The effect of grapevine leaf physiology on development of powdery mildew

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Introduction

Powdery mildew, caused by *Erisyphe necator*, is an important and widespread fungal disease of *Vitis vinifera* grapevines. The pathogen attacks leaves as well as fruit and can result in serious losses in grape yield and quality in most years if uncontrolled. Wine made from Chardonnay grapes with as little as 1–5% of bunches affected by powdery mildew can have altered composition and sensory characteristics (Stummer et al. 2005). In general, there is a low tolerance of infected grapes in the winery meaning that a high level of disease control is required, often by multiple applications of protective fungicides. With current economical and environmental pressures, there is increased grower and consumer interest in reducing inputs of synthetic pesticides. The threat of pathogen resistance to fungicides, for example the DMI fungicides (Erickson and Wilcox 1997), is another incentive to minimise their use. In order to time fungicides strategically, a sound knowledge of disease epidemiology, in relation to vine development and environmental conditions, is required.

There are two separate but closely related epidemics of powdery mildew occurring on grapevines: one occurs on leaves and the other on bunches. The frequency and spatial distribution of diseased leaves at flowering has been related to the severity and distribution of powdery mildew on bunches at bunch closure (Calonnec et al. 2006). *E. necator* can colonise green floral pedicels and caps (calyptrae) and will infect the developing fruit at a high frequency during early fruit set (Gadoury et al. 2003). The leaf epidemic influences the amount of inoculum available for infection of highly susceptible flowers and immature fruit (Calonnec et al. 2008). The status of the leaf epidemic at flowering depends on when primary infection occurred and the proportion of the leaves in the canopy that were highly susceptible to *E. necator* during each infection event.

Erysiphe necator is a biotrophic fungus that infects green tissue and obtains its nutrition from living plant cells. Both leaves and berries of *Vitis vinifera* express ontogenic resistance to infection by *E. necator*, which means that infection efficiency declines as the plant organ ages (Doster and Schnathorst 1985; Gadoury et al. 2003). This type of resistance is recognised among many biotrophic pathogens that infect healthy, green tissue of woody, perennial plants. The consequence is that the proportion of all leaves on a grapevine that are highly susceptible to infection by *E. necator* varies during the growing season (Calonnec et al. 2008).

Fungicides are often applied at regular intervals, not taking into account temporal variation in the appearance of new, mildew susceptible leaves. In the interval after a fungicide application, rapid leaf emergence can result in new leaves that are unprotected by fungicide residue. Conversely, application of the subsequent spray might occur when there has been no increase in susceptible leaf area and existing fungicide residues continue to provide an effective dose. In Germany, protective fungicides for downy mildew are timed according to the amount of unprotected leaf area emerged since the last spray, based on models that describe shoot development as a function of thermal time (degree days; Schulz 1992). Smith *et al.* (2007) determined rates of leaf appearance and leaf area development in Tasmania for cane pruned Chardonnay vines trellised by vertical shoot positioning (VSP) and for Pinot Noir vines on Scott Henry trellis. There is potential to use of this information to time fungicides or novel approaches to crop protection, such as chemical elicitors of induced disease resistance (Kuć 1982), when the grapevine canopy is at greatest risk of infection. However, application of shoot development models depends on a better understanding of the susceptibility of individual leaves to *E. necator* as they develop.

Doster and Schnathorst (1985) noted that older grapevine leaves had fewer, smaller powdery mildew colonies when compared with younger leaves, but the relationship between disease expression and leaf maturity was not quantified precisely. The mechanism of ontogenic resistance in leaves is also unknown. During development, each leaf passes through an initial stage of carbohydrate sink before gradual transition to carbohydrate source (Turgeon and Webb 1973). Along with the associated physiological changes, obvious morphological changes also occur. Relative to young leaves, older leaves are more robust with heavier wall lignification, have lower concentrations of nitrogen, water and other nutrients (Coleman 1986) and generally have an increase in chemical and physical defences to pathogen invasion. In a preliminary report, Smith and Evans (2007) described the development of powdery mildew on maturing grape leaves. Expanding leaves that were approximately 50–90% of their full leaf area expressed more disease than younger or older leaves.

The objective of this study was to quantify the rate of development of leaf ontogenic resistance to *E. necator*, in relation to two different rates of leaf emergence. In addition, the hypothesis that maximum expression of powdery mildew occurs when leaves are infected during the photosynthetic sink to source transition was tested.

Materials and methods

Plant material and determination of leaf position

Own rooted grapevines, *Vitis vinifera* L. cv Cabernet Sauvignon, were grown in 15 cm-diameter pots in a glasshouse with a 16 h day length supplemented by 400 W mercury halide lights. Vines were pruned to one bud at the time of planting and grown until there were approximately 20 leaves. Twenty plants were grown at 25°C (±5°C) and 16 plants were grown at a later date at approximately 18°C (±10°C) in the glasshouse. Ambient temperature was monitored from budburst for calculation of daily thermal time above a base temperature of 10°C (Schultz 1992). Shoots were maintained free from powdery mildew using vapours of penconazole (Topas®, Syngenta Crop Protection Pty Limited, Szkolnik 1983). Topas® was removed from the glasshouse 7 days prior to treatment.

Immediately before treatment, leaves were numbered according to position from the apex using an ordinal measure starting at 0 for immature leaves, 1 for the first leaf with a lamina length of at least 30 mm, and then 2, 3, etc, for older leaves. Leaves at leaf position 0–2, 3–5 or ≥6 were referred to as immature, expanding or mature to indicate that leaves in each category had expanded to approximately <50%, 50% to 90% and >90%, respectively, of their mature size.

To determine the rate of leaf appearance for each temperature regime, lamina lengths on all leaves of each shoot were measured every 3–4 days until treatment, for the calculation of the plastochron index (PI, Erickson and Michelini 1957). A reference length of 30 mm was chosen based on earlier results in grapevine (Freeman and Kliewer, 1984; Schultz 1992). The PI denotes the number of leaves on a shoot. The rate of leaf emergence for each shoot was calculated from linear regressions of PI against calendar day or cumulative thermal time.

Treatment 1: Inoculation with *E. necator* and disease assessment

Half of the batch of plants per pre-conditioning temperature for shoot development were used to test if the rate of leaf appearance, before inoculation of all leaves per shoot with *E. necator*, influenced disease development and hence the expression of ontogenic resistance.

Erysiphe necator was collected, as a bulk isolate, from vineyards in southern Tasmania and cultured on detached grape leaves as described by Evans et al. (1996). Twelve-day old cultures were used to prepare a suspension of 10^5 conidia per mL water according to the method of Gadoury et al. (2001). The conidial suspension was applied to the adaxial side of all leaves using a hand held atomizer. Leaves were dried of moisture with fans immediately after inoculation. Plants were then maintained at 25°C to provide optimum conditions for fungal infection and colonisation of leaves (Chellemi and Marois 1992). After 14 days, disease severity per leaf, defined as the percentage of leaf area colonised by *E. necator*, was determined with the aid of a standard area diagram (B. Emmett, Department of Primary Industries, Victoria, pers. comm.). The incidence of powdery mildew was defined as the presence or absence of powdery mildew at each leaf position. To describe ontogenic resistance, mean disease severity or percentage incidence was calculated for each leaf position across shoots and plotted as a function of leaf position for each pre-conditioning temperature.

Treatment 2: $^{14}CO_2$ labeling and autoradiography

The other half of the batch of plants per pre-conditioning temperature for shoot development were used to identify the position of leaves on each shoot undergoing the transition from carbohydrate sink to source.

Two fully expanded leaves on opposite sides of each shoot were enclosed within a polyethylene bag and $^{14}CO_2$ released by the addition of lactic acid to ^{14}C-labeled sodium carbonate. Treatments were done at 08:00 and photosynthesis allowed to continue for 2 h before the polyethylene bag was removed. Twenty four hours later, exposed leaves and leaves apical to the exposed leaves were cut from the shoot and dried for 7 days in a plant press at room temperature. Dried leaves of each shoot were then enclosed within a cassette on X-ray film (Agfa) for 3 weeks prior to film development. The leaf position for the sink/source transition at the cessation of carbohydrate import, as determined by lack of ^{14}C accumulation, was estimated visually from the autoradiographs.

Data analyses

The modal leaf position at maximum disease severity for each shoot was identified by a bootstrap approach (Efron and Tibshirani 1993) that estimates the mode. The mean of modal leaf positions for maximum disease severity was then calculated for each temperature regime. This mean was then compared for similarity with the mean leaf position for the transition from photosynthetic source to sink for each pre-conditioning temperature.

Results

The rate of leaf emergence varied for each pre-conditioning temperature; on average, 0.45 leaves emerged per day at 25°C, while only 0.18 leaves emerged per day at 18°C. There was, however, no difference between temperature regimes in the mean rate of leaf emergence per unit of thermal time, which was 0.028 leaves emerged per degree (°C) day.

At both preconditioning temperatures, disease expression was more severe on expanding leaves (positions 3–5) than on immature (positions 0–2) or mature leaves (position 6 or higher; Figure 1). Disease severity decreased as leaf maturity increased beyond leaf position 3–4 (Figure 1). The mean modal leaf position for maximum disease severity was estimated to be 3.7 and 4.7 for pre-conditioning temperatures of 18 and 25°C, respectively (Table 1).

Overall, disease expression of powdery mildew was more severe on shoots preconditioned at 25°C (Figure 1a) than on shoots preconditioned at 18°C (Figure 1b), with a greater mean leaf area per shoot infected. Disease was expressed on a greater number of leaves on shoots preconditioned at the higher temperature (Figure 2). No powdery mildew was observed at leaf positions ≥17 on shoots preconditioned at 25°C (Figure 2a), nor at leaf positions ≥11 for shoots preconditioned at 18°C (Figure 2b).

The sink to source transition occurred on average at leaf positions 3.8 and 4.7 for pre-conditioning temperatures of 18 and 25°C, respectively (Table 1). There was no difference between the mean modal leaf position at maximum disease expression and the mean leaf position when leaves were in the transition from a sink to a source leaf for either temperature regime (Table 1).

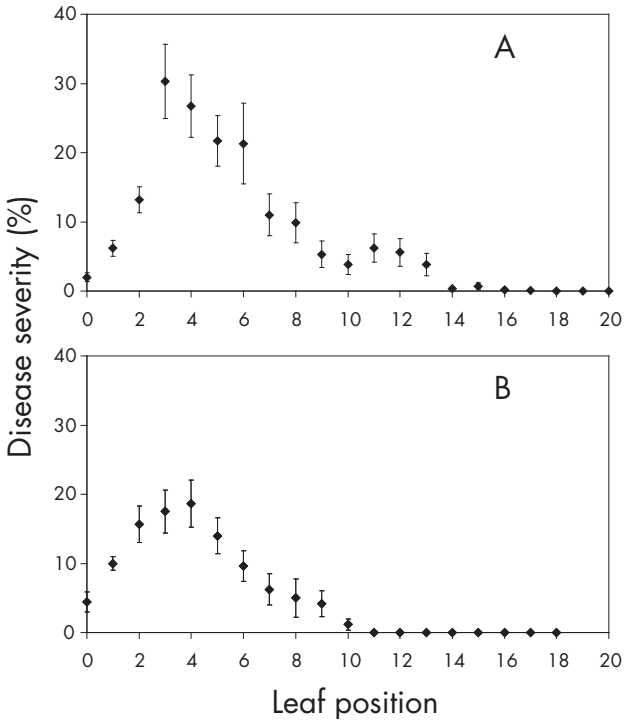

Figure 1. The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on mean severity of powdery mildew (± standard error) 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Values for leaf position increase with greater leaf maturity. Plants grown at **(A)** 25°C or **(B)** 18°C prior to inoculation.

Discussion

The results of this study demonstrate that a grapevine leaf becomes increasingly resistant to colonisation by the powdery mildew fungus as it ages, as reported previously (Doster and Schnathorst 1985, Singh and Munshi 1993). Unlike previous studies, the non-linear change in disease severity for immature leaves was quantified precisely so that the leaf position for maximum disease severity could be identified. This is the first report of the association between leaf position for maximum severity of powdery mildew and the position of the leaf in the sink to source transition, immediately after it had ceased importing carbohydrates. Moreover, this correlation was maintained when the rate of leaf emergence varied, as influenced by ambient temperature. Furthermore, the rate of leaf emergence pre-inoculation affected the incidence and severity of powdery mildew for the entire shoot, with a higher rate of leaf emergence leading to more disease per shoot.

Although the results clearly indicate a peak in leaf susceptibility to disease, the severity of powdery mildew observed on immature and expanding leaves may be influenced by the rate of leaf expansion after inoculation. When compared with the use of detached leaves for inoculation studies, rapid leaf expansion after inoculation may have resulted in an apparently lower severity per area of leaf when compared with leaves with slower or completed expansion.

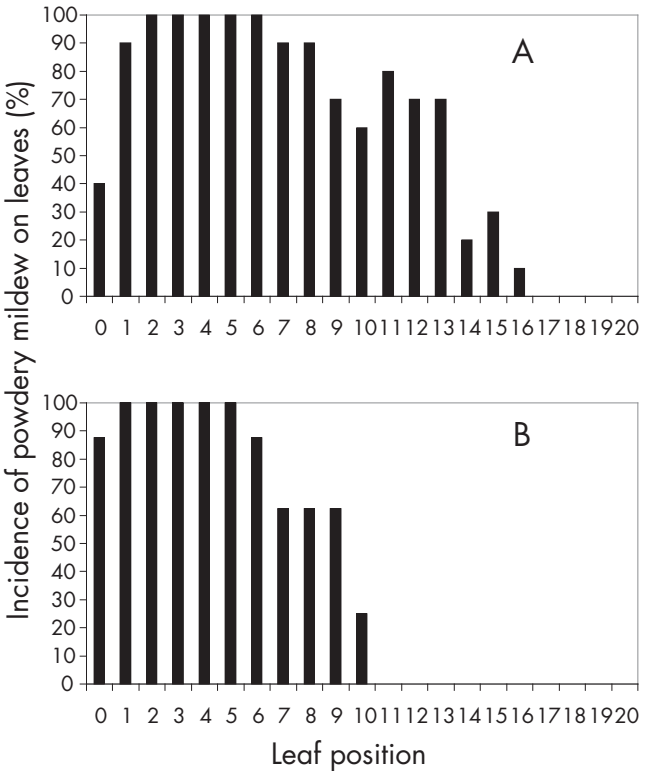

Figure 2. The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on mean severity of powdery mildew (± standard error) 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Values for leaf position increase with greater leaf maturity. Plants grown at **(A)** 25°C or **(B)** 18°C prior to inoculation.

Table 1. The mean and range for leaf position of maximum powdery mildew severity and of the leaf in the sink to source transition as determined by cessation of import. Data presented for shoots pre-conditioned at two temperature regimes.

	18°C		25°C	
	Mean	**Range**	**Mean**	**Range**
Leaf position for maximum disease severity	3.7	2.1–4.8	4.7	3.0–6.6
Leaf position for sink to source leaf	3.8	3.0–5.0	4.7	3.0–7.0

However, Reuveni (1998) reported a similar responset curve using a detached grapevine leaf disc assay, where there was no expansion in leaf area between inoculation with the downy mildew pathogen, another biotroph, and assessment of disease severity.

The decline in susceptibility to powdery mildew, with increasing leaf position, beyond the position of maximum disease severity, suggests a concurrent increase in the defence response of leaves. Physical and biochemical defences that might contribute to ontogenic resistance include cell wall and cuticle thickness, the amount of cell wall lignification and the activity of antifungal enzymes. With regard to cuticle thickness, Heintz and Blaich (1989) found a negative linear relationship between cuticle thickness of young grape leaves and the intensity of *E. necator* sporulation on these leaves. Leaves taking a longer time to develop, for example at the 18°C pre-conditioning regime, may have formed a thicker cuticle than leaves at an equivalent position on shoots developing at a higher temperature. Changes in cuticle thickness and cell wall lignification as leaves mature needs to be quantified as a function of the temperature at which shoots develop.

In the downy mildew-grapevine pathosystem, leaf ontogenic resistance was correlated positively with increases in peroxidase and β-1, 3-glucanase activities (Reuveni 1998). Enhanced peroxidase activity is a marker for leaf senescence (Thomas and Stoddart 1980; Takahama et al. 1999) and enzymes associated with leaf senescence may also be involved in plant defence mechanisms against pathogens (Lamb and Dixon 1997). Separating biochemical effects relating to leaf senescence and pathogen defence is likely to be a challenging research problem. Nevertheless, the activation of secondary biochemical pathways required for induced defence could be constrained in immature leaves that are a strong sink for carbohydrate. These leaves rely on other plant parts for their carbohydrate supply and concentrations of free sugars are low as they get used up immediately to build the new lamina. The severity of disease observed on immature leaves may be the result of a number of factors including rapid expansion of leaf area, a lack of secondary metabolism for defence and possibly a pathogen that is less able to compete with the sink strength of the host (Cole 1966).

Coleman (1986) suggested that leaves that are making the transition from sink to source are more susceptible to abiotic or biotic stress. This study provides further evidence that just after leaves have lost the ability to import carbohydrate the leaf is most susceptible to infection by the powdery mildew fungus. At this stage of physiological development, export begins as import ceases (Turgeon and Webb 1973) and there are many structural and metabolic changes associated with accumulation and export of photosynthate in the transition to a source organ (Dickson and Larson 1981). When leaves are expanding and changing from sink to source they have two sources of photosynthate, *in situ* and imported. An ideal ecological niche for infection by *E. necator* might be created at this time, considering that the powdery mildew fungi are classed as high-sugar pathogens (Horsfall and Dimond 1957). The powdery mildew fungi co-evolved intimately with their host plants and appear to have found a mechanism to acquire available photosynthate for obligate parasitism. Additionally, leaves in the sink-source transition are not yet physiologically mature, resulting in weak defence response to pathogen attack if the leaf is allocating more resources into growth, rather than defence. Once the mildew pathogen has established its feeding structures called haustoria, then the fungus itself becomes a strong sink for carbohydrate and redirects the carbohydrate metabolism of the plant (Brem et al. 1986).

This study has clearly demonstrated the effect ontogeny of leaves has on the ability of the powdery mildew fungus to express disease.

Past research has focused on how the environment affects the pathogen, not how the environment affects the host's susceptibility to the pathogen. Consequently grapegrowers may have greater control over the disease from cultural management than previously thought. The results of this study suggest that growers might be able to manipulate vigour to create more robust leaves and a smaller proportion of the canopy susceptible at any given time. As disease susceptibility of leaves represents the inoculum load for grape infection (Calonnec et al. 2008), small changes in management practices may have profound effects on disease expression on the berries.

This work is ongoing to examine which component of the infection and colonisation process by *E. necator* is inhibited as ontogenic resistance develops, including spore germination and hyphal development. A mathematical model describing the development of powdery mildew on maturing grapevine leaves is being developed to gain further insight into the nature and expression of ontogenic resistance in different environments. Ultimately, this knowledge will be related to shoot development in the vineyard towards developing cultural measures or better timing of crop protection for improved management of powdery mildew. .

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