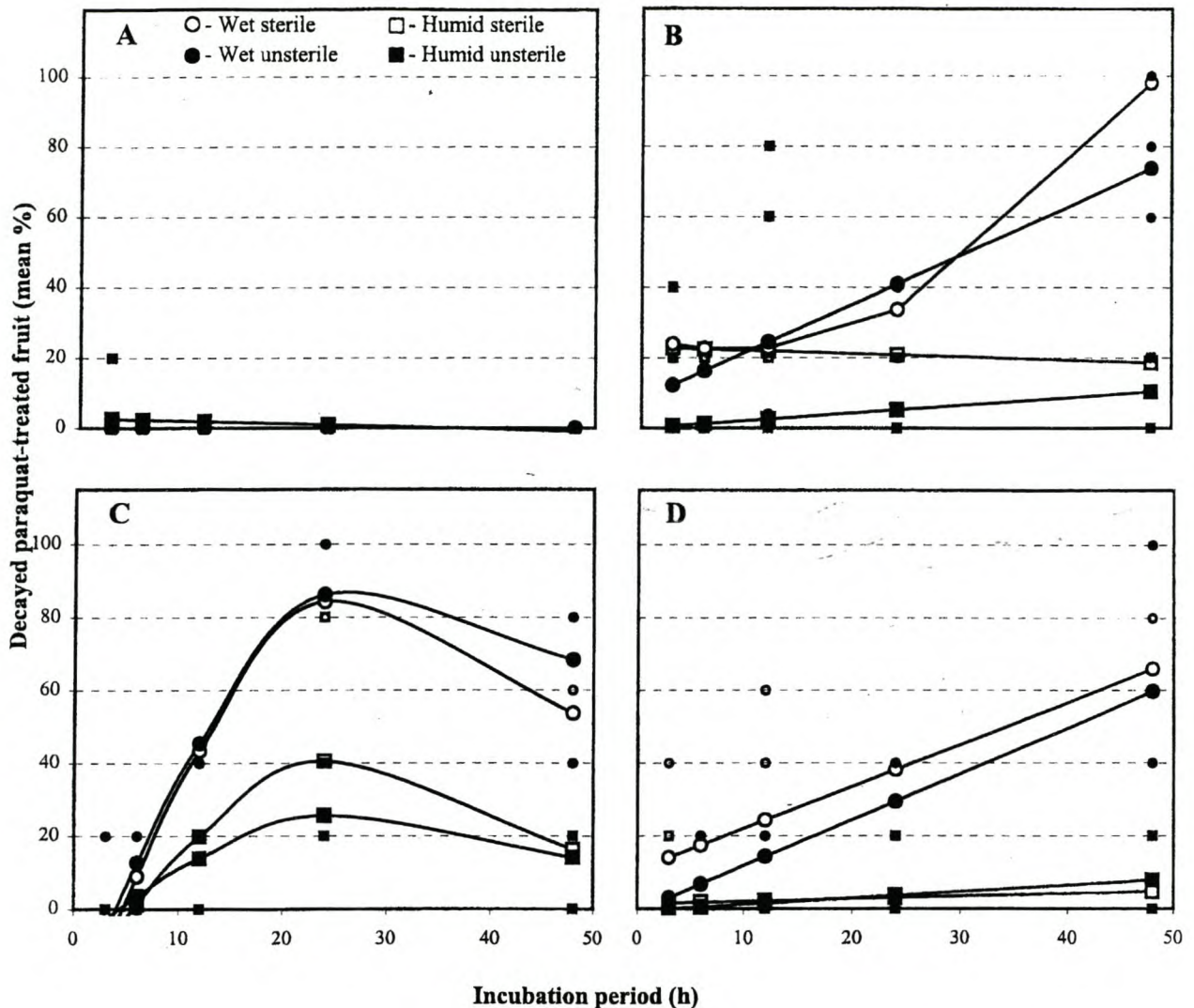


Figure 4. Effect of wetness regime and incubation period on disease expression by solitary *Monilinia laxa* germlings on Laetitia plum fruit at pit hardening (A), 2 wk before harvest (B), harvest stage (C) and after cold storage (D). Fruits were removed after each incubation period at a given wetness regime, immersed in 3% paraquat and rinsed in water, then kept dry. Points represent mean percentages decayed fruit recorded in three experiments, whereas lines represent predicted values based on linear or quadratic regression equations derived from the point data.

5. WOUND INFECTION OF PLUM FRUIT BY AIRBORNE CONIDIA OF *MONILINIA LAXA*

ABSTRACT

Plum fruit (cultivar Laetitia) at pit hardening, 2 wk before harvest, harvest stage and after cold storage were dusted with dry conidia of *Monilinia laxa* in a settling tower. Infection of nonwounded and freshly wounded fruit by the airborne conidia on dry, humid and wet plum fruit surfaces, and by conidia and germlings that had been established on fruits under these wetness regimes, was then investigated. Nonwounded immature and mature fruit remained mostly asymptomatic, whereas nonwounded cold stored fruit decayed readily. Wounding drastically increased infection by airborne conidia. Immature fruits were less susceptible to wound infection by the airborne conidia than mature fruits. Conidia that were dispersed freshly were more successful in infecting fresh wounds than conidia that were deposited, on germlings that established, on fruits 4 days prior to wounding. This decrease in infectivity was especially pronounced on humid- and even more on wet incubated fruit. This study clearly showed that in order to reduce the incidence of brown rot, inoculum levels on fruit approaching maturity should be reduced by sanitary practices and fungicides. Furthermore, it is essential to protect fruit, especially near-mature fruit, from being wounded.

INTRODUCTION

In the stone fruit producing regions of the Western Cape province of South Africa, brown rot of nectarine and plum is considered the most destructive phase of the *Monilinia laxa* disease syndrome (Fourie & Holz, 1985a; Schlagbauer & Holz, 1987; Part 2). Several reports indicated that on both fruit types, infection is established when fruits are approaching maturity (Kable, 1971; Schlagbauer & Holz, 1989a; 1989b; Part 2; Part 3; Part 4). Fruit generally remained free of latent infection at the shuck fall and pit hardening stages and only developed symptoms on mature fruit. Infection studies with solitary conidia provided evidence for the importance of short-term latency of *M. laxa* on nectarine (Part 3), but not on plum (Part 4). The studies furthermore showed that the barrier capacity of skins of the two

fruit types differed drastically later in the season. On nectarine, fruits skins were more readily penetrated and disease expression became more pronounced when fruit approached maturity. Maturing plum fruit, on the other hand, did not display this drastic change in the barrier capacity of fruit skins observed on nectarine. Collectively, the findings indicate that *M. laxa* fruit rot epidemics on plum and nectarine may be driven by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period. The role of latent contamination (Jerome, 1958) may thus be underestimated in the epidemiology of *M. laxa* on plum and nectarine fruit.

The mode of penetration of fruits by the brown rot fungi *M. fructicola*, *M. laxa* and *M. fructigena*, and subsequent disease expression, have not been well documented. Infection by these pathogens is mainly associated with wounds on fruit (Byrde & Willetts, 1977; Fourie & Holz, 1985b; Xu & Robinson, 2000). However, in the laboratory, entry of fruits inoculated with conidial suspensions has been observed through undamaged surfaces, including direct penetration of the cuticle by appressoria of *M. fructicola* (Cruickshank & Wade, 1992b) and structures such as hair sockets, lenticels and stomata (Curtis, 1928; Smith, 1936; Hall, 1971; Byrde & Willetts, 1977; Willetts & Bullock, 1993). By working with airborne conidia of *M. laxa*, it was demonstrated that germlings do not penetrate nectarine (Part 3) or plum (Part 4) fruit directly, but entered through stomata, lenticels and microfissures in the fruit skin. It was furthermore suggested that as the skin of plum acts as an effective barrier to *M. laxa*, more emphasis should be placed on the careful handling of fruit and the prevention of injury during harvest and handling practises. However, information describing wounding and fruit rot epidemics of stone fruit by *M. laxa* is lacking. The aim of this study was (i) to determine the infection of fresh wounds by airborne *M. laxa* conidia on dry, humid and wet plum surfaces, and (ii) to investigate whether conidia and germlings that have been established on fruits under this range of wetness regimes can infect fresh wounds. The inoculation, incubation and wounding techniques used simulate infection under natural conditions.

MATERIALS AND METHODS

Fruit. A plum orchard (cultivar Laetitia) with a history of low levels of brown rot incidence was selected in the Blaauwklippen valley, Stellenbosch. Four weeks prior to the pit hardening stage, a section of this orchard was demarcated and no fungicides were applied.

Sound, unblemished fruit were selected at pit hardening, 2 wk before harvest, and at the harvest stage from these trees. Fruit obtained at harvest stage were either used, or kept under conditions simulating overseas shipment and marketing before being used (10 days at -0.5°C , 18 days at 7.5°C followed by 1 wk at 23°C at $\pm 56\%$ RH). Before usage, fruits were surface sterilised (30 s in 70% ethanol, 2 min in 2% sodium hypochlorite, 30 s in 70% ethanol), packed on sterile, epoxy-coated steel mesh screens ($53 \times 28 \times 2$ cm) and allowed to air-dry. Picking wounds at or near the peduncle-end were covered with petroleum jelly. In order to recognize the inoculated cheek of the fruit at a later stage, a 0.5 cm mark was made near the peduncle-end with a soft-tipped koki pen. Preliminary studies showed no phytotoxic effect. Before inoculation, surface sterilised fruit were kept for at least 24 h in ethanol-disinfected perspex (Cape Plastics) chambers ($60 \times 30 \times 60$ cm) at 22°C at $\pm 56\%$ RH to allow re-establishment of surface nutrients.

Inoculation. A virulent *M. laxa* isolate, sensitive to triforine, iprodione and benomyl and obtained from a naturally-infected nectarine fruit, was maintained in the laboratory at 22°C on a synthetic agar medium amended with sugars, minerals and malic acid at concentrations occurring in grape berry exudates (1.85 g glucose; 1.95 g fructose; 0.25 g sucrose; 0.15 g malic acid; 5 g peptone; 5 g sodium chloride; 15 g agar; and 2 g yeast extract per liter deionised water), or was kept on malt extract agar (MEA) slopes at 5°C in the dark. Inoculum was prepared by inoculating ripe surface-sterilised nectarines with mycelium discs or conidia obtained from fresh cultures growing on potato dextrose agar (PDA). Inoculated fruit were incubated for 10 to 14 days at 22°C on screens in humid perspex chambers (see below) to allow infection, colonisation and profuse sporulation by *M. laxa*. The mummified fruit were then kept in dry chambers at $\pm 56\%$ RH. For inoculation, a mummified fruit was placed on a shelf 10 cm below the ceiling of a spore settling tower ($1.5 \times 1.0 \times 1.5$ m [length x width x height]). Conidia were blown for 1 s from the mummy with a pressure pump (Rietzchle VTE 3 [$3.5\text{--}4.2$ m³/h]) and the lid in the ceiling closed. The conidia were allowed 10 min to settle onto the fruit, which were positioned on three screens on the floor of the tower. Petri dishes with 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).

Infection of fresh wounds by fresh conidia. Wounds (10 wounds per fruit, 30 fruits per sampling) were made on the marked side of fruit with a wound maker, consisting of a cork stopper, dome shaped to fit onto the fruit cheek, through which five insect needles protruded in a criss-cross pattern. The needles were 5 mm apart and inflicted wounds 1.5 mm deep. After each wounding, the wound maker was sterilised by pressing it for five seconds onto an ethanol drenched cotton wool swab. After wounding, the fruit were kept for 1 h at low humidity ($\pm 65\%$ RH). Preliminary microscopic examinations showed that within this 1 h period exudates exuded from the wound onto a small fringe of the surrounding skin and then retracted. Control fruit (30 fruit per sampling) were left nonwounded. The wounded and nonwounded fruit were then inoculated and incubated at 22°C under dry, humid or wet conditions. For dry incubation, perspex chambers were kept dry ($\pm 65\%$ RH). For humid incubation, perspex chambers were lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity ($\geq 93\%$ RH). For wet incubation, perspex chambers were lined with a sheet of chromatography paper as described before, and fruit were overlaid with sterile paper towels soaked with sterile deionised water. These conditions provided three different moisture regimes for the pathogen; i.e. dry conidia on dry fruit at low humidity (dry), dry conidia on dry fruit at high humidity (humid), and dry conidia on fruit covered in a film of water (wet). After 24 h the fruit were removed from the chambers and the wet paper towels carefully removed from the wet incubated fruit. The fruit were packed into cartons and kept for a further 10 days at 23°C under dry conditions ($\pm 56\%$ RH).

Infection of fresh wounds by established inocula. Sound unblemished fruit (30 per sampling) were inoculated and incubated in the perspex chambers under the set of wetness regimes described above. After 24 h the fruit were removed from the chambers, the wet paper towels carefully removed from the wet incubated fruit and the fruit air-dried. The air-dried fruit were packed into cartons, incubated for an additional 72 h at 23°C under dry conditions ($\pm 56\%$ RH) to establish germling growth and penetration and then wounded as described above. Control fruit (30 per sampling) were left nonwounded. The wounded and nonwounded fruit were kept for a further 10 days before decay was assessed.

Decay assessment and statistical analyses. Inoculated fruit were inspected daily, the number of infected wounds recorded and the percentage decayed wounds per fruit (decay

severity) was calculated. The incidence of decay (percentage of decayed fruit) was recorded for the nonwounded fruit. The trials were repeated twice. Analysis of variance of a completely randomised split-plot design was done using SAS. Least significant difference values were obtained and the means compared using Student's *t*-test.

RESULTS

Analyses of variance were done on the mean decay incidence on nonwounded and wounded fruit (Table 1) and on the percentage wounds that developed decay (Table 2). Three-factor interactions were observed between stage, wound and incubation treatment for decay incidence and percentage decaying wounds.

Conidia used at each inoculation were highly viable and germinated freely on PDA. Germination usually varied between 98–100% at 6 hpi. Decay incidences and percentage wounds infected are given in Tables 3 and 4, respectively. On the nonwounded fruit no decay developed at any growth stage, irrespective of incubation conditions. An exception was found at the harvest stage, where 6.7% of fruit decayed after wet incubation. Cold stored fruit, on the other hand, were susceptible and a minority of the fruit decayed in both the dry and humid treatments. Wet incubation markedly increased decay incidence.

The ability of fresh conidia to infect fresh wounds was influenced by fruit phenology and wetness (Tables 3 and 4). On immature fruit, decay at the wound sites became visible at approximately 48 hpi and on mature fruit at 24 hpi. Lesions on immature fruit were restricted and leathery, but soft and expanded fast on mature fruit. At pit hardening none of the fruits decayed when incubated in the dry regime. Incubation under high humidity or wet conditions facilitated decay at the wound sites. Decay levels were however relatively low under both wetness regimes and decay developed at a minority of the wound sites. Wounding drastically affected decay levels when fruits were wounded 2 wk before harvest or at harvest stage. Incidences were unaffected by wetness regime and nearly all the fruit incubated under the dry, humid and wet conditions developed decay. Wetness had no effect on the percentage wound sites that developed decay on fruit inoculated 2 wk before harvest, but the percentage was significantly increased on fruit inoculated at harvest by incubation under wet conditions. A similar trend was found on cold stored fruit.

The ability of established inocula to infect fresh wounds differed from the trend described for fresh inoculum. Firstly, on immature fruit decay at the wound sites became visible approximately 72 hpi and on mature fruit 60 hpi. Secondly, at pit hardening a relatively high proportion of fruits that were dry incubated decayed and decay levels were significantly lower on fruit that were humid- or wet incubated. Furthermore, significantly more wound sites on fruits kept dry, than on humid or wet fruit, developed decay. Thirdly, a similar behaviour was found on fruit inoculated at the other ripening stages and on cold stored fruit. However, significantly more fruit developed decay at the latter stages than at pit hardening and significantly more wound sites became infected.

DISCUSSION

The inoculation technique used in the present study simulates natural dispersal of airborne conidia, and allows for the deposition of separate, dry conidia on fruit surfaces. Approximately 9 conidia per mm² were deposited on the upward facing cheeks (approximately 400 mm²) of plum fruits. The fruits were either nonwounded, or had 10 minute, artificially inflicted wounds. Ample opportunity for direct penetration or contact between wounds and solitary conidia or germlings was therefore provided on dry, humid or wet plum fruit, conditions which normally prevail in the orchard, fruit bins or storage cartons. Under the conditions provided in the study nonwounded immature and mature fruit mostly remained asymptomatic, whereas nonwounded cold stored fruit decayed readily. Wounding drastically increased infection by airborne conidia of *M. laxa*, which confirmed previous observations (Fourie & Holz, 1985b; 1987a) on the necessity of wounds for infection made with spore suspensions in the laboratory. Although it was shown that airborne conidia (Part 4) and conidia suspended in droplets (Curtis, 1928; Smith, 1936; Hall, 1971; Byrde & Willetts, 1977; Willetts & Bullock, 1993) penetrate plum fruits directly through natural openings, it was found that these infections not always led to fruit decay.

Immature fruits were less susceptible to wound infection by airborne *M. laxa* conidia than mature fruits. There are several possible explanations for this. Firstly, the inability of solitary *M. laxa* conidia to infect immature plum fruits has been correlated with poor growth caused by substances in exudates, wax layers or other skin components (Part 4). Phenols, particularly chlorogenic and caffeic acids, are high in resistant immature peach genotypes and

decline with fruit maturity (Bostock *et al.*, 1999). Working with *Botrytis cinerea*, Fourie and Holz (1998) showed that prior to the period of rapid cell enlargement, growth of this fungus on raised slides was inhibited by plum exudates. Secondly, germlings of *M. laxa* need an external supply of nutrients for germ tube differentiation and penetration of host surfaces since conidia of the brown rot fungi contain insufficient reserves (Willetts & Bullock, 1993). Sugar concentrations in the exudates of plum fruit are low prior to pit hardening (Fourie & Holz, 1998). At corresponding concentration, glucose, fructose and sucrose did not influence growth of *B. cinerea* in a mineral medium. Fungal growth was only enhanced when either of the reducing sugars or sucrose was supplied in excess of 0.27 and 0.14 mM, respectively. During the last 2 wk prior to harvest, total sugar in plum exudates was near these values. These effects were shown by solitary *M. laxa* conidia grown on humid and wet fruit (Part 4).

Conidia dispersed freshly were more successful in infecting fresh wounds than conidia that were deposited, or germlings that had established on fruits 4 days prior to wounding. This decrease in infectivity was especially pronounced on humid- and even more on wet incubated fruit. Fluorescence microscopy studies of the behaviour of *M. laxa* on nonwounded nectarine (Part 3) and plum (Part 4) surfaces showed that solitary conidia formed germ tubes within 3 hpi on both humid and wet fruit. Germination rates were higher and germ tube growth was more extensive on wet fruit. Wetness, however, had a negative effect on survival of the pathogen. Different criteria showed that free water on the fruit surface drastically reduced the viability of conidia and germlings. The deleterious effect of increased wetness on the survival of conidia therefore resulted in lower decay levels on fruit that were wounded 72 h after wet- or humid incubation. Decay levels on dry incubated fruit that were wounded after incubation were comparable to that observed on fruit wounded prior to inoculation, except on plum fruit from the pit hardening stage. These results therefore agree with conclusions by Naqvi and Good (1957) that very humid conditions were more detrimental to conidium survival than very dry conditions.

Germination and germ tube growth on fruit incubated under relatively dry conditions ($\pm 65\%$ RH) were not microscopically studied. Previous studies with nectarine (Part 3) and plum fruits (Part 4) have shown that airborne *M. laxa* conidia seldom land on stomata, lenticels or micro-fissures. Germlings usually entered these structures when they grew in close proximity to them. The tendency to grow towards a specific site and to penetrate was

furthermore enhanced by fruit wetness. Given the fact that wounded fruits that were kept dry decayed, the event of germination and germ tube growth at the wound site on these fruits must be accepted. Preliminary microscopic examinations showed that exudates exuded from the wound onto a small fringe of the surrounding skin and then withdrew within an hour. Wound infection on dry fruit indicates that the microclimate around the wound site may be conducive to germination. In the event of conidia deposited prior to wounding, exudate withdrawal may also carry the ungerminated conidia into the wound site, thereby enhancing infection. Wound exudates may therefore negate the need for free water and the readily available carbon sources, such as glucose, which are most important for successful infection (Wade & Cruickshank, 1992; Xu & Robinson, 2000).

It has been suggested (Parts 2,3,4) that *M. laxa* fruit rot epidemics on plum and nectarine are driven by inoculum levels on fruit approaching maturity and by weather conditions prevailing during the preharvest and harvest period. Latent contamination (Jerome, 1958) may therefore be of major importance in the epidemiology of *M. laxa* on plum fruit. This study clearly showed that in order to reduce the incidence of brown rot, inoculum levels on fruit approaching maturity should be reduced by sanitation practices and fungicide applications. Furthermore, it is essential to protect fruits, especially near-mature fruits, from being wounded. Careful handling during harvest and postharvest practices is therefore needed to prevent fruit from being damaged and infected.

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Table 1. Analysis of variance for effects of growth stage (G), wounding (I) and wetness regime (wet[W]/humid[H]/dry[D]) on decay (%) caused by airborne conidia of *Monilinia laxa* on surfaces of Laetitia plum fruit

Source of variation	Df	SS	MS	SL
Model	35	196166	5605	0.0001
G	3	53203	17734	0.0001
I	2	98941	49470	0.0001
W/H/D	2	2941	1470	0.0002
G x I	6	23511	3919	0.0001
G x W/H/D	6	1533	256	0.1275
I x W/H/D	4	9426	2356	0.0001
G x I x W/H/D	12	6611	551	0.0002
Error	72	10667	148	
Corrected total	107	206832		

Table 2. Analysis of variance for effects of growth stage (G), wounding (I) and wetness regime (wet[W]/humid[H]/dry[D]) on wound infection (%) caused by airborne conidia of *Monilinia laxa* on surfaces of Laetitia plum fruit

Source of variation	Df	SS	MS	SL
Model	23	23906	1039	0.0001
G	3	9603	3201	0.0001
I	1	7565	7565	0.0001
W/H/D	2	172	86	0.1573
G x I	3	2567	856	0.0001
G x W/H/D	6	542	90	0.0809
I x W/H/D	2	2248	1124	0.0001
G x I x W/H/D	6	1211	202	0.0010
Error	48	2143	45	
Corrected total	71	26049		

Table 3. Percentage plum fruit (cultivar Laetitia) at different growth stages that developed *Monilinia laxa* decay after being subjected to a differential set of inoculation, wounding and incubation treatments

Treatment ^{x,y}	Pit hardening ^z	2 wk before harvest	Harvest	Cold stored fruit
Nonwounded				
Dry	0.0 aA	0.0 aA	3.3 aA	6.7 aA
Humid	0.0 aA	0.0 aA	0.0 aA	3.3 aA
Wet	0.0 aA	0.0 aA	6.7 aA	23.3 abAB
Fresh wounds and fresh conidia				
Dry	26.7 abA	90.0 cB	100.0 cB	96.7 dB
Humid	6.7 aA	93.3 cB	100.0 cB	96.7 dB
Wet	13.3 aA	100.0 cB	100.0 cB	100.0 dB
Fresh wounds and established inocula				
Dry	0.0 aA	96.7 cB	83.3 cB	93.3 dB
Humid	3.3 aA	60.0 bB	93.3 cC	66.7 cB
Wet	0.0 aA	10.0 aA	56.7 bC	33.3 bB

^xNonwounded = unblemished fruit were dusted with conidia in a spore settling tower and incubated; fresh wounds and fresh conidia = fruits were wounded, dusted with conidia and incubated; fresh wounds and established inocula = fruits were dusted with conidia, incubated and freshly wounded.

^yDry = fruit incubated dry ($\pm 65\%$ RH); humid = fruit incubated at high humidity ($\geq 93\%$ RH); wet = fruit overlaid with wet paper towels.

^zMeans (%) followed by different small letters indicate significant difference between treatments (within columns), whereas capital letters indicate differences between stages (within rows). Least significant difference ($P = 0.05$) = 19.78.

Table 4. Percentage wounds infected by *Monilinia laxa* on plum fruit (cultivar Laetitia) at different growth stages after being subjected to a differential set of inoculation, wounding and incubations treatments

Treatment ^{x,y}	Pit hardening ^z	2 wk before harvest	Harvest	Cold stored fruit
Fresh wounds and fresh conidia				
Dry	4.3 aA	27.3 bB	40.3 cBC	31.3 bB
Humid	0.7 aA	31.0 bB	41.3 cBC	28.3 bB
Wet	1.7 aA	34.0 bcB	68.3 dD	52.0 cC
Fresh wounds and established inocula				
Dry	0.0 aA	22.3 bB	17.0 aB	23.7 bB
Humid	0.3 aA	8.3 aA	19.7 abB	11.3 aAB
Wet	0.0 aA	1.0 aA	7.6 aA	3.3 aA

^x Fresh wounds and fresh conidia = fruits were wounded, dusted with conidia in a spore settling tower and incubated; fresh wounds and established inocula = fruits were dusted with conidia, incubated and freshly wounded.

^y Dry = fruit incubated dry ($\pm 65\%$ RH); humid = fruit incubated at high humidity ($\geq 93\%$ RH); wet = fruit overlaid with wet paper towels.

^z Means (%) followed by different small letters indicate significant difference between treatments (within columns), whereas capital letters indicate differences between stages (within rows). Least significant difference ($P = 0.05$) = 10.97.