



The effect of grape berry growth stage on germination of *Botrytis cinerea* in New Zealand

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Abstract

The presence and germination rate of *Botrytis cinerea* conidia on ‘Sauvignon blanc’ grape berries from Pukekohe, New Zealand, was determined over the 2010–11 and 2011–12 growing seasons by means of inoculated berries. Conidia on the inoculated berries were able to germinate and enter the grape berries at any time during the season, albeit at low rates during the early season. In both years, the proportion of germinated conidia increased with berry age/growth stage. Microscopic examinations revealed that *B. cinerea* conidia germinated on the grape berry surface and hyphae entered the berries directly either between adjoining cells, by penetrating directly through the cell wall, or via appressoria. Penetration of the grape berry surface was highly variable, with some germ tubes penetrating immediately beneath the conidium and others having extended germ tubes. While most germinated conidia appeared to penetrate the berry, a proportion were seen to germinate to form spermatia (phialomicroconidia). Conidial anastomosis (two conidia connected by a short germ tube) was seen on the surface of some berries.

Keywords Botrytis bunch rot · Conidial germination · Spermatia · Conidial anastomosis · *Vitis vinifera*

Introduction

Botrytis bunch rot of grapes (BBR), caused by *Botrytis cinerea*, is a serious fungal disease in all major grape-producing regions of the world, particularly in regions with cooler climates (Beresford et al. 2006). The disease is exacerbated by frequent periods of rain and has been correlated with wetness duration and temperature (Broome et al. 1995).

The teleomorph state of *B. cinerea*, *Botryotinia fuckeliana*, was first documented by Groves and Drayton (1939), but is rarely seen in field situations and has not been recorded in the field in New Zealand (Beever and Parkes 1993). Thus the conidia of *B. cinerea*, rather than ascospores, remain the primary means of inoculum dispersal and berry infection (Choquer et al. 2007).

Elmer and Michailides (2007) identified five potential pathways for infection including infections via the stigma and style, via the pedicel and receptacle, via the berry skin from flowering through véraison to ripening, and via

wounds. The first infections of inflorescences occur prior to, and during, capfall from conidia originating on plant debris from the previous season. This stage is considered a critical period for the infection of berries by conidia lodged between the base of the ovary and receptacle in the region of the abscission zone of the cap (fused corolla), and the bases of the stamens (Cadle-Davidson 2008; Keller et al. 2003; Viret et al. 2004). Although infection via stigmas and styles at flowering has been shown to be a major pathway of infection for mid-season bunch rot in table grapes (McClellan and Hewitt 1973), it is considered to be of less importance in late-season BBR in wine grapes (Viret et al. 2004).

Following flowering, *B. cinerea* can colonise flower debris (dead caps, spent anthers) and aborted berries that become trapped between enlarging berries, providing an ongoing spore source for further infections of rachis and berries. Most early-season infections do not progress to symptoms. The pathogen typically remains latent in the tissues until the fruit begin to ripen, a phenomenon mediated by constitutive and induced resistance compounds such as proanthocyanidins, stilbenes, flavanol-glycosides and hydroxyl-cinnamic acid derivatives (Cadle-Davidson 2008; Hill et al. 1981; Keller et al. 2003; Kretschmer et al. 2007). While there is uncertainty over the specific mechanisms

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involved, there is general agreement that as the resistance mechanisms weaken during véraison, the latent infections become active and the fungus aggressively invades ripening berries (Cadle-Davidson 2008). Thereafter sporulation on early-rotting berries provides further inoculum for new infections.

Various studies have shown that spore germination and the ability of *B. cinerea* to penetrate intact plants is influenced by the availability of exogenous nutrients, particularly sugars (Benito et al. 1998; Cotoras et al. 2009; Kosuge and Hewitt 1964). Over the weeks leading up to harvest there is an increase in sugar content and a decrease in organic acids and antifungal compounds in the berries, and a corresponding increase in sugar exudates through the skin, particularly hexoses, glucose and fructose (Doehleemann et al. 2006; Kretschmer et al. 2007). Prior to those changes associated with ripening, the capacity of conidia to germinate, penetrate and form latent infections is likely to be governed by the availability of nutrients on the skin surface.

The various infection scenarios in grapes that ultimately lead to BBR have led to some uncertainty over the definition of ‘infection’ by *B. cinerea* in grape. Studies of *B. cinerea* infection of leaves of broad bean (*Vicia faba*) (McKeen 1974) and of tomato fruit (*Solanum lycopersici*) (Rijkenberg et al. 1980) have identified two distinct stages of infection, the first being the passage through the cuticle (by an enzymatic process) and the second being the penetration of the epidermal cell wall. In both those hosts there was no visible response from the underlying epidermal cell until the cuticle had been breached. Thereafter there were rapid changes in the epidermal cell including swelling, discolouration and degradation of the cell wall, formation of vesicles in the cytoplasm below the infection site and sometimes movement of the nucleus closer to the site of penetration. That was followed by entry of the infection hypha into the lumen of the cell. Rijkenberg et al. (1980) defined ‘successful penetration’ as the presence of fungal cells in both the swollen epidermal cell wall and cell lumen.

Autofluorescence is a common plant cell response to infection by fungi and oomycetes and is frequently associated with hypersensitive responses to infection; for example Bennett et al. (1996) working with *Bremia lactucae* on lettuce (*Lactuca sativa*), Ersek et al. (1982) with *Peronospora manshurica* on soybean (*Glycine max*) and Koga (1994) with *Pyricularia oryzae* on rice (*Oryza sativa*). Ersek et al. (1982) postulated that the compounds responsible for autofluorescence were associated with a hypersensitive resistance reaction by the cell when in intimate contact with the fungus. As cellular reactions to infection by *B. cinerea* do not occur until the cuticle has been penetrated and the infection peg is in contact with the wall of the epidermal cell (McKeen 1974; Rijkenberg et al. 1980), autofluorescence and discolouration of cells below the appressorium or infecting hypha may be

taken as an indication of successful breach of the cuticle and hence ‘infection’.

During the course of studies on BBR it was noted that the conidia of different strains of *B. cinerea* isolated from BBR affected bunches in New Zealand would germinate in water while others required sugar (1% sucrose) to germinate (unpublished data). This raised the question of whether strains that do not require sugar to germinate would be more likely to germinate and infect during the early stages of berry development, when surface nutrients are minimal, than those that do. In this study, changes in conidial germination rate and infection rate of two strains of *B. cinerea* (sugar-dependent and sugar-independent for germination) were measured during the period of development from flowering to berry ripeness.

Materials and methods

Botrytis cinerea isolates and conidial harvest

Two strains of *B. cinerea* originally isolated from naturally infected grape berries in New Zealand were used in this study. Strain cc487 was chosen for its low germination rate (c. 5%) in water, hereafter termed ‘nutrient-dependent’, and cc488 for its ability to germinate in water (c. 70%), hereafter termed ‘nutrient-independent’. Strain cc487 was able to germinate when sucrose was added to the water at a rate of 1%. Both isolates were from Pukekohe, New Zealand and are held in long-term storage in the New Zealand Institute for Plant & Food Research (Mt Albert, Auckland) Plant Pathology culture collection.

Both strains were maintained on Difco® Potato Dextrose Agar (PDA) at c. 21 °C under warm white fluorescent/near-UV light with a 12 h photoperiod. At each inoculation date, 18-day-old cultures were used for the conidial suspensions. To obtain clean, dry conidia of *B. cinerea*, the method of Cotoras and Silva (2005) was used. Petri dishes containing *B. cinerea* were inverted and tapped to dislodge conidia into the lids. Conidia deposited in the lids were collected and immediately suspended in sterile reverse-osmosis (RO) water. The concentration of the conidial suspensions were estimated with a haemocytometer and adjusted to $1\text{--}6 \times 10^6$ conidia/mL. At each inoculation date, the inoculum suspensions of the nutrient-independent and nutrient-dependent strains were re-checked for the ability to germinate in water and in 1% sugar solution.

Grape berry sample collection

Grape inflorescences, and subsequently bunches of berries, were collected weekly over the 2010–11 growing season, and fortnightly over the 2011–12 growing season, from an

experimental vineyard of ‘Sauvignon Blanc’ at the Pukekohe Research Centre, Plant & Food Research, Pukekohe, New Zealand. Single bunches were taken at random from individual vines at approximately 15 m intervals along the entire lengths of three rows of grapevines (c. 200 m long). The first three sampling times were pre-capfall. At each sampling date the soluble solids content (°Brix) of five grape berries was measured using an Atago® hand-held refractometer and the growth stage of the bunches was recorded using the numbered growth stages established by Eichhorn and Lorenz (1977). The °Brix, berry growth stage (Eichhorn and Lorenz 1977) and phenological stage at each harvest time are shown in Table 1. Fungicides specifically for the control of *B. cinerea* had not been applied to these vines. The fungicides mancozeb and sulphur were applied for downy mildew and powdery mildew control, respectively.

Laboratory inoculation

At each sampling date, five detached bunches of inflorescences or berries were inoculated with either sterile RO water (control, to assess background natural infection), the nutrient-dependent strain or the nutrient-independent strain. The bunches were not surface-sterilised prior to inoculation, firstly to allow natural inoculum to survive on the controls,

and secondly to replicate those conditions that would be encountered by natural infection. Berries were inoculated on the day they were collected.

The water control and the conidial suspensions were applied to the grape berries using an airbrush, which allowed an even application of inoculum. Inoculated grape bunches were inverted individually in jars, with stems in approximately 1 cm of water; lids were not completely sealed to allow a small amount of air exchange. Bunches were incubated at c. 20 °C for 3 days and then preserved in 95% ethanol.

Assessments

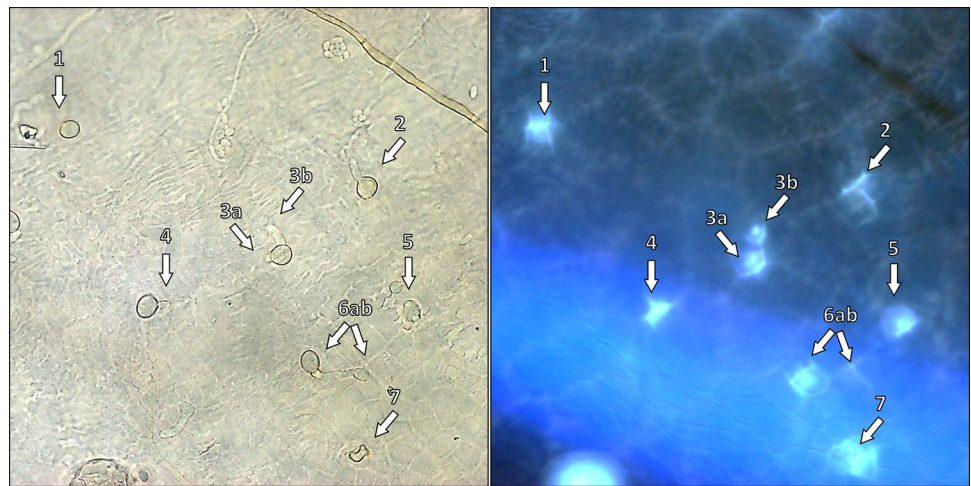
For histological examination, thin strips of the berry epidermis were excised by hand, stained with Trypan blue and examined under a light microscope at 60×10 magnification. Ten berries were examined from each bunch and, where possible, at least 100 conidia were examined on each berry. For the 2010–11 assessments, conidia were recorded as either germinated or not-germinated. Germinated conidia were defined as those that had a conspicuous germ tube whether an appressorium had formed or not and those that had initiated infection directly below or immediately beside the conidium, as indicated by brown discoloration of the

Table 1 Flower/berry bunch harvest date, °Brix and grape berry growth stage over the 2010–11 and 2011–12 growing seasons

Season	Date	°Brix	Grape berry growth stage*	Grape berry phenological stage	
2010–11	2/11/2010	-	12	inflorescence visible	
	16/11/2010	-	15	-	
	23/11/2010	-	16	-	
	30/11/2010	3.0	17	flowers separating	
	14/12/2010	4.0	25	80% of caps fallen	
	5/01/2011	4.0	31	-	
	11/01/2011	3.8	33	berry touch	
	18/01/2011	4.0	34	-	
	8/02/2011	8.3	34	-	
	22/02/2011	13.2	36	véraison	
	22/03/2011	17.7	38	-	
	2011–12	25/10/2011	-	12	inflorescence visible
		8/11/2011	-	15	-
22/11/2011		-	17	flowers separating	
6/12/2011		2.2	19	-	
20/12/2011		2.5	25	80% of caps fallen	
3/01/2012		4.5	27	-	
17/01/2012		3.6	30	-	
30/01/2012		4.2	33	berry touch	
14/02/2012		5.8	34	-	
28/02/2012		9.6	34	-	
13/03/2012	13.9	35	véraison		
27/03/2012	16.2	37	-		

* numbered growth stages established by Eichhorn and Lorenz (1977)

Fig. 1 *Botrytis cinerea* conidia infection of epidermal strip of grape berry. Left: View under transmitted normal light, Right: the same field of view under epifluorescence



subtending epidermal cell. For the 2011–12 assessments, in addition to recording germ tubes and infection as above, data were gathered on conidia germinating to form spermatia (phialomicroconidia). In both seasons the first three assessments for all parameters (incidence of natural inoculum, incidence of natural infection, incidence of germination) were made on flower caps and subsequent assessments made

on developing berries. Parallel observations were made on uninoculated berries (water control).

Autofluorescence of infection sites

Destructive sampling of the epidermis of the grape berries to observe conidial germination and infection prevented observation

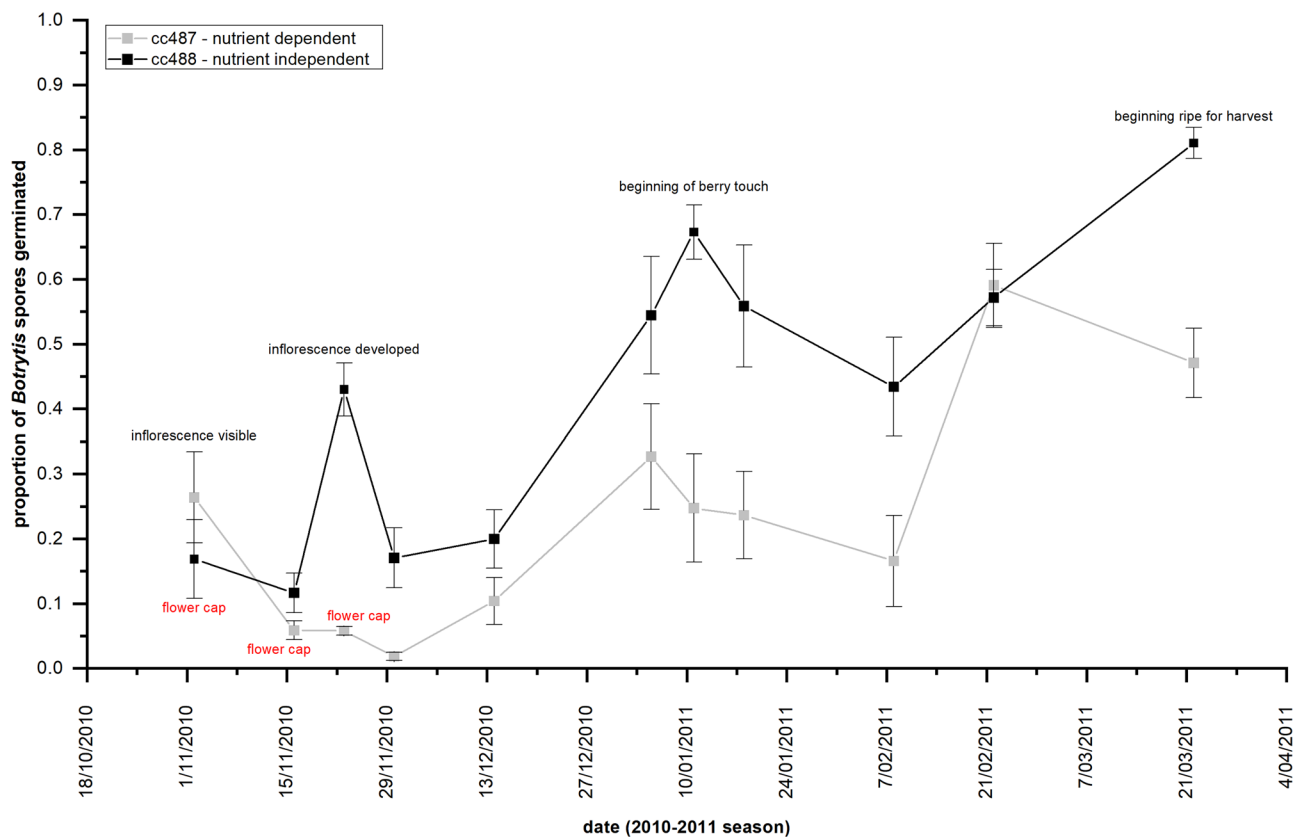


Fig. 2 Proportion of *Botrytis cinerea* conidia germinating on inoculated grape flowers or berries (cc487, cc488) throughout the 2010–11 season. Counts of spore germination were made on flower caps for

the first three time points and on berries for the remainder of the season. Values are means \pm standard errors of at least 100 conidia assessed on each of 50 replicate grape berries

of the extent to which the pathogen had penetrated and established in the epidermal cell. In the absence of visual confirmation of infection, autofluorescence of cells in the vicinity of infection structures was used as an indicator of successful infection.

In order to correlate visual surface features of conidial germination and appressorium formation with successful penetration and contact with epidermal cells ('infection'), a series of excised epidermal strips of inoculated berries were mounted in 70% ethanol and examined using UV epifluorescence (Excitation 330–385 nm, emission 420 nm) on an Olympus Vanox AHB3 microscope (Olympus Optical, Tokyo). Fields of view were first examined using transmitted normal light, then the same field examined under epifluorescence. With experience it was possible to recognise, from visual features, which surface structures (appressoria, germ tube tips, direct infection below or beside a conidium) had resulted in a successful infection.

Confirmation of natural infection of berries

A 'freeze and incubate' technique (Holz et al. 2003) was used over the 2011–12 growing season to assess the amount of prior infection (internal) and external inoculum (on the grape

berry surface) at each growth stage. At each sampling date, 10 berries from each of five grape bunches (collected as above) were surface-sterilised with 0.1% NaOCl for 15 min, rinsed twice in sterile RO water and dried in a laminar flow cabinet. A second set of berries was rinsed twice in sterile RO water and dried, serving as the non-sterilised control. All berries were frozen for 2 h, then incubated in humid conditions in separate containers (ice-cube trays in a plastic bag with damp paper towels underneath) at c. 21 °C, under near UV lights with a 12 h light/dark photoperiod. Berries were assessed for the presence of *B. cinerea* sporulation after 10–14 days.

Statistical analysis

The statistical analysis was carried out in SAS 9.2 using the Glimmix procedure. Series (date), treatment and their interaction were treated as fixed effects while bunch and berry within bunches were classified as random effects. A logit link function with a binomial distribution for the response was specified. The effect slice option was used to test for differences in the log-odds of germination between the two treatments at each time point. All statistical tests were carried out at the 5% level of significance.

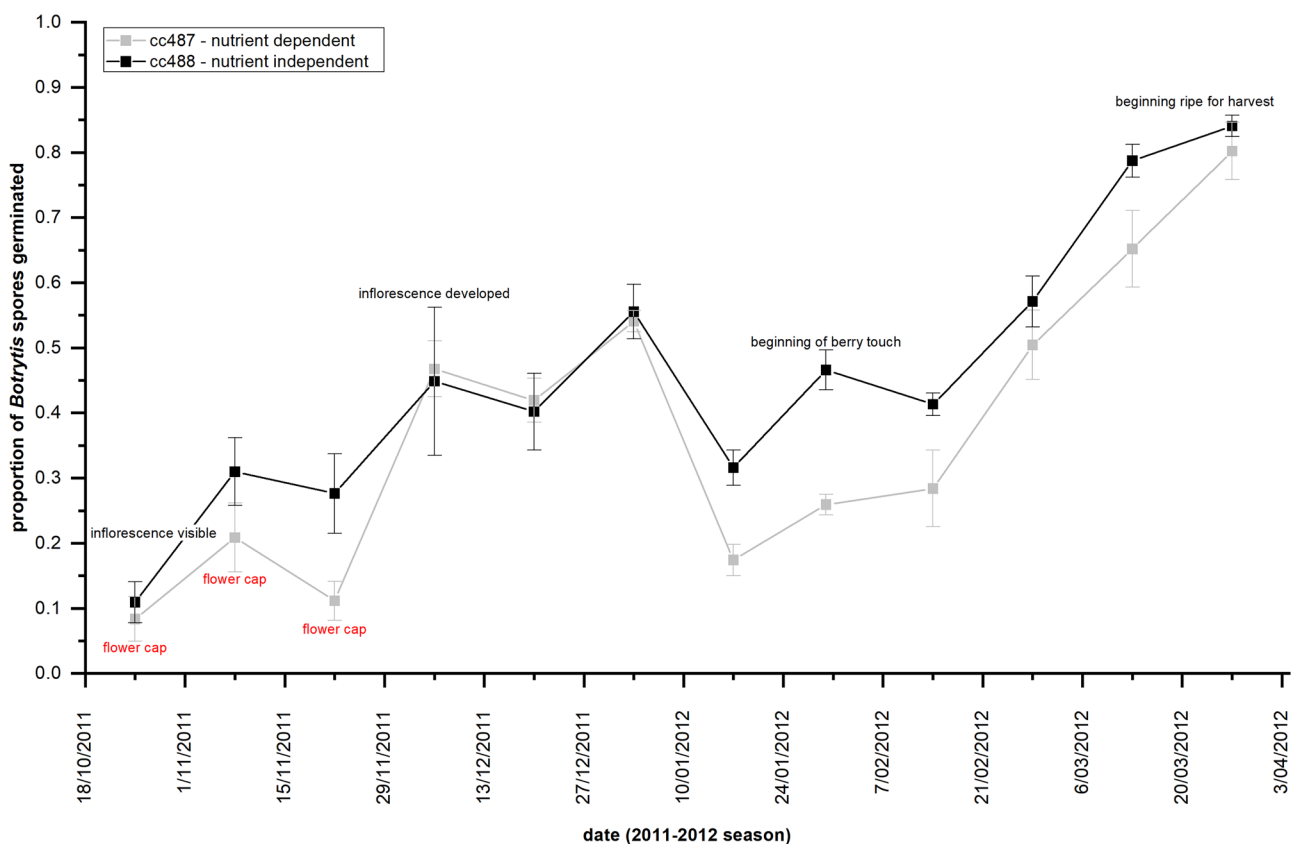


Fig. 3 Proportion of *Botrytis cinerea* conidia germinating on inoculated grape flowers or berries (cc487, cc488) throughout the 2011–12 season. Counts of spore germination were made on flower caps for

the first three time points and on berries for the remainder of the season. Values are means \pm standard errors of at least 100 conidia assessed on each of 50 replicate grape berries

Results

Autofluorescence of infection sites

A comparison of microscope fields by transmitted light and by epi-UV illumination showed that virtually every site of an appressorium or germ tube penetration was matched by fluorescence of the underlying

and surrounding epidermal cells (Fig. 1). Following the interpretations of McKeen (1974) and Rijkenberg et al. (1980), fluorescence of cells in the vicinity of an appressorium was taken as evidence of a successful infection. Most infection sites that showed autofluorescence also had brown discoloration of the epidermal cells below the appressorium. Thereafter the presence of appressoria with clearly defined germ pores and/or discoloration of the

Fig. 4 *Botrytis cinerea* conidia germinating on the berry surface to form **A**) direct germ tubes (top row), **B**) appressoria (middle row), and **C**) spermatia (bottom row)

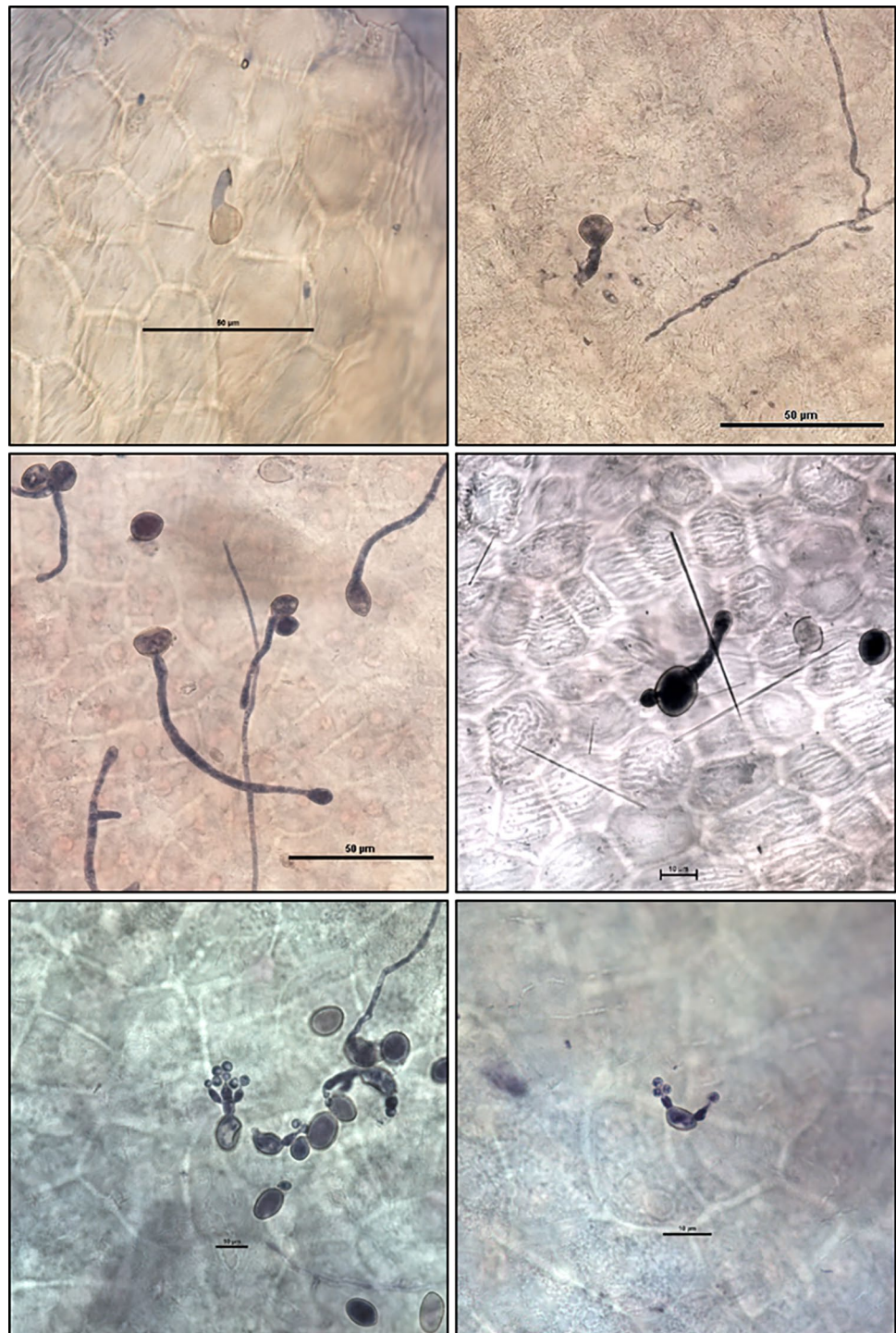




Fig. 5 Multiple infection points from a single spore of *Botrytis cinerea* on skin surface of a ripe grape berry

epidermal cells beneath or beside a germinating conidium were considered to be ‘infections’.

Germination and infection behaviour

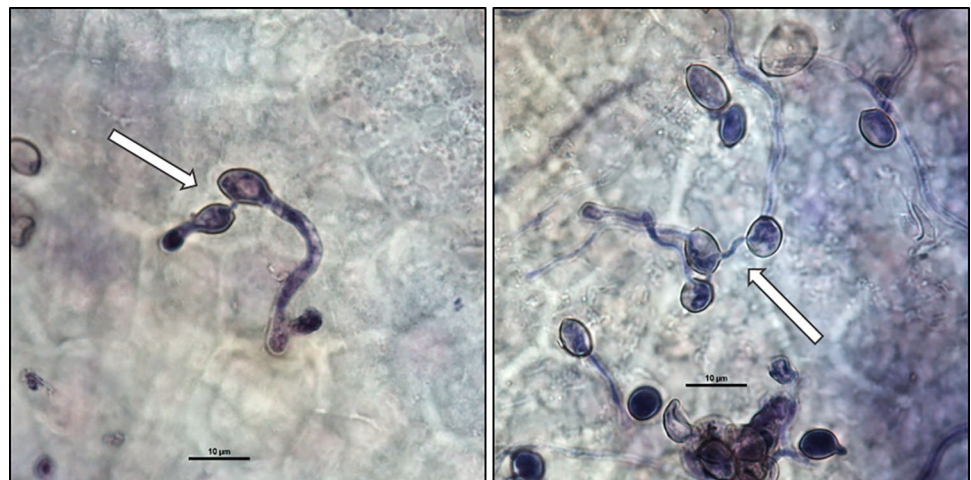
The pattern of germination of the nutrient-dependent and the nutrient-independent strains, initially on the flower caps and subsequently on berry skin for the 2010–2011 and 2011–2012 grape seasons (approximately October – April each year), are shown in Figs. 2 and 3. Although there was considerable variability in germination rates between sampling times in each season, particularly the 2010–2011 season, there were features consistent for both strains in both seasons. Conidia were able to germinate and infect

at any time during the season. The germination rate was relatively low pre-flowering (on flower caps) and increased as the season progressed. The pattern is best illustrated in the 2011–2012 season (Fig. 3) when germination of both the nutrient-independent and nutrient-dependent strains increased from approximately 10% on caps at the first sampling to approximately 80% at harvest ripeness. Allowing for a few unexpectedly high germination rates of the nutrient-dependent strain, both strains followed a similar pattern over the course of the season with the germination rate of the nutrient-dependent strain significantly lower than that of the nutrient-independent strain.

The extent of germ tube growth on the surface of the berry before appressorium formation differed according to the phenological stage of the berry. On young berries, pre-véraison, germination gave rise to predominantly single germ tubes with or without appressoria at their tips. In many cases infection was initiated directly from the tip of the germ tube without formation of an appressorium. In some cases infection occurred via direct penetration beneath or just beside the conidium (Fig. 4). On ripe berries there was extensive growth and branching of ‘germ tubes’ over the surface of the berry with a single spore giving rise to multiple appressoria and infection sites (Fig. 5). Conidial anastomosis (two conidia connected by a short germ tube) was seen on the surface of some berries (Fig. 6). Infections occurred anywhere on the berry, both directly through the tangential walls of the epidermal cells or between adjoining cells and no infections were seen on stomata or wounds. No differences in conidial germination behaviour were observed between the naturally infected (control) and inoculated (cc487, cc488) berries.

While most conidia germinated to form germ tubes and appressoria, a proportion germinated to form spermatia (microconidia). During the 2011–12 assessments the numbers of spores germinating to form microconidia were recorded (Fig. 7). Microconidial formation was found at

Fig. 6 Anastomosis between two *Botrytis cinerea* conidia on the grape berry surface (arrows)



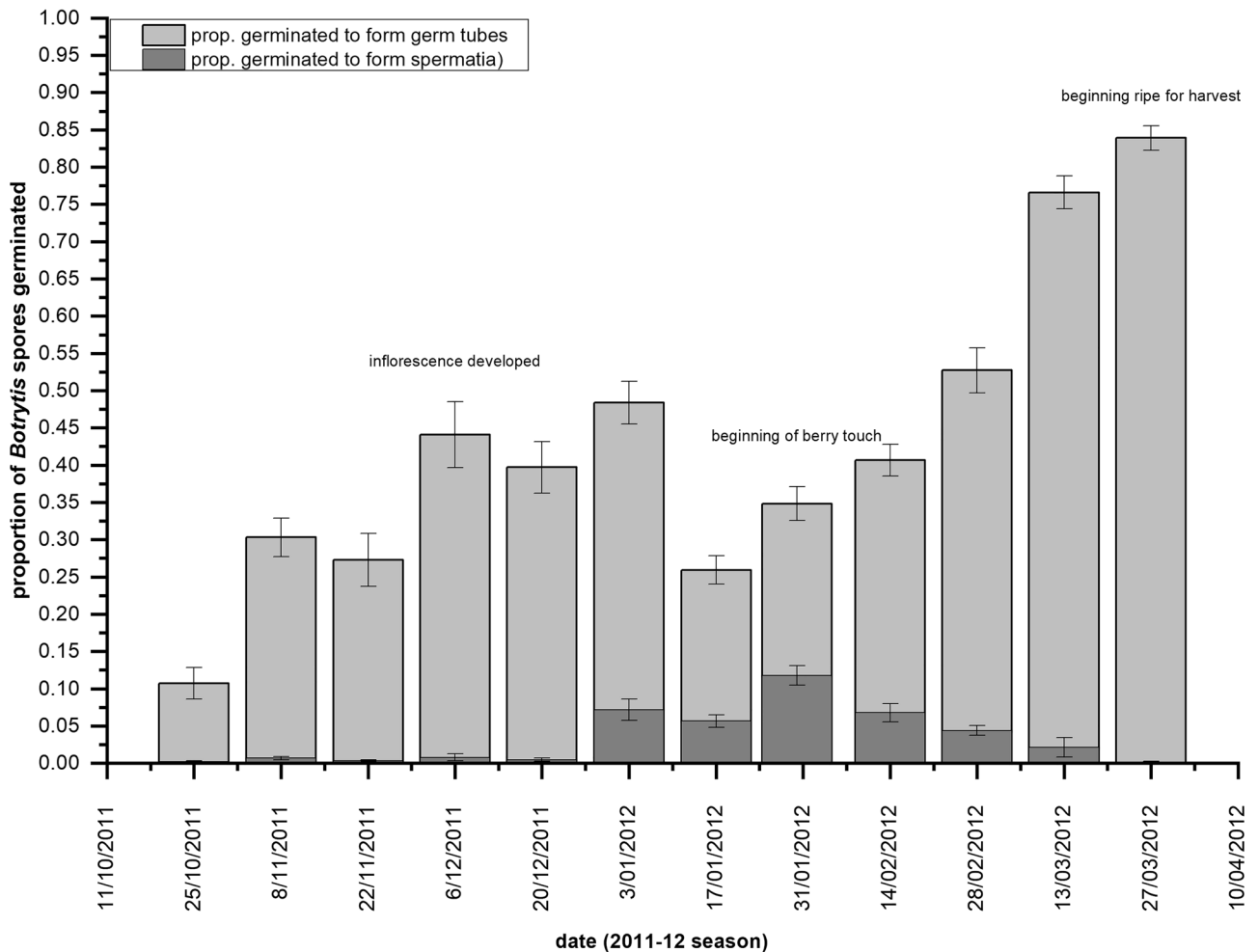


Fig. 7 Proportion of *Botrytis cinerea* conidia germinating to form germ tubes, or to form spermatia, on grape berries inoculated with isolate cc488 throughout the 2011–12 season. Values are means \pm standard errors of at least 100 conidia assessed on each of 50 replicate grape berries

each time of inoculation but most frequently over the mid- to late-season period January to March 2012. The formation of microconidia was more abundant in the nutrient-independent strain cc488, peaking at approximately 12% of total conidia in late January 2012. Very few conidia of the nutrient-dependent strain cc487 germinated in this manner.

Incidence of natural infection in the field

Numbers of naturally deposited conidia and the conidial germination rates on non-inoculated bunches at different sampling times are shown in Figs. 8 and 9. *B. cinerea* were present on berries throughout both seasons. In each season there was significant variability in the number of conidia present on the surface at the different sampling times, reflecting the amounts of natural inoculum prevailing at the time. In general the numbers of naturally deposited spores were low, ranging from 0.1 to 5.9/ ~ 5 mm² in 2010–11, and 0.06 to 4.1/ ~ 5 mm² in 2011–12. Nevertheless there was a general

pattern of very low numbers at the start of bunch development and an increase as the season progressed. The frequency of germination and infection was also highly variable between sampling times but conidia were able to germinate and infect the caps and berries throughout the season with a sometimes very high proportion of conidia germinating.

In the surface sterilisation and freezing assay, *B. cinerea* was recovered from a greater proportion of non-sterilised berries than from surface sterilised berries over almost all of the season (Table 2) confirming that there was a proportion of natural conidia on the surface of the berries at the time of sampling.

Discussion

This study involved observations on 3600 grape berries and 360,000 conidia over the course of two growing seasons (2010–2011 and 2011–2012) and provides new insights

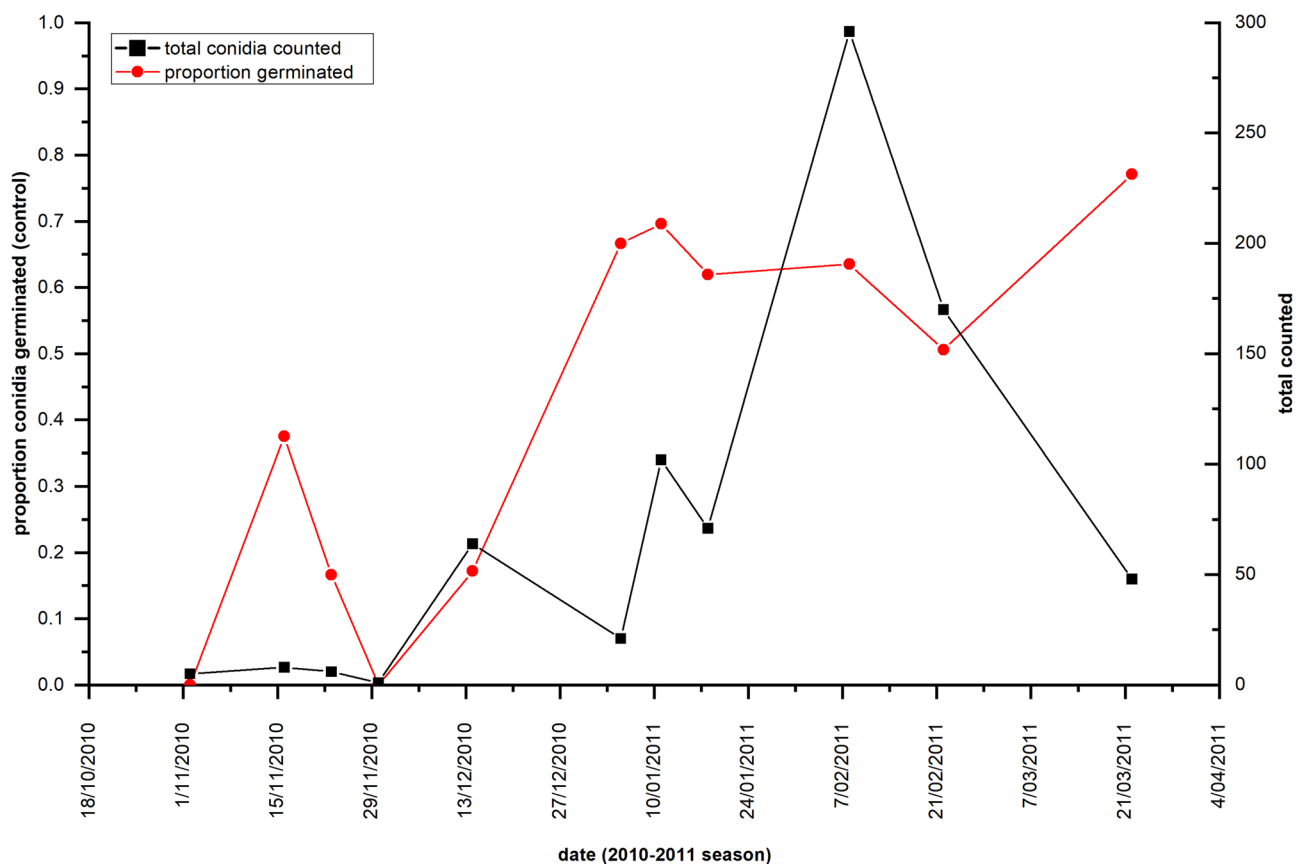


Fig. 8 Number of *Botrytis cinerea* conidia counted per c. 5 mm² at each sampling date on non-inoculated control berries, and the proportion of conidia germinating throughout the 2010–11 season

into the dynamics of germination and berry infection by *B. cinerea* during bunch development of wine grapes. Previous studies (Cadle-Davidson 2008; Keller et al. 2003; Viret et al. 2004) concluded that the early stages of flowering were a critical period for infection originating from spores lodged between the base of the ovary and receptacle, and in the abscission zone of the cap. In this study, artificial inoculations made on surface-sterilised berries at approximately two-weekly intervals from pre-cap fall to fruit ripening showed that the pathogen can germinate and infect berries at any time during the season. This has been confirmed in a subsequent study by Hill et al. (2014). Using three identifiable nitrate non-utilising (nit) mutants of *B. cinerea*, that study demonstrated that the pathogen could infect at three different times (flowering, pre-bunch closure and véraison), remain latent during berry development and that all three strains could be retrieved from rotted bunches after ripening.

It is widely accepted that germination and infection of berries is influenced by the availability of exogenous nutrients, particularly sugars, and that the risk of infection increases as surface nutrients increase later in the season (Benito et al. 1998; Cotoras et al. 2009; Kosuge and Hewitt

1964). Despite some apparently anomalous data points in both years, this study has shown that there is an overall trend of an increase in conidial germination over the whole season which is apparently not related to the typical increase in nutrient rich exudates on the berry surface post-véraison. A strain of *B. cinerea* dependent on exogenous sugar for germination under laboratory conditions was able to germinate and infect berries throughout the season albeit at a lower rate than the sugar-independent strain. This result corresponds to previous work showing that conidia were able to germinate equally well in washings (Kosuge and Hewitt 1964) or on skin (Hill et al. 1981) of both young and maturing berries. Collectively these results demonstrate that the nutrient requirement for successful germination and infection of *B. cinerea* is minimal and that nutrients on the berry surface, possibly from external sources, are normally adequate for *B. cinerea* infection even for nutrient-fastidious strains.

The stage of berry development had a substantial effect on the post-germination behaviour of the germ tube. On caps and early stage berries, conidia produced predominantly single germ tubes which infected directly from their tips or from haustoria. Others infected directly from below or immediately adjacent to the conidium. It has been

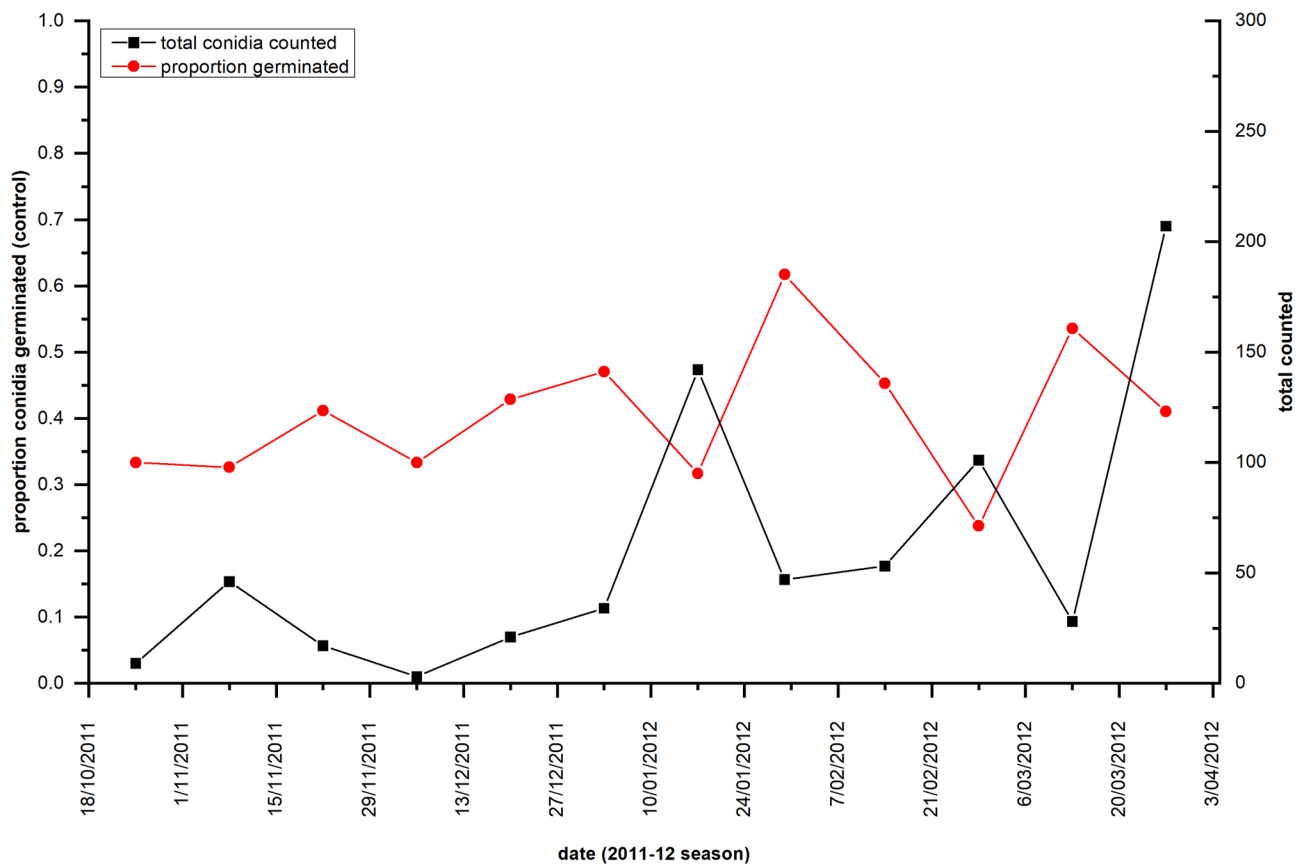


Fig. 9 Number of *Botrytis cinerea* conidia counted per c. 5 mm² at each sampling date on non-inoculated control berries, and the proportion of conidia germinating throughout the 2011–12 season

suggested that for *B. squamosa* immediate penetration by a short germ tube reduces the dependency on exogenous nutrients (Clark and Lorbeer 1976). As the berries ripened the germ tubes often branched and made extensive growth over the surface of the berry, resulting in multiple

haustoria being generated from a single spore. This behaviour, a response to a richer nutrient environment of the germinating spores (Clark and Lorbeer 1976; Cole et al. 1996; Garcia-Arenal and Sagasta 1980), greatly increased the infection capacity of each spore. Observations on the

Table 2 Percentage of non-sterilised (NS) and surface-sterilised (SS) grapes exhibiting *Botrytis cinerea* sporulation after freezing and incubation for 10–14 days (2011–12 growing season)

Date	°Brix	Grape berry growth stage*	Grape berry phenological stage	NS % <i>Botrytis</i>	SS % <i>Botrytis</i>
25/10/2011	-	12	inflorescence	60%	0%
8/11/2011	-	15	inflorescence	100%	40%
22/11/2011	-	17	inflorescence	100%	20%
6/12/2011	2.2	19	-	14%	0%
20/12/2011	2.5	25	80% of caps fallen	34%	20%
3/01/2012	4.5	27	-	74%	34%
17/01/2012	3.6	30	-	48%	24%
30/01/2012	4.2	33	berry touch	14%	10%
14/02/2012	5.8	34	-	8%	4%
28/02/2012	9.6	34	-	76%	30%
13/03/2012	13.9	35	véraison	20%	12%
27/03/2012	16.2	37	-	2%	10%

* numbered growth stages established by Eichhorn and Lorenz (1977)

sites of infection concur with those of Coertze et al. (2001) that infection occurs by direct penetration of the cuticle and not through stomata or wounds.

Flower debris trapped within bunches and colonised by *B. cinerea* are a major source of inoculum for bunch rots both during berry development and at ripening (Elmer and Michailides 2007). This, and previous research (Calvo-Garrido et al. 2014; Holz et al. 2003) has shown that although berries were exposed to a low but increasing spore load for most of the season, they are able to germinate and infect flower caps and young berries. The early infection of flower caps may be an important feature of the disease cycle. The flower caps are, in effect, 'pre-infected' and able to begin colonisation immediately after dehiscence and senescence. When trapped within the bunches they serve as an immediate and localised source of inoculum to the parent bunch from early berry development through until ripening.

Previously it was considered that the conidia either infected the juvenile grape berries at flowering and remained latent until ripening, or infected the berries post-véraison (Keller et al. 2003; Saito et al. 2013). This study has confirmed that not only is there a progressive accumulation of latent infections in the berries throughout the season, there is also the potential for an extremely rapid increase in the numbers of latent infections over time as a result of both an increasing number of spores as the season progresses, an increased proportion of spores germinating and, from multiple appressoria forming on branched germ tubes, from each conidium on maturing fruit.

The latency of infections in berries has been widely studied and is considered to be associated with a range of constitutive antifungal compounds, particularly stilbene derivatives such as resveratrol (Doehlemaun et al. 2006; Kretschmer et al. 2007). While these compounds may have a significant defence role in grapes they may not always prevent infection but have a major role in maintaining the fungus in a latent form (Keller et al. 2003; Pezet et al. 2003). The weakening and loss of these defence mechanisms during ripening allows the accumulated latent infections in the skins to become active and invade the berries over a very short period. In addition, late-season infections can immediately progress to active infections. Wilcox (2002) considered that late-season direct infections of that kind were just as important as the activated latent infections in contributing to the rate and severity of berry rot. The combination of activated latent infections and direct infections from high, late-season spore loads can result in a very rapid transition from apparently healthy to severely rotting bunches.

In both years of this study, the germination of conidia to form spermatia on the surface of the grape berries was observed. The spermatia were small, 1 µm diam., hyaline microconidia that formed from phialides formed on the

macro-conidia. In several species of the family Sclerotiniaceae, the microconidia function as spermatia in the sexual cycle (Willets 1997). This was demonstrated for *B. cinerea* by Groves and Drayton (1939) and confirmed more recently by Fukumori et al. (2004). Apothecial production by *B. cinerea* has been demonstrated under laboratory conditions (Beever and Parkes 1993; Fukumori et al. 2004; Groves and Drayton 1939). However the teleomorph of *B. cinerea* is rarely seen in the field and has not been recorded in the field in New Zealand (Beever and Parkes 1993). Hahn (2014) however, maintains that although the sexual structures of *B. cinerea* are rarely observed, sexual recombination is likely to be important to maintain the high level of genetic variability that is found in the field.

Many aspects of the interaction between *B. cinerea* and host species have been well studied over time. This study of the germination rate of *B. cinerea* conidia on grape berries over two seasons has shown that conidia can germinate and infect berries and accumulate as latent infections throughout the season. With the weakening of resistance mechanisms at maturity, accumulated latent infections will be able to contribute significantly to the initial phases of bunch rot development. Thus the weather conditions and the amount of natural inoculum within the vineyard throughout the entire season are important factors in governing the potential for bunch rots at maturity. This study is also likely to have implications for fungicide timing throughout the growing season.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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