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Effect of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot (*Botrytis cinerea* PERS.: FR.) with 3 *Vitis vinifera* L. cultivars

by

D. C. PERCIVAL[1]), J. A. SULLIVAN[1]) and K. H. FISHER[2])

[1]) Department of Horticultural Science, University of Guelph, Ontario, Canada.

[2]) Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada

Summary: Leaf removal treatments were applied to Riesling during 1990 and 1991, and to Cabernet franc and Optima in 1991. Shaded and exposed clusters of Riesling were sampled prior to veraison and shaded and exposed clusters of all 3 cultivars were sampled prior to their respective harvest dates. Clusters were sampled to examine the effect of berry exposure, berry contact, time of sampling, and cultivar on cuticular membrane formation and the occurrence of bunch rot (*Botrytis cinerea* PERS.: FR.). Non-contact and contact cuticle proper and epicuticular wax berry samples were examined qualitatively using cryogenic scanning electron miscroscopy and quantitatively using enzymatic separation and chloroform extraction.

Exposed Riesling samples had 18.6 and 35.0 % more epicuticular wax and cuticle proper respectively than shaded samples. Non-contact Riesling samples had 15.7 and 35.0 % more epicuticular wax and cuticle proper than contact samples, and although significant, epicuticular wax and cuticle proper weights of Riesling increased by only 5.7 and 4.5 % respectively, between veraison and harvest. Although exposed cuticular membrane samples from all 3 cultivars had more epicuticular wax and cuticle proper than shaded samples, large differences between cultivars were present. Clusters from the hand leaf removal (i.e. exposed) treatment of all 3 cultivars examined had significantly less bunch rot than clusters from the control (i.e. shaded). Cabernet franc was the most tolerant and Optima the most susceptible of the 3 cultivars to bunch rot. Time of sampling and differences between years also influenced the occurrence of bunch rot for Riesling with less rot present at veraison compared to harvest and less rot present at the 1990 compared to the 1991 harvest. Exposure, cultivar and level of contact within the cluster are all important factors in the cuticular membrane formation process and contribute greatly to determining the overall susceptibility of a grape cultivar to bunch rot when grown in a temperate climate.

Key words: Leaf removal, cuticular membrane, epicuticular wax, cuticle proper, Botrytis cinerea.

Introduction

The cuticular membrane consists of insoluble cutin polymers (the cuticle proper) which form the framework of the membrane, and soluble waxes deposited on the surface as epicuticular wax (MARTIN and JUNIPER 1970; HOLLOWAY 1982 a, 1982 b; GAY and PEARCE 1984). In addition to physically confining tissues to maintain a firm, compact form, the cuticular membrane serves to reduce water loss due to transpiration, contributes to controlled gaseous exchange, restricts the leaching of essential compounds and nutrients, protects the plant from injuries (e.g. physical abrasion, frost and harmful radiation) (MARTIN and JUNIPER 1970) and provides the main constitutive (i.e. performed) defense mechanism against pathogens such as *Botrytis cinerea* PERS.: FR. (HEATH 1984; MAROIS *et al.* 1986).

The epicuticular wax develops after bloom in the form of overlapping platelets which increase in size and number as the fruit develops and matures (RADLER 1965; AIST 1984; ROSENQUIST and MORRISON 1988). The epicuticular wax layer of a mature

Correspondence to: D. C. PERCIVAL, Department of Horticultural Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

grape berry cuticular membrane is semi-crystalline to crystalline in structure (POSSINGHAM *et al.* 1967). It influences the retention of pesticides, the wettability of the berry surface and the adhesive ability of plant pathogens (HOLLOWAY 1969; BAKER 1982; HALLAM 1982; GAY and PEARCE 1984; HEATH 1984; NICHOLSON 1984).

The grape berry cuticle proper is present on the pre-anthesis pistil as a well developed, continuous, multi-layered structure (RADLER 1965; AIST 1984; ROSENQUIST and MORRISON 1988). The cuticle proper provides the primary physical barrier to pathogen invasion. For berry infection to occur from an external origin, a pathogen must either find a weakness on the berry surface where it can bypass the cuticle proper, or directly penetrate these layers of insoluble, polymeric material (BESSIS 1972; KOLATTUKUDY 1984; BULIT and DUBOS 1988). Penetration of *B. cinerea* through the cuticle proper has been postulated to be facilitated by the use of fungal cutinases (KOLATTUKUDY 1984). However, as a physical barrier, the quantity of the cuticle proper present on a grape berry may play an important role in increasing the resistance to pathogen penetration and subsequent colonization.

Although predominantly genetically controlled, the quantity of epicuticular wax and cuticle proper and the appearance of epicuticular wax is strongly influenced by environmental factors (MARTIN 1964; GEISE 1975; REED and TUKEY 1982; AIST 1984; GAY and PEARCE 1984; ROSENQUIST and MORRISON 1989). Epicuticular wax formation occurs only in the presence of light and thus its formation is increased by conditions of high light intensity (MARTIN 1964). Epicuticular wax and cuticle proper weight and deposition is increased with the presence of low relative humidity and high temperature (GEISE 1975; AIST 1984).

Cluster architecture also affects the formation of the cuticular membrane (ROSENQUIST and MORRISON 1989). Cuticle proper deposition is reduced and normal wax layer platelet formation is hindered in areas of berry contact (ROSENQUIST and MORRISON 1989). Contact areas between berries are more susceptible to infection by *B. cinerea* than non-contact areas and take up dyes which cannot penetrate wax, suggesting a thin, incomplete epicuticular wax layer in these areas (BLAICH *et al.* 1984; ROSENQUIST and MORRISON 1989).

This evidence suggests an apparent relationship between the formation of the cuticular membrane and the occurrence of the disease bunch rot (*B. cinerea*). The objective of this study was to examine the effect of cluster exposure, berry contact and cultivar on the formation and distribution of berry epicuticular wax and cuticle proper and occurrence of bunch rot.

Materials and methods

Experimental vineyard: The experiment was conducted at the Horticultural Research Institute of Ontario vineyard located at the base of the Niagara escarpment west of Vineland, Ontario.

Plant Material: Vines consisted of 8-year-old, tight clustered, disease susceptible, *V. vinifera* Riesling clone 239 on SO 4 rootstock (1990, 1991) and 9-year-old, disease prone, *V. vinifera* Optima on *V. riparia* Gloire de Montpellier rootstock, and 9-year-old, loose clustered, disease tolerant, *V. vinifera* Cabernet franc on SO 4 rootstock (1991).

The vines had similar levels of vigour, were cane pruned and trained to a pendelbogen (i.e. double half bow) system with a head height of approximately 1 m. Vineyard spacing was 1.5 m × 2.2 m (within row × between row) and row orientation was north-south. Full bloom occurred on June 16, 1990 and June 9, 1991.

Treatments: Treatments consisted of : (i) shaded clusters from vines that had no leaf removal other than routine topping (control); (ii) exposed clusters by stripping 100 % of the leaves in the fruiting zone from the shoot origin to the most distal cluster of each fruiting shoot once the berries had reached pea size (i.e. July 11, 1990 and June 29, 1991). Treatments were arranged in a randomized complete block design of 4 replications with 4 vines per replicate of Cabernet franc and Optima and 16 vines per replicate of Riesling in which 4 vines per replicate were identified for collection of harvest data.

Cuticle proper/epicuticular wax disk preparation: Ten clusters per replicate were sampled from the middle of the fruiting zone. Riesling clusters were sampled at veraison (Sept. 4, 1990: 10.2 °Brix; Aug. 30, 1991, 9.8 °Brix) and harvest (Oct. 6, 1990: 17.7 °Brix; Sept. 24, 1991: 17.8 °Brix) and Cabernet franc and Optima clusters were sampled only at harvest (Cabernet franc — Sept. 4, 1991, 18.0 °Brix; Optima — Aug. 24, 1991, 16.2 °Brix). Clusters were then frozen to facilitate the cuticle proper/epicuticular wax extraction process.

Cuticular membrane disks were removed from the outer layer of berries in the central 1/3 portion of frozen clusters using a 7 mm cork borer. From Riesling: 10 from non-contact (i.e. surface) portion of the berries and 10 from the contact portion of the adjacent berries, for a total of 100 disks for each level of exposure and contact. With few to no areas of berry contact, only 10 non-contact disks per cluster were removed from Cabernet franc and Optima. The cuticle proper/epicuticular wax layer was then separated from the underlying epidermal cell tissue by incubating the disks in an enzyme isolation medium consisting of 2 % (w/v) polygalacturonase (Sigma Chemical Co., St. Louis, MO) and 0.1 % cellulase (Sigma Chemical Co., St. Louis, MO) (Norris and Bukovac 1965; Rosenquist and Morrison 1989) in a 100 mM sodium acetate/acetic acid buffer solution (pH 3.8), with gentle agitation for 60 h.

Cuticle proper and epicuticular wax isolation and extraction: Disk samples were skimmed from the buffer solution and placed in test tubes. Epicuticular wax was separated from the cuticle proper by chloroform extraction overnight. The epicuticular wax solution was then transferred to Erlenmeyer flasks where 0.5 g sodium sulphate anhydride were added to remove any remaining water in the wax/chloroform solution. After 4 h, the wax solution was transferred to a tarred, round-bottomed evaporator flask. The chloroform was removed with the use of a roto-evaporator and the remaining wax residue was dried in a drying oven for 4 h at 60 °C, followed by 24 h at 105 °C and weighed. Once the epicuticular wax solution was removed from the cuticle proper disks, the disks were then placed in a drying chamber (criteria as above) and weighed.

Epicuticular wax morphology: At harvest small skin samples (2 mm^2), consisting of the epicuticular wax layer, cuticle proper, epidermis and underlying tissue, were sampled from non-contact berry portions of all cultivars. In addition, exposed and shaded non-contact and contact skin samples of Riesling were sampled at veraison. The samples were mounted on cryo-sample holders with Tissue Tek® mounting medium. The samples were frozen in liquid nitrogen slush (–210 °C), sublimated at –80 °C for 50 min, coated with 30 nm gold at –160 °C and were scanned at 10 kV using a Hitachi S-570 scanning electron microscope at –150 °C.

Cultivar susceptibility to bunch rot (*Botrytis cinerea*): At harvest in 1991, 20 clusters per cultivar with no visible symptoms of bunch rot were randomly sampled from each replication of the control treatment. Ten clusters were misted with distilled water and the remainder sprayed with a spore suspension of *B. cinerea* (10^6 conidia/ml water). The 10 clusters were placed on a raised rack, over a moist paper towel, sealed in a plastic bag, kept at room temperature (22 °C) for 48 h and then evaluated for the presence of bunch rot.

Occurrence of bunch rot (*B. cinerea*): To examine the effect of improved air and sunlight exposure on the occurrence of bunch rot, 10 Riesling clusters from each replication of the control (no leaf removal) and the HLR plots were randomly sampled at veraison, placed on a raised rack, over a moist paper towel, sealed in a plastic bag, kept at room temperature (22 °C) for 48 h and then evaluated for the presence of bunch rot.

At harvest, all plots were harvested on a per vine basis and graded for the presence of bunch rot. For each vine, individual clusters were placed in one of four categories representing the percent surface area of a cluster infected by bunch rot (i.e. no disease symptoms, slight: 0.5—5.9 %, moderate : 6.0—49.9 %, and severe: 50.0—100 %).

Statistical analysis: Analysis of variance (ANOVA) was completed using the General Linear Models procedure of SAS (SAS Institute, Cary, NC). Where appropriate, mean separation was completed using Duncan's procedure.

Results

Epicuticular wax weight: Although significant, epicuticular wax deposition of Riesling increased less than 9.2 % from 1990 to 1991. Exposed samples of Riesling had more epicuticular wax than shaded samples and non-contact samples had more epicuticular wax than contact samples (Fig. 1). Time of sampling (veraison *vs.* harvest), although significant, resulted in only a slight increase of epicuticular wax deposition (Fig. 1).

Similarly, exposure significantly increased epicuticular wax deposition with all 3 cultivars in 1991 (Fig. 2). A significant cultivar effect was also present with Cabernet franc and Optima having 208 % resp. 108 % more non-contact epicuticular wax than Riesling (Fig. 2).

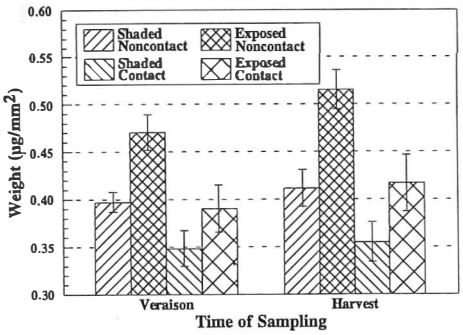

Fig. 1 (left): Effect of exposure and berry contact on epicuticular wax deposition of Riesling grape berries. Error bars represent SE of the means.

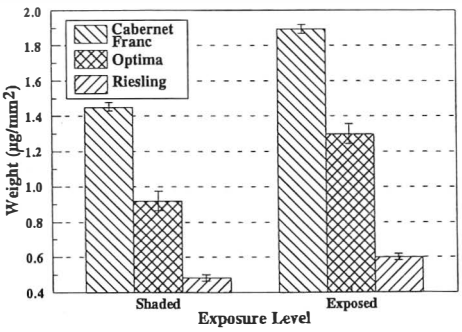

Fig. 2 (right): Effect of exposure and cultivar on non-contact grape berry epicuticular wax deposition. Error bars represent SE of the means.

Epicuticular wax appearance: No visible differences in epicuticular wax structure were present upon examining Riesling micrographs between the years 1990 and 1991, between veraison and harvest, or between shaded and exposed contact samples. However, a very noticeable difference between the contact and the non-contact region was apparent (Fig. 3: E, F and G). The wax layer formation pro-

cess had been hindered as a result of berry contact and hence, was less dense than the non-contact samples and lacked the platelet structures apparent in the non-contact samples (Fig. 3: E, F and G).

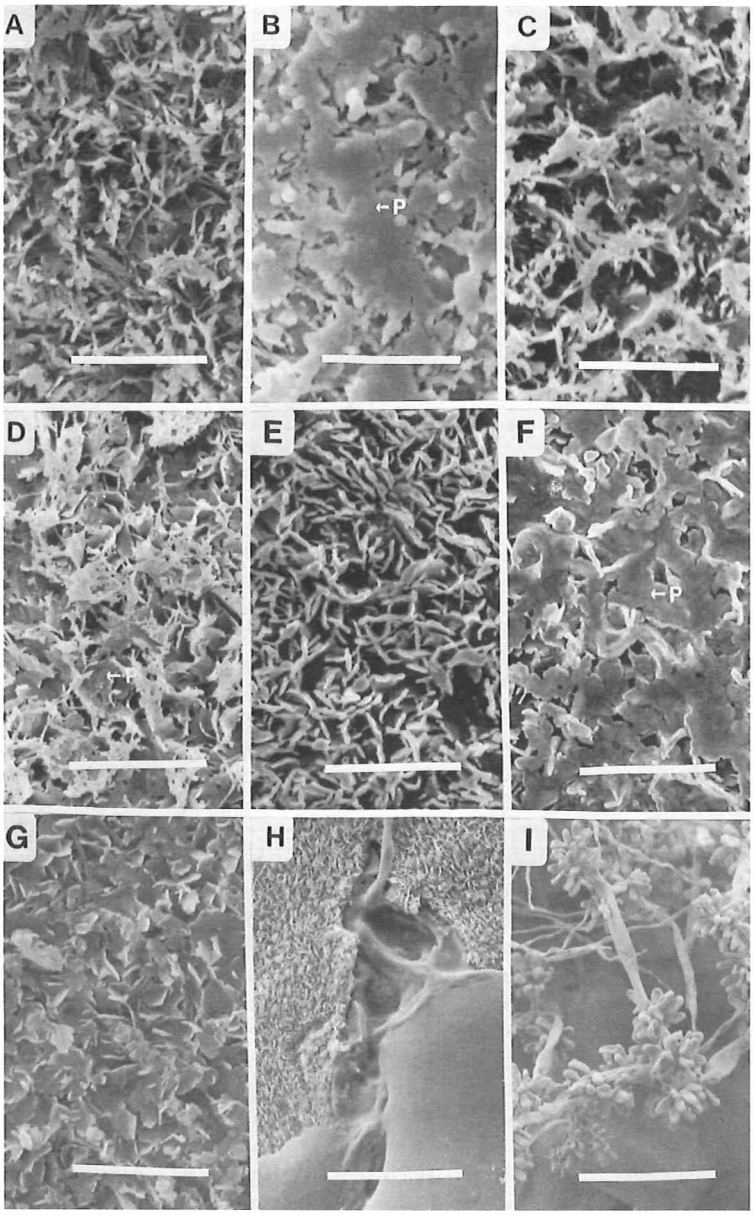

Fig. 3: Effect of exposure, berry contact and cultivar on epicuticular wax morphology. A: Shaded non-contact Cabernetfranc; B: Exposed non-contact Cabernet franc with platelet structures (P); C: Shaded non-contact Optima; D: Exposed non-contact Optima with platelet structures (P); E: Shaded non-contact Riesling; F: Exposed non-contact Riesling with platelet structures (P);. G: Contact Riesling; H: Crack in contact sample of Riesling with pathogen infection; I: Colony of *B. cinerea*. — A-G: Bar = 6.0 µm; H: Bar = 30 µm; I: Bar = 100 µm.

The exposed, non-contact, epicuticular wax layer of all 3 cultivars was characterized by larger and more numerous platelets than the shaded, non-contact samples (Fig. 3: A and B, C and D, E and F). Upon examining the non-contact exposed samples, cultivar differences were present with large, numerous platelets being present on Cabernet franc (Fig. 3: B), smaller platelets being present on Riesling (Fig. 3: F) and very small platelets being present on the Optima samples examined (Fig. 3: D).

Cuticle proper weight: Unlike the epicuticular wax samples, the 1991 Riesling cuticle proper weights were significantly less than the 1990 values (Fig. 4). Exposed samples had more cuticle proper than shaded samples and non-contact samples had more cuticle proper than contact samples. A significant increase of only 4.5 % was observed from veraison to harvest.

Exposure significantly increased cuticle proper deposition in the 3 cultivars examined at harvest in 1991 (Fig. 5). However, differences in the magnitude of the effect of exposure existed between cultivars. Differences in non-contact cuticle proper weight between cultivars were present with Cabernet franc and Riesling having 45.7 and 9.1 % more cuticle proper, respectively, than Optima.

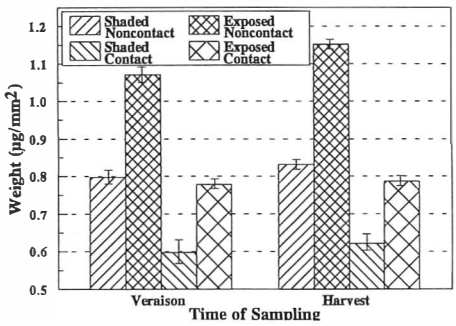

Fig. 4 (left): Effect of exposure and berry contact on cuticle proper deposition of Riesling grape berries. Error bars represent SE of the means.

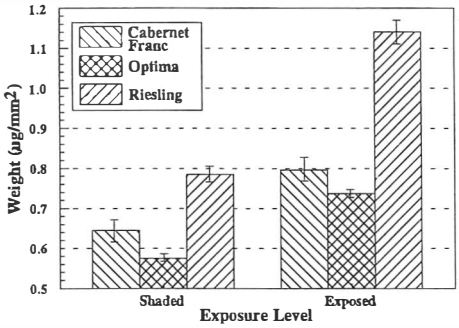

Fig. 5 (right): Effect of exposure and cultivar on non-contact grape berry cuticle proper deposition. Error bars represent SE of the means.

Cultivar susceptibility to bunch rot (*B. cinerea*): The 3 cultivars misted with only water had no infected clusters after the 48 h incubation period. The 3 cultivars misted with the spore suspension of *B. cinerea* however, exhibited varying levels of susceptibility of *B. cinerea*. Cabernet franc had the lowest number of diseased clusters, fewest disease foci per cluster and the lowest percentage of the clusters showing symptoms of bunch rot compared to Riesling and Optima which was the most susceptible cultivar (Tab. 1).

Occurrence of bunch rot (*B. cinerea*): There was no difference in the percentage of Riesling clusters with symptoms of bunch rot or the number of disease foci per cluster between veraison in 1990 and 1991. Large differences however, were present in the percentage of the cluster surface area with symptoms of bunch rot.

Exposed Riesling clusters had fewer diseased clusters and fewer disease foci than shaded clusters (Tab. 2) and had only 6.0 resp. 0.9 % of the surface area of the control showing symptoms at veraison of 1990 and 1991. At harvest of 1990, there was no effect of exposure on the occurrence of bunch rot in Riesling (Fig. 6). However, in 1991, the shaded clusters had more diseased clusters than the exposed treatment.

There was also no influence of exposure on the percentage of clusters showing slight symptoms at harvest in 1990 (Fig. 6). In 1991 however, there was an influence

Table 1

Relative susceptibility of 3 *V. vinifera* cultivars to *B. cinerea*

Cultivar[1]	Percent of clusters with symptoms[2] of bunch rot	Number of disease foci per cluster	Percent of cluster area with symptoms of bunch rot[3]
Cabernet franc	27.5 (a)[4]	0.125 (a)	12.87 (a)
Optima	82.5 (b)	1.875 (c)	43.48 (b)
Riesling	67.5 (b)	1.375 (b)	37.47 (b)

[1] Harvest dates:
Cabernet franc: September 4, 1991 (18.0 °Brix); Optima: August 24, 1991 (16.2 °Brix); Riesling: September 24, 1991 (17.8 °Brix).
[2] Clusters were sprayed with a *B. cinerea* solution (10^6 spores/ml water).
[3] Bunch rot severity measured on percentage surface of a cluster with symptoms of bunch rot.
[4] Mean separation by Duncan's multiple range test. Means within a column followed by a different letter are significantly different at the 5 % level of significance.

Table 2

Incidence and severity of bunch rot *(B. cinerea)* at veraison of control (i.e. shaded) and hand leaf removal (i.e. exposed) Riesling grapes

Treatment	Percentage of clusters with symptoms[1] of bunch rot	Number of disease foci per cluster	Percentage of cluster surface area with symptoms of bunch rot[2]	
	1990 and 1991	1990 and 1991	1990	1991
Control (Shaded)	16.3	0.213	10.8	1.13
Hand LR (Exposed)	3.8	0.038	0.63	1.01
Significance[3]	***	*	*	NS

Sampling dates: September 4, 1990 (10.2 °Brix), August 30, 1991 (9.8 °Brix).

[1] Clusters were sprayed with a *B. cinerea* solution (10^6 spores/ml water).
[2] Bunch rot severity measured on percentage surface of a cluster with symptoms of bunch rot.
[3] NS, *, ***: Not significant and significant at the 0.05 and 0.001 levels of significance, respectively.

of leaf removal with exposed clusters having 20.2 % fewer clusters with slight symptoms than the control (i.e. shaded). During both years the percentage of clusters examined with moderate to severe symptoms was low. As a result there was no significant effect of exposure or year on the percentage of clusters exhibiting moderate to severe symptoms.

There was both an exposure and cultivar effect on the occurrence of bunch rot in the 1991 cultivar trial (Fig. 7). Upon averaging the 3 cultivars, exposed clusters had 17 % fewer diseased clusters than shaded clusters. Differences between cultivars were apparent with Cabernet franc and Riesling having 20 resp. 11 % fewer diseased clusters than Optima.

Exposure and cultivar also reduced the percentage of clusters with slight symptoms of bunch rot (Fig. 7). Exposed clusters had, on average, 9 % fewer clusters with slight symptoms than the control. Differences between cultivars were evident with

Cabernet franc and Riesling having 16.0 resp. 5.3 % fewer clusters with slight symptoms than Optima. The percentage of clusters with moderate to severe bunch rot was low in 1991. Exposed clusters had 3.0 % fewer clusters with moderate symptoms than the control and Cabernet franc had 3.1 % fewer clusters with moderate symptoms than both Optima and Riesling. Although cultivar influenced the percentage of clusters with severe symptoms of bunch rot, so few clusters fit into this category (i.e. < 1 %) than the contribution to the overall incidence and severity of bunch rot was minimal.

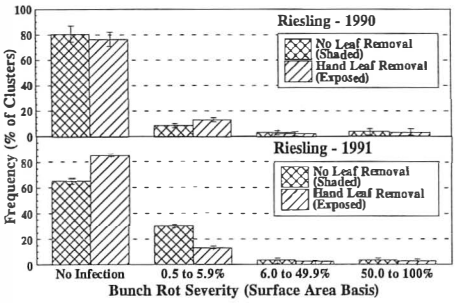

Fig. 6 (left): Effect of year and exposure on the occurrence of bunch rot (*B. cinerea*) with Riesling grapes at harvest of 1990 and 1991. Error bars represent SE of the means.

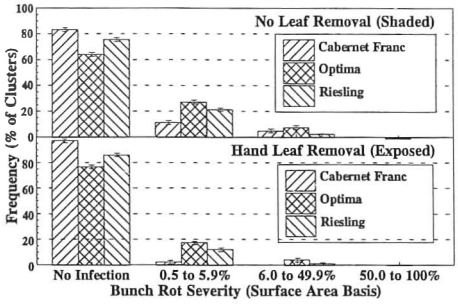

Fig. 7 (right): Effect of exposure and cultivar on the occurrence of bunch rot (*B. cinerea*) with 3 *V. vinifera* cultivars at harvest of 1991. Error bars represent SE of the means.

Discussion

In other reports, increased epicuticular wax and cuticle proper deposition is favoured by conditions of reduced relative humidity, increased light intensity and elevated temperature (MARTIN 1964; AIST 1984; GAY and PEARCE 1984). The leaf removal treatments used in the present study exposed clusters to sunlight, increased berry temperature and reduced relative humidity around the clusters resulting in an increase in epicuticular wax and cuticle proper deposition (MARTIN 1964; AIST 1984; GAY and PEARCE 1984; PERCIVAL 1992).

Cultivar differences in epicuticular wax deposition were also evident with Cabernet franc and Optima which had 104.3 and 207.3 % more non-contact epicuticular wax deposition, respectively, than Riesling. Epicuticular wax deposition in the tight clustered cultivar Riesling was further reduced due to abundant areas of berry contact, and there were no platelet structures which were present in the contact portions examined (Fig. 3: G). These semi-crystalline platelet structures assist in repelling water from the grape surface and may reduce the adhesiveness of plant pathogens to the cuticular membrane (HOLLOWAY 1969). Therefore, the susceptibiltiy of the tight clustered cultivar Riesling to bunch rot is partially due to a reduced water repellency, increased pathogen adhesiveness to the cuticular membrane, and a slower cluster drying rate (HOLLOWAY 1969; HEATH 1984). This relationship becomes more relevant since the successful conidia germination and direct infection through the cuticular membrane is dependent upon cluster saturation for at least 15 h (BULIT and DUBOS 1988) and bunch rot of Riesling clusters was observed to first occur in contact areas.

Similarly, the size and distribution of platelet structures differed on non-contact portions of the 3 cultivars examined (Fig. 3: A—F). The presence of very large, dis-

tinct, platelet structures on the surface of the epicuticular wax layer of Cabernet franc (Fig. 4: B) along with the open non-contact nature of the cluster may partially explain the tolerance of this cultivar to bunch rot. The susceptible cultivar Optima had smaller platelet structures than the other cultivars (Fig. 3: B, D, F). Therefore, Optima may not have possessed the same level of constitutive plant defence mechanism against pathogen infection as Cabernet franc.

The epicuticular wax weights obtained in the present study were considerably higher (0.35—1.9 μg/mm²) than those reported by ROSENQUIST and MORRISON (1988) for Thompson Seedless (0.11—0.14 μg/mm²) (Figs. 1 and 2). There are two possible reasons which may account for differences between the two studies: (1) minor improvements in methodology may have recovered higher amounts of epicuticular wax and (ii) the existence of a thinner cuticle proper in this study which may have permitted the diffusion of greater amounts of epicuticular wax to the berry surface (GAY and PEARCE 1984).

Cuticle proper weights also increased as a result of cluster exposure. Cuticle proper deposition on non-contact portions of exposed Riesling berries was 36 % greater than on shaded non-contact samples examined (Fig. 4) and these differences were 30 and 46 % greater for Cabernet franc and Optima (Fig. 5), respectively. The increased amount of cuticle may be due to decreased relative humidity, increased temperature or a combination of the two factors around the clusters (ROSENQUIST and MORRISON 1989). Although these values are similar to those reported for cuticles isolated from the leaves of grapevines which range from 0.8 to 1.1 μg/mm² (RADLER 1965), they are far below the 4.2—5.2 μg/mm² obtained by ROSENQUIST and MORRISON (1989) for similar tight clustered cultivars susceptible to bunch rot.

Contact areas of Riesling had significantly less cuticle proper than non-contact areas (Fig. 4). Since cuticle proper deposition is a diffusion process (HOLLOWAY 1982 a; GAY and PEARCE 1984), these large differences between contact and non-contact areas (Fig. 4) may be due to the suppression of cuticle proper deposition in contact areas and the reallocation of the cuticle proper precursors to non-contact areas. As a result surface cracks often occurred in contact areas which are of importance to the epidemiology of *B. cinerea* because they provide an unhindered entrance for infection past the cuticular membrane and also provide exudate which may stimulate germination of spores (BESSIS 1972; BLAICH *et al.* 1984; BULIT and DUBOS 1988). Therefore, cluster infection in contact areas was enhanced by longer wetness durations compared to non-contact portions, and the presence of open wounds and exudate which lessened the dependency of *B. cinerea* on long wetness conditions (BULIT and DUBOS 1988).

Large differences in non-contact cuticle proper deposition existed between cultivars with Riesling and Cabernet franc having 45.7 and 9.1 % more cuticle proper, respectively, than Optima (Fig. 6). However, due to flaws in contact areas of Riesling, this probably did not improve the tolerance of this cultivar to bunch rot. Despite having an open, loose, cluster architecture, Optima had the largest berry size and the least cuticle proper of the 3 cultivars examined. Not being able to provide adequate mechanical support after berry expansion, cracks often occurred in the cuticular membrane. This was particularly apparent with shaded clusters which had less berry cuticle proper (Fig. 6) and a higher incidence of bunch rot (Fig. 7). The presence of these cracks provided open wounds and exudate through which *B. cinerea* infection could occur more rapidly (BULIT and DUBOS 1988). Having a medium sized berry and an open, loose, cluster architecture, Cabernet franc had no physical flaws in the cuticular membrane structure. Therefore, successful infection was mostly dependent on infection directly through the cuticular membrane which requires a prolonged wetness period.

At veraison in 1990 and 1991, there was a significant influence of exposure on reducing the incidence and severity of Riesling clusters showing symptoms of bunch rot (Tab. 2). By harvest in 1991 (but not in 1990), these differences became even more pronounced with the HLR (i.e. exposed) treatment having a greater percentage of uninfected clusters and fewer clusters showing slight symptoms of bunch rot (Fig. 6). These results are consistent with those of GUBLER *et al.* (1991) who found cluster exposure to reduce the incidence and severity of bunch rot by 31 and 23 %, respectively.

Infection directly through the cuticular membrane is dependent on wetness periods being present for at least 15 h (BULIT and DUBOS 1988). Fairly dry conditions were experienced prior to the 1990 harvest with clusters rarely getting saturated for prolonged periods of time. Therefore, exposed clusters had a cuticular membrane structure which was more resistant to *Botrytis* infection but this improved tolerance was never expressed due to the dry 1990 season. In 1991 however, rainfalls occurred prior to harvest saturating clusters for prolonged periods of time (i.e. > 15 h). This period of cluster wetness duration was more than sufficient for *Botrytis* infection to occur (BULIT and DUBOS 1988). Under these conditions differences in cuticular membrane structure between exposed and shaded treatments played an important role on increasing the incidence and severity of bunch rot in shaded clusters.

Cultivar susceptibility can also be attributed to cuticular membrane structure. Although Riesling had the thickest non-contact cuticle proper, the tight, compact Riesling clusters consisted of a large proportion of contact surface area which contributed to a flawed epicuticular wax and cuticle proper structure and longer periods of cluster wetness than Optima or Cabernet franc. These factors contributed to Riesling being susceptible to bunch rot. Optima clusters had a fairly open architecture but had a large berry size and minimal quantities of cuticle proper, abundant berry splitting occurred which made this cultivar the most susceptible to bunch rot. Cabernet franc clusters were very loose which allowed for uninhibited cuticular membrane formation and a fast drying rate. With a thick epicuticular wax structure, large platelets and ample cuticle proper to mechanically support the medium sized berry, there were no apparent flaws in the cuticular membrane of Cabernet franc. Since the quantity and quality of epicuticular wax and cuticle proper are known to hinder the infection process (HOLLOWAY 1969; BAKER 1982; HALLAM 1982; GAY and PEARCE 1984; HEATH 1984; NICHOLSON 1984), Cabernet franc possessed the best constitutive defense mechanism against bunch rot infection of the 3 cultivars.

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