UNIVERSIDAD DE LA REPÚBLICA

FACULTAD DE AGRONOMÍA

DESHOJADOS EN COMBINACIÓN CON APLICACIONES DE CAOLINITA Y SUS EFECTOS SOBRE LA SANIDAD DE LA UVA Y COMPOSICIÓN DE MOSTOS Y VINOS DE VITIS VINIFERA L. CV SAUVIGNON BLANC

por

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TESIS presentada como uno de los requisitos para obtener el título de *Magister* en Ciencias Agrarias opción Ciencias Vegetales.

MONTEVIDEO

URUGUAY

2011

PÁGINA DE APROBACIÓN

Tesis aprobada por el tribunal integrado por la Dra. Milka Ferrer, el Dr. Eduardo Boido y la Dra. Silvana Vero el 15 de noviembre de 2011. Autor: Andrés Coniberti Mundy. Director: MSc. Edgardo Disegna. Co-directora: MSc. Vivienne Gepp

AGRADECIMIENTOS Y DEDICATORIA

A INIA, Viñedos Juan Toscanini e Hijos, Pedro Toscanini, Nelson Toscanini, Edgardo Disegna, Eduardo Dellacassa, Vivienne Gepp, Virginia Ferrari, Alan Lakso, Gerardo Casella, Luis Silvera, Lilian Cabrera, Richard Franco, Estela de Frutos, José Lez, Eduardo Boido. Este trabajo esta dedicado a Juan Ferreri, mentor de la idea.

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RESUMEN

Las enfermedades de racimos y en particular *Botrytis cinerea* presentan importancia primaria en la viticultura de clima húmedo, siendo en Uruguay la principal restricción para la obtención de un producto de calidad sostenida. Se visualiza como alternativa emplear prácticas culturales como el deshojado basal comúnmente no utilizada en variedades de uva blancas debido al riesgo que presenta la sobre-exposición solar de sus racimos. El presente trabajo se planteó con el objetivo de evaluar como el momento e intensidad de desojado inciden sobre la sanidad y calidad de uvas y vinos del cv. *Sauvignon blanc*. La gran susceptibilidad a la oxidación y quemado de la uva de la variedad motivó que los tratamientos fueran evaluados en combinación con aplicaciones de caolinita. El trabajo fue realizado durante las temporadas 2007/08 a 2009/10 proponiéndose diferentes intensidades y momentos de deshojado en combinación con aplicaciones de Caolinita (Sorround ®) en diseño de bloques al azar con seis repeticiones. Todos los tratamientos de deshojado y fundamentalmente aquellos realizados en postcuajado redujeron significativamente la ocurrencia de podredumbres de racimo y presentaron un impacto determinante sobre los metabolitos primarios y secundarios responsables del sabor final del vino. Si bien teóricamente una reducida exposición solar contribuiría a la complejidad aromática de la variedad, durante la evaluación sensorial los vinos provenientes de tratamientos de desojados fueron mejor evaluados. Aunque los deshojados redujeron significativamente el contenido de IBMP, estos mayores puntajes se asociaron a una mayor intensidad de sus notas frutadas o tropicales más que a la ausencia de sus notas verdes. La aplicación de caolinita redujo significativamente la temperatura de las bayas, el quemado de sol y otros daños asociados a la sobre-exposición solar de los racimos.

Palabras clave: Deshojado, caolinita, Sauvignon Blanc, IBMP.

FRUIT ZONE LEAF REMOVAL AND KAOLIN APPLICATIONS ON BUNCH ROT AFECTIONS AND MUST AND WINE COMPOSITION OF VITIS VINIFERA L. CV SAUVIGNON BLANC L.

SUMMARY

Bunch rot diseases and primarily *Botrytis cinerea* is one of the main constrain to obtain high quality wines in Uruguay. Some years, rainfalls near harvest time as well as high relative humidity promote bunch rot, not allow obtaining full maturity and so far to get sustained quality through the time. Basal leaf removal is a practice employed in wine red varieties to modify bunch microclimate, improve grape quality and control bunch rot. However this practice is not commonly used in white grape varieties because of the risk of cluster sun over-exposure causing berries sunburn. In order to assess how the timing and intensity of leaf removal affects grapes and wines quality of cv. *Sauvignon blanc* the present study was performed. Due to the susceptibility to oxidation and sun burning of the variety, treatments were evaluated in combination with kaolin applications. The work was done during the 2007/08 to 2009/10 period evaluating different intensities and times of defoliation in combination with kaolin (Surround ®) applications in a randomized block design with six replications. All defoliation treatments and mainly those made after fruit set, significantly reduced the occurrence of bunch rots and had a critical impact on primary and secondary metabolites responsible for the final taste of wine. Contrary to previous founding where theoretically a low sun cluster exposure should contribute to the aromatic complexity of the variety, our trial showed that wines from leaf removal treatments were better sensory evaluated by a trained panel. Although defoliation treatments significantly reduced IBMP grape and wine contents, these higher sensory scores obtained were associated more with to a greater intensity of tropical fruity notes than to the absence of green notes. Kaolin application significantly reduced berries temperature, sunburn and other berry damages associated with clusters solar overexposure.

Key words: Leaf removal, Kaolin, Sauvignon Blanc, IBMP.

1. INTRODUCCIÓN Y ANTECEDENTES

1.1. IMPORTANCIA DE BOTRYTIS EN VITIVINICULTURA

En zonas con climas templado-húmedos como el del Uruguay las condiciones de cultivo son muy favorables al desarrollo de podredumbres de racimo. Si bien la "podredumbre ácida de los racimos" asociada a la presencia de un complejo de bacterias y levaduras representa un problema de relevancia, *Botrytis cinerea* Pers., agente causal de la podredumbre gris de la vid, es considerado uno de los principales problemas en la viticultura de clima húmedo (Ferrer *et al.* 2009, Disegna *et al.* 2005b, La Torre y Vásquez 1996, Bulit y Dubos 1988).

El daño producido por *Botrytis cinerea* no sólo se restringe a las serias mermas de productividad ocasionadas bajo condiciones favorables a su desarrollo, sino fundamentalmente a la grave alteración de las características químicas y organolépticas de la uva (Nicolosi *et al.,* 1996). Se mencionan en la bibliografía: reducción del contenido de sólidos solubles, alteración del equilibrio ácido por aumento de la síntesis de ácido glucónico, oxidación de compuestos fenólicos como consecuencia de la acción polifenoloxidasa de la laccasa, modificación del perfil aromático, aumento de la producción de polisacáridos dificultando la clarificación del vino y aumento de la tasa de combinación del anhídrido sulfuroso (Cordonnier, 1987).

En consecuencia en regiones de clima húmedo su nivel de incidencia se transforma en uno de los indicadores más importantes a considerar al definir la cosecha (Disegna *et al.* 2005b, La Torre y Vásquez 1996, Bulit y Dubos 1988, La Torre 1986). Por tanto bajo estas condiciones en general la uva es cosechada sin haber completado su óptima madurez y los vinos producidos presentan un bajo tenor alcohólico, una menor concentración de compuestos fenólicos, una inadecuada relación de ácidos orgánicos, baja intensidad aromática y de aromas frutados, carácter herbáceo y una marcada astringencia, dificultando seriamente las posibilidad de producir vinos de alta calidad en forma regular a través de los años (Disegna *et al.,* 2005b).

1.2. EL MICROCLIMA DEL RACIMO Y LA INCIDENCIA DE BOTRYTIS

Botrytis cinerea afecta a un gran número de especies vegetales encontrándose distribuida en un amplio rango de condiciones microclimáticas (Elad *et al.,* 2007). Este patógeno representa un problema mayor de la viticultura en todo el mundo, causando importantes daños inclusive en regiones de clima árido (Savage y Sall, 1984). Aún cuando *Botrytis* afecta todos los órganos de la vid, salvo bajo circunstancias particulares los daños producidos en hojas, pámpanos y sarmientos no presentan relevancia en comparación con aquellos ocasionados sobre los racimos (Hidalgo, 1993).

Aunque en presencia de heridas la infección podría ocurrir en cualquier estado de desarrollo de la baya (Savage y Sall, 1984), esta es originada comúnmente en floración y precosecha (Morales, 1990). Asociado a una profusa esporulación y la gran habilidad de dispersión de los sus conidios por viento, la ocurrencia de la enfermedad se encuentra generalmente mas relacionada a condiciones microclimáticas favorables a su desarrollo que a la cantidad de inoculo presente (Shtienberg y Elad, citados por Elad *et al.,* 2007). Aunque la severidad del daño se ve incrementada en años donde precipitaciones coinciden con el periodo precosecha, grandes pérdidas pueden ocurrir en ausencia de las mismas (Gubler *et al.,* 1987). Sin embargo, los conidios son propágulos con vida corta en condiciones de campo, estando su sobrevivencia determinada por la temperatura, la humedad, la actividad microbiana y la exposición solar (Elad *et al.,* 2007). La germinación promedio de los conidios de *Botrytis* sp. después de la exposición directa a los rayos solares durante cuatro horas a condiciones de campo en Nueva Zelanda, fue mayor al 80%, mientras que luego de ocho horas esta se había reducido al 50%. Luego de diez minutos de re-exposición en un segundo día, la germinación promedio se vio reducida al 25% (Seyb, citado por Elad *et al.,* 2007). Si bien *Botrytis cinerea* es capaz de permanecer activa en un amplio rango de humedad y temperatura, la temperatura moderada (18 a 23 ºC) y humedad relativa por sobre 90% son favorables a su infección (Savage y Sall, 1984).

Existe una compleja relación entre la duración de las condiciones ambientales favorables, la producción de propágulos y la dispersión de la enfermedad (Elad *et al*., 2007). En condiciones de campo es común utilizar la evaporación potencial como una simplificación de la compleja interacción entre la temperatura, la humedad relativa y el viento que gobierna su desarrollo (Thomas *et al.,* 1988). Sin embargo la predicción del nivel de daño asociado, es complejo debido a los múltiples factores involucrados en su epidemiología (variedad, clon, portainjerto, fertilización nitrogenada, sistema de conducción, manejo en verde, fuente de inóculo, especies y cepas patogénicas, presencia de heridas, estado fenológico de la vid, entre otros) (English *et al.,* 1989).

Aún cuando el signo y síntoma en general se hacen visibles próximo a la cosecha, gran parte de estas infecciones se encuentran ligadas a la colonización primaria de restos florales senescentes producida meses antes de la madurez (McClellan y Hewitt, 1973). Sin embargo aunque la correlación entre las infecciones latentes de *Botrytis* sp. y el nivel de daño a cosecha ha sido reportado (Chebil *et al.* 2003, Mundy y Beresford 2003), ello no siempre es observado (Fermaud *et al.* 2003, Coulon 2002). De cualquier forma, la incidencia y severidad de la enfermedad se encontrará siempre fuertemente influenciadas por las condiciones microclimáticas de la canopia, determinante de la frecuencia y duración de los periodos favorables para su desarrollo (Savage y Sall, 1984).

La vid presenta hábito de crecimiento indeterminado por lo cual en regiones vitícolas como Uruguay donde la fertilidad del suelo, la temperatura y la disponibilidad de agua inducen altas tasas de crecimiento vegetativo, es común observar canopias densas y desequilibradas (Reynolds, 2000). El vigor de la planta juega un rol determinante en el desarrollo de podredumbres de racimo. En plantas vigorosas los racimos serán grandes y compactos, los brotes vigorosos con largos entrenudos, hojas grandes y múltiples brotaciones laterales (feminelas) (English *et al.,* 1989). Esto resulta en un ambiente favorable al desarrollo de podredumbres dado que en canopias densas gran proporción de los racimos se encontrarán excesivamente sombreados, la velocidad del viento reducida y en consecuencia la humedad sobre la superficie del racimo incrementada (Emmett *et al.,* 1994). En Uruguay donde el viñedo es tradicionalmente no irrigado, la estrategia comúnmente utilizada para hacer frente a los frecuentes e impredecibles períodos de déficit hídrico, es mantener plantas de buena expresión vegetativa capaces de tolerar dichos eventos produciendo rendimientos aceptables, lo que en definitiva acentúa aún más este problema en temporadas normales (Coniberti *et al.,* 2011). Por otro lado, la efectividad de control de los productos fungicidas en canopias compactas se ve sumamente disminuida a razón de una reducida capacidad de penetración (Gubler *et al.,* 1987).

1.3. PROBLEMAS ASOCIADOS AL CONTROL QUÍMICO DE BOTRYTIS

Si bien en algunas regiones vitícolas la utilización de insecticidas es frecuente, los fungicidas dirigidos al control de *Plasmopara viticola, Erysiphe necator* y *Botrytis cinerea* son los pesticidas más utilizados en vitivinicultura. Siendo *Plasmopara viticola* agente causal de la peronospora y la *Botrytis cinerea* responsable de la podredumbre gris de los racimos, los patógenos más importantes en regiones húmedas (Edder *et al.,* 2009).

En particular el control de *Botrytis cinerea* representa un gran desafío debido al gran potencial de daño bajo estas condiciones productivas (Edder *et al.,* 2009). En general la estrategia de control químico recomendada se basa en proteger los estados fenológicos definidos como críticos (floración, cerrado de racimos y envero) mediante la pulverización dirigida a los racimos con fungicidas específicos (Edder *et al.,* 2009, Moraga 2003) siendo en algunos casos bajo autorización permitida una cuarta aplicación en el periodo precosecha cuando ésta se ve retrasada (Edder *et al.,* 2009).

Debido al gran riesgo de generación de resistencia del patógeno a estos principios activos, se recomienda evitar la pulverización repetida de fungicidas de un mismo grupo químico, alternando con productos de diferente mecanismo de acción (Edder *et al.,* 2009). Sin embargo la sensibilidad disminuida a algunos productos ampliamente utilizados como los Benzimidazoles, Dicarboximidas, Pyrimetanil y Boscalid +Pyraclostrobin ha sido ya reportada a nivel nacional (Gepp *et al.,* 2011).

Por su lado, varios trabajos reportan la presencia de residuos de pesticidas en uvas a cosecha, alguno de los cuales continuaban presentes en el vino (carbendazim, fenhexamid, azoxystrobin, cyprodinil, pyrimethanil, tebuconazol, dimethomorph, miclobutanil, thiophanate-methyl, carbaryl, iprovalicarb y fluodoxinil) (Cabras *et al.,* 2001) existiendo actualmente estrictos reglamentos como por ejemplo las directivas de la UE (76/895/EEC, 86/362/EEC, 86/363/EEC y 90/642/EEC) o el Codex Alimentarius FAO/WHO, quienes establecen límites máximos de residuos en vino (Edder *et al.,* 2009).

1.4. PRÁCTICAS CULTURALES DIRIGIDAS AL CONTROL DE BOTRYTIS

En un contexto donde cada vez son menos los fungicidas permitidos y en donde además *Botrytis cinerea* ha mostrado sensibilidad disminuida a algunos de los principios activos mas utilizados, los países productores visualizan la necesidad de desarrollar estrategias de control no químicos. Estas, están dirigidas a obtener racimos pequeños, bien distribuidos y poco compactos y reducir el vigor y compacidad de la canopia, lo que resulta en un microclima menos favorable para el desarrollo de podredumbres (Emmett *et al*., 1994).

Sin embargo en virtud del hábito de crecimiento de la vid, éste es un objetivo difícil de alcanzar en condiciones productivas como la de la tradicional zona vitivinícola uruguaya, caracterizada por un volumen de precipitaciones proximos a los 1100 mm anuales y 650 mm durante el periodo vegetativo (datos promedio del periodo 1970-2004 de la EE-INIA-Las Brujas) y suelos fértiles (fundamentalmente Brunosoles y Vertisoles) con una elevada capacidad de retención de agua, que dependiendo de la profundidad y grado de erosión podrían superar los 120 a 170 mm (Molfino y Califra, 2001). Sin embargo, los frecuentes períodos de déficit hídrico y el riesgo potencial que implica manejar niveles de estrés en viñedos no irrigados, ha llevado a que la estrategia productiva comúnmente utilizada en la tradicional zona de producción vitivinícola uruguaya, sea utilizar portainjertos de vigor medio a alto (SO4, 3309C, 1103P), la aplicación de herbicida en la fila como forma de eliminar competencia por malezas y la excesiva fertilización fundamentalmente nitrogenada, acentuando este problema en temporadas sin restricción hídrica (Coniberti *et al.,* 2011).

Esta estrategia es sustentada tanto en el conocimiento empírico, como en resultados de investigación nacional, donde portainjertos poco vigorosos como Riparia han mostrado una limitada capacidad de reducción de vigor en temporadas lluviosa y una elevada sensibilidad a períodos de estrés hídrico (Disegna *et al.,* 2001). Por otro lado, en concordancia con aquello ampliamente reportado (Disegna *et al.,* 2005a, Howell *et al.,* 1991, Shaulis *et al.,* 1966) una sensible reducción del vigor individual de brotes y mayor exposición de racimos, fue observado en plantas conducidas en sistemas de canopia dividida (Lira) frente a aquellas conducidas en Espaldera (VSP). Sin embargo, la respuesta de plantas conducidas en Lira respecto al régimen hídrico, es comparable a la respuesta de plantas injertadas sobre portainjertos de vigor reducido (Disegna *et al.,* 2005a), por lo que si bien este es un sistema altamente difundido en general el vigor de las plantas es mantenido relativamente alto en base a fertilización nitrogenada.

Es así que la mayoría de los trabajos de investigación nacionales han enfocado la resolución del problema a través de la aplicación de prácticas correctivas como el corte apical de pámpanos (Coniberti *et al.,* 2007a), los deshojados parciales (Coniberti *et al.,* 2007a, Disegna *et al.,* 2005b) y los deshojados a nivel de los racimos (Coniberti *et al.,* 2007b, Disegna *et al.,* 2005b, Felix 2003, Ferrer y González 2000), estando estas prácticas correctivas en mayor o menor medida adoptadas por productores de avanzada.

Estudios tanto nacionales como internacionales han reportado la efectividad de los deshojados a nivel de los racimos en la reducción del desarrollo de podredumbres de racimo, siendo reportado en general como más efectivo la aplicación temprana de la práctica (Disegna *et al.,* 2005b, Reynolds *et al.,* 1996, Zoecklein *et al.,* 1992, Gubler *et al.,* 1987, Savage y Sall 1982). En Uruguay esta práctica es comúnmente aplicada en envero y fundamentalmente en variedades tintas, donde el riesgo de quemado por sobre exposición de racimos son menores y otros beneficios como el aumento de la concentración de antocianos son perseguidos (Disegna *et al.,* 2005a). En las variedades blancas, cuya sensibilidad al ataque de *Botrytis* sp. es mayor (Fregoni, 1998), las prácticas de deshojado no son frecuentes. Las principales causas de la no aplicación de esta práctica de manejo radican en la mayor sensibilidad de estas variedades al quemado del sol y su impacto negativo sobre la oxidación y composición aromática de la uva (Lee y Jaworski, 1988).

Asociado a la compacidad de sus racimos *Sauvignon blanc* es una de las variedades más sensibles a *Botrytis cinerea*, situación que se ve agravada en condiciones de excesivo vigor (Savage y Sall, 1984). Sin embargo pese a la reconocida efectividad de los deshojados basales sobre el control de las podredumbres de racimo, existe una gran controversia respecto al riesgo que implica su aplicación por su efecto en la expresión de sus caracteres varietales. Dry (2009) no recomienda bajo ningún concepto la aplicación de esta práctica en *Sauvignon blanc* a razón de la excesiva reducción en los contenidos de 2-Methoxy-3-isobutylpyrazine (IBMP) observados a consecuencia de la sobre exposición de la fruta. Este compuesto es responsable de los aromas típicos de morrón verde de los *Sauvignon blanc* de regiones frescas e importante para distinguir sus vinos de aquellos producidos con otras variedades (Allen *et al.,* 1991). No obstante Marais (2000) afirma que el manejo de la canopia contribuye positivamente a la calidad de los vinos *Sauvignon blanc*. Esta diferencia posiblemente esté establecida por un lado en opiniones dispares respecto a la aceptabilidad del típico aroma a morón verde del *Sauvignon blanc* (Marais, 1994) y por otro en las condiciones productivas en que los trabajos fueron realizados. Si bien la exposición de las racimos podría ser contraproducente en regiones cálidas con alta radiación solar incidente, en climas moderados y situaciones productivas favorables al desarrollo vegetativo, la producción de IBMP en bayas podría ser excesiva y la exposición de sus racimos ser favorable atenuando su presencia dominante en el aroma final del vino (Allen y Lacey 1999, Marais 1994).

1.5. EL MICROCLIMA DEL RACIMOS Y LA CALIDAD ORGANOLÉPTICA

Canopias excesivamente compactas no solo significan un microclima más propicio para el desarrollo de podredumbres de racimo (Emmett *et al.,* 1994) sino también se encuentran asociadas a un pobre contenido de sólidos solubles, concentración de antocianos, relación tartárico/málico y compuestos aromáticos de la uva (Ojeda *et al.,* 2002, Spayd *et al.,* 2002, Dry *et al.,* 2001, Keller *et al.* 1998, Dokoozlian y Kliewer 1996, Dry *et al.,* 1996, Champagnol 1984), así como a la excesiva acumulación de potasio en bayas impactando negativamente sobre el pH de las uvas y vinos producidos (Dokoozlian y Kliewer 1995, Rojas-Lara y Morrison 1989, Archer y Straus 1989, Smart *et al.,* 1985).

1.5.1. El microclima del racimo y el pH

El pH del vino es resultado del equilibrio de los diversos ácidos incluidos en su composición (fundamentalmente el tartárico y málico) y la influencia del K sobre la reducción de ácido tartárico libre (Gawel *et al.,* 2000).

El microclima del viñedo jugará un rol determinante en el pH final del vino (Smart, 2005). El grado de exposición a la luz, o sombreado y posiblemente la demanda evaporativa influyen significativamente en la acumulación de potasio y ácidos orgánicos en las bayas. Un excesivo sombreamiento, inhibe la acción de las enzimas (PEP-carboxilasa y tartrato sintetasa) reduciendo la síntesis de ácidos orgánicos. Al mismo tiempo, la fotosíntesis en uvas verdes es responsable de aproximadamente el 50% de la acumulación de los ácidos orgánicos hasta envero, por lo que un excesivo sombreamiento influirá directamente sobre su contenido (Winkler *et al.,* 1974).

La concentración de ácidos se reduce durante la maduración debido fundamentalmente a una reducción en la habilidad de la baya de sintetizar ácidos orgánicos, una reducción en la translocación de ácidos desde hojas hacia racimos (Hardy, 1969), la dilución de los ácidos asociado al incremento del volumen de la baya (Winkler *et al.,* 1974) y al metabolismo de los ácidos orgánicos a azúcares (Ribereau-Gayon, 1968). La tasa de respiración del málico se ve progresivamente incrementada hacia la madurez debido a incremento en la permeabilidad de las membranas de las vacuolas donde este es acumulado (Kliewer 1971, Hardy 1968). Esta respiración ocurre más fácilmente en climas cálidos y uvas expuestas a insolación directa (Butzke y Boulton, 1997).

A su vez, cepas excesivamente vigorosas y sombreadas presentan una mayor acumulación de potasio en bayas y mostos (Dokoozlian y Kliewer 1995, Rojas-Lara y Morrison 1989, Archer y Straus 1989, Smart *et al.* 1985). Entre los cationes minerales que neutralizan los ácidos, el potasio es el más abundante y el que determina la mayor parte de los equilibrios ácido-base, siendo los niveles excesivos del mismo, el motivo fundamental de un pH elevado en al vino (Gawel *et al.* 2000, Boulton 1980). Un elevado contenido de potasio también puede incidir en la tasa de degradación del ácido málico, pues impide la transferencia de malato de los depósitos de almacenamiento de las vacuolas al citoplasma (medio de degradación del malato) (Hale, 1977). Por consiguiente un alto contenido de potasio en bayas promoverá la elevación del pH y una reducción de la proporción tartrato/malato en el vino, situación generalmente indeseable en la producción de vinos finos.

El pH tiene gran influencia no sólo en la calidad organoléptica de los vinos sino también en su evolución química y microbiológica, siendo éste un parámetro fundamental para garantizar la calidad y la estabilidad del mismo (Beelman, 1984).

Un pH elevado en el mosto provoca inestabilidad en el mosto y el vino resultante exponiéndolos a una mayor incidencia de daños biológicos y oxidativos (Beelman 1984, Somers 1977). El desarrollo de bacterias lácticas y las levaduras pertenecientes al género *Brettanomyces* sp. se acelera considerablemente a partir de 3,8, al tiempo que el poder antiséptico del dióxido de azufre se reduce progresivamente conforme el pH del vino incrementa por encima de 3,6 (Chatonnet *et al.,* 1999). El porcentaje de antocianinas presentes en sus formas coloreadas, disminuye a medida que aumenta el pH (Beelman 1984, Somers 1975), evolucionando el color de estos vinos hacia notas amarillas-anaranjadas (indeseables) tanto más intensa y rápidamente cuanto más alto sea el pH (Beelman 1984, Sims y Morri 1984). En consecuencia, la velocidad de envejecimiento oxidativa de los vinos se acelera claramente cuando aumenta el pH, lo que permite suponer que podría influir en el mismo sentido sobre la estabilidad de los compuestos aromáticos que determinan tanto el carácter "afrutado" de los vinos jóvenes, como el "bouquet" del envejecimiento, altamente sensibles a la oxidación (Tominaga *et al.,* 2003, Tominaga *et al.,* 2000).

1.5.2. El microclima del racimo y el aroma

Numerosos estudios han demostrado la importancia de los monoterpenos, C13-norhisoprenoides y methoxipirazinas sobre el carácter y calidad de uvas y vinos (Marais, 1996). Si bien el macro y mesoclima tendrán un determinante impacto en la calidad enológica de la uva, el microclima de la canopia y en especial el de los racimos jugará un papel igualmente importante (Smart *et al.,* 1990). Parámetros como la cantidad e intensidad de luz incidente y la temperatura dentro de la canopia, inciden significativamente sobre la concentración de los distintos componentes responsables de los aromas florales y frutales (monoterpenos y C13-norisoprenoides) así como aromas típicos varietales y de carácter herbáceos (IBMP y compuestos C6) (Allen y Lacey 1993, Marais *et al.,* 1992, Lacey *et al.,* 1991, Morrison y Noble 1990).

El efecto de la insolación directa sobre el metabolismo de la baya es complejo viéndose la actividad fotosintética en general acelerada; proceso acompañado de una modificación de los tenores de pigmentos fotosintéticos como los carotenoides precursores de los C13–norisoprenoides (Bogoard 1976, Lichtenthaler 1975). Los C13–norisoprenoides presentan interesantes propiedades aromáticas, definiendo para muchas variedades el potencial aromático del vino, particularmente durante el proceso de envejecimiento (Williams *et al*., 1992, Enzell 1985).

El contenido de carotenoides en los granos de uva es variable (15 a 2000 µg/Kg) según la variedad, zona de cultivo, clima, manejo, etc., disminuyendo su concentración hacia la madurez (Fariña *et al.* 2010, Guedes de Pinho *et al*. 2001, Razungles *et al.* 1993, Razungles *et al.* 1988). Estudios realizados por Razungles *et al.* (1993), sobre el efecto del sombreamiento en los contenidos de azúcares y precursores aromáticos sobre las bayas, encontraron que el tenor de dicha disminución durante el proceso de maduración, está también relacionada con el nivel de sombreamiento de los racimos. Los resultados indicarían que sombreamientos de racimos superiores al 70%, se traducen en menor contenido de carotenoides en las bayas previo al envero. Por el contrario, en cosecha las bayas desarrolladas en la sombra presentan tenores de carotenoides más elevados que aquellos más expuestos. Los autores atribuyen este comportamiento al diferencial de temperatura máxima (-5ºC) en las bayas crecidas a la sombra en comparación con aquellas expuestas a un mayor grado de insolación.

Ristic *et al.* (2007), quienes compararon vinos de la variedad *Shiraz* producidos en viñas de canopias excesivamente sombreadas versus los producidos en viñas con buen grado de exposición, demostraron como los vinos provenientes de viñedos sombreados, presentaban menores caracteres “licorosos” deseados y un mayor carácter “herbáceo”, además de una mayor astringencia, que aquellos producidos con uvas provenientes de viñedos de canopias expuestas.

La persistencia en el crecimiento vegetativo, asociada a una buena disponibilidad de agua y nutrientes, resulta en un excesivo sombreamiento de racimos y establece una fuerte competencia hoja/fruta por asimilados, atrasando el inicio de la acumulación de azucares en el grano y la cosecha. Recientes evidencias, indican que el excesivo crecimiento vegetativo en sí mismo, elevaría el contenido residual de los compuestos aromáticos herbáceos (C6, IBMP) (Lakso y Sacks, 2009). Por tanto, las relaciones de concentración entre compuestos aromáticos frutales y otros que imprimen caracteres herbáceos indeseables en los vinos, se reportan ligados tanto a la luz recibida por las bayas como al excesivo crecimiento vegetativo (Riberau-Gayon *et al.,* 1998) siendo su contenido mayor en canopias vigorosas y excesivamente sombreadas (Allen y Lacey, 1993).

En función del ambiente y el manejo, la vid tendrá la capacidad de producir uvas y por tanto vinos de características particulares (Parr *et al.,* 2007). Características únicas e inherentes a la combinación ambiente-cultura, concepto del cual se sostiene gran parte de la política vitivinícola europea (Barker, 2006). Estas características propias distintivas y esperables en el producto obtenido en una determinada área geográfica le otorgan al mismo "tipicidad" (Parr *et al.,* 2007).

Sauvignon blanc es producido en muchas regiones vitícolas del mundo, siendo algunas de ellas reconocidas por producir vinos de características propias o distintivas como Sudáfrica (Marais *et al.,* 1999), Francia o Nueva Zelanda (Parr *et al.,* 2007). El estilo dominante de estos vinos enfatiza sus intensos caracteres varietales más que aquellos provenientes de la manipulación enológica (Parr *et al.,* 2007), siendo posible diferenciar dos estilos marcados en vinos *Sauvignon blanc* (Marais *et al.,* 1999). Aquellos provenientes de climas frescos donde los descriptores como el morrón verde o el espárrago se expresan en forma dominante y los producidos en regiones más cálidas donde en general descriptores como la guayaba y otros frutos tropicales serán característicos (Parr *et al.,* 2007). La concentración de las pirazinas y fundamentalmente la 2-Methoxy-3-isobutylpyrazine (IBMP), es la responsable de los aromas típicos de morrón verde de los vinos *Sauvignon blanc* de regiones frescas (Allen *et al.,* 1991), mientras que las concentraciones de otros componentes aromáticas varietales como los monoterpenos, norisoprenoides son los responsables de los aromas florales y frutales típicos de regiones más cálidas (Marais *et al.,* 1999).

En particular las metoxipirazinas son compuestos característicos de variedades como *Cabernet Sauvignon* y *Sauvignon blanc* siendo su presencia importante para distinguir sus vinos, de aquellos producidos con otras variedades. La intensidad del aroma a "pimiento verde" característico del *Sauvignon blanc* se encuentra correlacionado a la presencia de 3-isobutyl-2-methoxypyrazine (IBMP) (Roujou de Boubee *et al.,* 2000). Este compuesto presenta umbrales de percepción extremadamente bajos [1-2 ng/L (Mega, 1989)] pudiendo incluso a muy bajas concentraciones tener un impacto marcado en el aroma final del vino (Allen *et al.,* 1991). Si bien existen diferentes opiniones respecto a la aceptabilidad del típico aroma a morrón verde del *Sauvignon blanc* (Marais, 1994) en regiones como Sudáfrica y Nueva Zelanda su presencia es considerada importante en la definición del estilo, siempre y cuando éste no sea dominante en el vino (Allen y Lacey 1999, Marais 1994).

La ocurrencia de IBMP muestra una clara y consistente relación con la variedad y las condiciones en que esta fue producida (Allen y Lacey, 1999). El contenido de metoxipirazinas disminuye durante la madurez, siendo la temperatura y la incidencia de la luz en el racimo factores determinantes en esta variación (Ryona *et al.,* 2008). En general en regiones frescas y plantas vigorosas con canopias excesivamente sombreadas los niveles de IBMP tenderán a mantenerse elevados hasta la cosecha (Allen y Lacey, 1993). Aunque los mecanismos que gobiernan su síntesis y degradación permanecen aún desconocidos la exposición de los racimos previo a envero reduciría sustancialmente el pico máximo de acumulación comúnmente observado días previos al envero, al tiempo que durante el periodo envero-cosecha incrementan su degradación (Ryona *et al.,* 2008, Hashizume y Samuta 1999).

Si bien tanto la iluminación como la temperatura incrementan la tasa de degradación de IBMP en post envero, el grado de exposición de los racimos durante el periodo previo a envero (Scheiner *et al.* 2010, Ryona *et al.* 2008, Sala *et al.* 2004, Marais *et al.* 1999) y el grado de madurez alcanzado a cosecha (Ryona *et al.,* 2008), serían los factores determinantes en los contenidos de IBMP en los vinos.

1.6. LA CAOLINITA

El desarrollo de productos de origen natural para uso en frutas y hortalizas ha surgido como una nueva tecnología alternativa para el manejo de plagas y enfermedades en lo Estados Unidos (Puterka y Reinke, 2007). Dentro de este grupo se encuentra la caolinita [Al2Si2O5(0H4)], arcilla químicamente inerte, blanca, poco expansiva, no porosa ni abrasiva y de fácil dispersión en agua (Glenn y Puterka, 2005). Ésta es fundamentalmente comercializada bajo el nombre de Sorround®, producto registrado en la OMRI (Organic Material Review Institute) y aceptada para su uso en producción orgánica de uvas y otros cultivos.

El uso de caolinita se ha hecho extensivo a gran cantidad de cultivos hortícolas y frutícolas, demostrando gran efectividad y prácticamente nulo impacto ambiental en el control de enfermedades y plagas (Braham *et al.*, 2007) como la psylla del peral, *Cacopssylla pyricola* (Foester) y *C. Pyri* (Linné) (Pasqualini *et al.,* 2002, Puterka *et al.,* 2000), mosca del olivo, *Bactrocera oleae* (Gmelin) (Saour y Makee, 2003), psyllido del pistachio, *Agonoscera targionii* (Lichtenstein) (Saour, 2005), mosca del mediterráneo, *Ceratitis capitata* (Braham *et al.,* 2007, Mazor y Erez 2004), vaquilla de la vid *Macrodactylus subspinosus* (Fabricius); y *Homalodisca coagulata* (Say) vector de la enfermedad de Pierce (Blua y Morgan 2003, Sorensen y Gill 1996). La película formada sobre el follaje actúa fundamentalmente evitando la ovoposición, multiplicación del insecto y su alimentación (Blua y Morgan 2003, Sorensen y Gill 1996), además presentaría efectos sinérgicos en combinación con insecticidas (Isaacs *et al.,* 2004).

Más allá de sus usos en el control de plagas su aplicación sobre las hojas ha demostrado gran utilidad para el control del estrés térmico-hídrico y sobre la eficiencia en el uso del agua (Shellie y Glenn, 2008), la productividad, el quemado, composición aromática y organoléptica de la uva (Ahmed *et al.* 2011, Glenn y Shelle 2010, Ou *et al.* 2010, Shellie y Glenn 2008). Otros autores sin embargo reportan un sensible retardo de la madurez, menor concentración de sólidos solubles y antocianas cuando ésta es aplicada sobre la fruta (Battany, 2006). La película blanca formada sobre los órganos aplicados reflejaría gran parte de la radiación ultravioleta e infrarroja y permitiría la transmisión de la radiación fotosintéticamente activa (PAR) (Glenn y Puterka, 2005), transformándola en una herramienta efectiva para la reducción del estrés calórico y el aumento de la asimilación neta de carbono (Glenn *et al.,* 2002).

A su vez, el efecto atenuante de la deshidratación y cracking de la uva (Engelhard Corporation, 2001), reduciría la aparición de heridas en bayas tratadas, las que representa la reducción de una importante vía de infección de *Botrytis* sp. y otras podredumbres (Greer *et al.,* 2003a)

Finalmente, por ser una arcilla de baja expansión su uso potencial como clarificante y agente estabilizante proteico de vinos ha sido también reportado (Sarmento *et al.,* 2000).

1.8. JUSTIFICACIÓN Y OBJETIVOS DEL TRABAJO

En Uruguay las podredumbres de racimos y en particular *Botrytis cinerea* presenta importancia primaria en la producción vitivinícola, siendo la principal restricción para obtener un producto con calidad sostenida a través de los años. Al mismo tiempo cada vez son menos los fungicidas autorizados, existen normativas cada vez más estrictas respecto a niveles permitidos de residuos en vino y *Botrytis cinerea* ha mostrado su sensibilidad disminuida a algunos de los principios activos más utilizados a nivel nacional.

En este contexto, se visualiza la necesidad de desarrollar estrategias de control no químicos. Tal como fuera mencionado, las prácticas de manejo cultural están dirigidas a la obtención de un microclima menos favorable al desarrollo de podredumbres. Sin embargo en virtud del hábito de crecimiento indeterminado de la vid, ello es difícil de conseguir en Uruguay donde la temperatura y la disponibilidad hídrica promueven un excesivo desarrollo vegetativo.

Por tanto la resolución del problema queda prácticamente restringida a la aplicación de prácticas correctivas, como son los deshojados basales. Estos son comúnmente aplicados en variedades tintas por productores de avanzada, por sus efectos positivos sobre la calidad de la uva y el control de podredumbres de racimo. Por el contrario debido a su mayor susceptibilidad a la oxidación y quemado de sol, éstos no son empleados en variedades de uva blanca.

Es axial que con el objetivo de ajustar una práctica de manejo que permita reducir la incidencia de *Botrytis cinerea* y otras podredumbres de racimos, sin afectar negativamente otros parámetros de calidad en uvas blancas se planteó durante las temporadas 2007/08 a 2009/10 el presente trabajo. La marcada susceptibilidad de la variedad a las podredumbres de racimos y la gran controversia internacional respecto al posible impacto del deshojado a nivel de los racimos sobre la expresión de sus típicos caracteres varietales, justificó el focalizarlo en esta variedad.

El estudio se basó en las siguientes 3 hipótesis: 1) es posible mediante deshojados basales disminuir la presencia de podredumbres de racimos; 2) la excesiva exposición de uvas blancas a la insolación directa aumenta la incidencia de quemado de sol, favoreciendo la degradación de ácidos orgánicos y compuestos aromáticos en *Sauvignon blanc*; 3) es posible mediante la aplicación de caolinita atenuar los efectos asociados a la sobre-exposición solar de los racimos, sin afectar negativamente la composición química y calidad organoléptica de los vinos.

Durante los dos primeras temporadas se propusieron nueve tratamientos con distintas intensidades y momentos de deshojado en combinación con aplicaciones de Caolinita (Surround®). Durante la temporada 2009/10 basados en los resultados parciales obtenidos, se comparó el testigo sin deshojar con el mejor de los tratamientos de deshojado propuestos originalmente. El tratamiento de deshojado fue también evaluado en combinación con la aplicación de caolinita.

Se evaluó el efecto de los tratamientos sobre: a) la sanidad de la uva b) el quemado de sol, la oxidación de los compuestos fenólicos durante la maduración y su influencia en el color final del vino, c) El pH de la uva y el vino, relacionándolo con el contenido de K y ácidos orgánicos de las bayas y d) la composición aromática de uvas y vinos.

2. FRUIT ZONE LEAF REMOVAL AND KAOLIN APPLICATIONS TO CONTROL BUNCH ROT AND IMPROVE QUALITY OF SAUVIGNON BLANC L. GRAPES IN TEMPERATE HUMID CLIMATES [1]

Abstract.

Botrytis bunch rot is a serious disease on grapes and probably the most important production issue under temperate–humid climate. In Uruguay favorable climatic conditions asociated with fertile soils and management practices, stimulate excessive vegetative growth, resulting in extremely dense and shaded canopies. These dense canopies determine an even more unfavorable canopy microclimate, leading to decrease grape and wine quality as well as increase *Botrytis cinerea* Pers. incidence. In Uruguay leaf removal is beginning to receive for many growers consideration as an important cultural practice for bunch rot control. It is normally applied after veraison and almost only on red varieties because of the high sunburn susceptibility of white varieties under our climatic conditions. The aim of this study was to determine how the moment and intensity of leaf removal impacts on bunch rot and grape quality, and if it is possible through Kaolin application to reduce the negative impact of excessive exposure to high temperatures on white grapes and wines. Different intensities and times of defoliation in combination with kaolin (Surround ®) applications were evaluated over 2007/08 and 2008/09 seasons. All defoliation treatments and mainly those made after fruit set significantly reduced the occurrence of bunch rots and had a critical impact on primary and secondary metabolites responsible for the final taste of wine. Kaolin application significantly reduced berry temperature, sunburn and other berry damages associated with cluster solar exposure.

Key words: Leaf removal, Kaolin, Bunch rot, IBMP, Browning, *Sauvignon blanc*.

2.1 INTRODUCTION

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[1] Este capítulo será enviado a la revista American Journal of Enologie and Viticulture para ser publicado como: Coniberti, A., Ferrari F., Gepp V., Boido E., Fariña L., Dellacassa E., Disegna E. Fruit Zone Leaf Removal and Kaolin Applications to Control Bunch Rot and Improve Quality of *Sauvignon Blanc L.* Grapes in Temperate Humid Climates.

Botrytis bunch rot is a serious disease on grapes and probably the most important production issue under temperate–humid climate. Also wines produced from bunch rot infected grapes have off-flavors and are considered fragile, sensitive to oxidation, susceptible to bacterial contamination, and unsuitable for aging (Bulit and Dubos 1989)

The disease can be severe when prolonged periods of moisture coincide with pre-harvest berry ripening. However, serious yield losses can still occur without free moisture in the presence of dense canopies or tight clusters (Gubler *et al.* 1991, Persival *et al.* 1994, Smith *et al.* 1988). In Uruguay favorable climatic conditions, fertile soils and management practices (plant material free from viruses, unsuited rootstock-scion combinations, soil management, trellis and vine spacing, severe toppings and in many cases excessive reduction in yield achieved through cluster thinning) stimulate excessive vegetative growth, which results in extremely dense and shaded canopies. These dense canopies determine an even more unfavorable canopy microclimate leading to decrease grape and wine quality as well as increase *Botrytis cinerea* Pers. incidence. Crop loss due to bunch rot in Uruguay can be as high as 100% in a given year and in many cases this is the main factor to determine harvest.

Under such conditions disease cannot be managed effectively only with fungicides (Gubler *et al.* 1987), so several canopy management practices are normally applied in order to reduce relative humidity at the fruit zone (shoot positioning, topping, suckering, partial defoliations and leaf removal). In particular, leaf removal is beginning to receive for many growers consideration as an important cultural practice for bunch rot control. This practice typically involves removal of leaves from the basal portions of shoots of grapevine canopies (Bledsoe *et al.* 1988, Stapleton and Grant 1992). It increases wind movement through the canopy and cluster sunlight exposure, enhances the drying conditions or evaporative potential in the fruit zone (English *et al.* 1990). In Uruguay leaf removal is normally applied after veraison and almost only on red varieties because of the high sunburn susceptibility that white grapes show under our climatic conditions.

Kaolin (Surrounds, Engelhard Corp, Iselin, NJ, USA) is beginning to receive consideration as a potential alternative pest management product (Puterka *et al.* 2000, Glenn *et al.* 1999) and is listed by the Organic Materials Review Institute (OMRI) for use in organic production. The white film formed after it application, protects fruits from sunburn (Glenn *et al.* 2002).

Although the effectiveness of leaf removal to reduce Botrytis/sour rot affections is well known, the aim of this study was to determined how the moment and intensity of leaf removal impact on bunch rot infections and grape quality, and at the same time to evaluate if it is possible through Kaolin application to reduce the negative impact of excessive exposure to high temperatures on white grapes and wines.

2.2 MATERIALS AND METHODS

2.2.1 Experimental site

This experiment was conducted over the seasons 2007/08 and 2008/09 in southern Uruguay (34º 44 S 56º 13 W). Uruguayan climate can be classified as temperate – humid without a notorious dry season (Köeppen 1931). Total rainfall in Southern Uruguay can rise up to 1100 mm in an average year, 650 mm of those during the growing season. Although yearly rainfall is even distributed, there is an historical tendency of slight increases in rainfall during early fall and spring. However rain accumulation from bloom to harvest and previous to harvest in 2007/08 and 2008/09 seasons were extremely dry for southern Uruguay climate conditions. Note how in 2008/09 season during bloom.fruit set no rain did occur (Table 1) Weather data details can be accessed at http://www.inia.org.uy/gras/agroclima/cara_agro/index.html

Table 1. Climate data for southern Uruguay (34º 44 S 56º 13 W)

	Rain accumulation (mm)		
Accumulation period	**Historical**	**2007/08**	**2008/09**
Budbreak to harvest (Sept-Feb)	583	505	257
Bloom to harvest (Nov-Feb)	386	219	205
Bloom-fruit set (Nov)	89	60	0
20 days before harvest	120	62	54
		GDD	
Accumulation period	**Historical**	**2007/08**	**2008/09**
Budbreak to harvest (Sept-Feb)	1616	1682	1740

Historical rainfall and growing degree-days base 10ºC (GDD) are presented as the average of 2000/01 - 2010/11 seasons.

2.2.2 Experimental vineyard

The experimental vineyard was a >12-years old *Vitis vinifera* L. *cv. Sauvignon Blanc* grafted onto SO4 rootstock. Vines were trained on vertical shoot positioning (VSP) system, spaced 1.2 m apart, in north-south rows 2.8 m wide. Cordon-trained plants were pruned to twelve two-bud spurs. The height of the cordon was 1.0 m and the top wire was located 1.8 m above ground. At approximately 30 cm shoot length all infertile shoots as well as shoots not located on spurs were removed. During the season shoots were positioned (by hand) vertically above the spurs and topped 30 cm above the top wire. Standard pest and disease control was applied and included combinations of Phtalimides, Iprodione, Ciprodinil + Fludioxonil and downy mildew fungicides. The vineyard was not irrigated and standard cover crop management also was applied. The soil of the site was classified as a Tipic Argiudolls (USDA 1998).

2.2.3 Treatments

Plots were selected at bloom on the basis of uniform vine size and canopy continuity. There were 4 hand defoliation treatments: 1) partial early leaf removal (EP) where leaves and laterals opposite and below of the most distal cluster of each cane were removed between stages 29 to 31 (Eichorn and Lorentz 1977). Care was taken to retain leaves which shaded clusters from the direct sunlight, theoretically giving 50% of sun exposure in the fruit zone; 2) total early leaf removal (ET) where all leaves and laterals located below to the second node above the most distal cluster from each cane, were removed between satges 29 to 31 (Eichorn and Lorentz 1977), giving a 100% of sun exposure in the fruit zone; 3) total veraison leaf removal (VT) where all the leaves and laterals located below to the second node above the most distal cluster in each cane were removed at veraison (stage 35 - Eichorn and Lorentz 1977), giving a 100% of sun exposure in the fruit zone and; 4) a combination of EP and VP treatments described above (EP+VT). Each defoliation treatment was also evaluated with (K) and without Kaolin application (No K). A control treatment (no leaf removal) was also evaluated giving a total of nine treatments. Treatments were arranged in a randomized complete block design consisting in nine treatments in six row replications. Plots comprised five adjacent vines.

2.2.4 Kaolin applications

A backpack air blast power sprayer Hatsuta Industrial Co. Ltd. CN-73 4-Gallon, was employed. Applications were performed at early morning at a rate of 10% (w/v) kaolin water suspension (Surround WP, Teessenderlo Kerley, US). To obtain thorough coverage, 250 mL per plot was sprayed at each application date (Equivalent to 15 kg of Surround® per hectare approximately). Grapes received three applications of kaolin during the post-veraison to harvest period. The first one was made at 12 Brix and the others after heavy rains occurred.

2.2.5 Canopy measurements

After defoliations and prior to harvest, total leaf area (TLA) per vine was estimated as follows: 1) The leaf blade of a sample of 500 leaf of different sizes of *Sauvignon blanc* plants from adjacent rows of the experiment were labeled and measured using a single rule, 2) the same leaves were scaned and individual leaf area was measured using an automatic area meter AAC-400 Hayashi Denkoh Co. Ltd., 3) the relationship between leaf blades length and leaf area was established, 4) later in the field leaf blade length was measured for all leaves of two representative shoots per vine of every plot, 5) finally Total leaf area (TLA) was estimated multiplying the average leaf area of these shoots by the total number of shoots of each vine. Exterior area of the canopy (ELA) was also measured according to Schneider (1989) and leaf index (LI) was calculated for each experimental plot using the ecuation: LI = (1 - t/d) EA/TLA where 1 –t/d estimates the gaps in the canopy (Schneider 1989). All these measurement were made at bloom and 20 days after defoliations. Ravaz index (RI) (fruit/pruning weight) per each experimental plot was calculated and averaged by treatment. Photosynthetic active radiation (PAR) of full sunlight and that received by the bunch zone was measured using a ceptometer (AccuPAR-LP80 Decagon Devices Inc). Measurements were taken two times during the growing seasons (20 days after each leaf removal treatmens were made) between 10:00 am and 3:00 pm. Ambient measurement were taken by positioning the ceptometer at the bunch zone height outside the canopy. Three readings per plot were made at the fruit zone on both sides of each vine with the ceptometer positioned parallel to the cordon and pointed upward. These three interior readings were averaged and divided by a single reading taken outside the canopy to determine the percentage of PAR available at the fruit zone. Between bloom and harvest air temperature (T) and percent relative humidity (RH) were registered at 30-min intervals using temperature and humidity data loggers (DS1923-F5# - Hygrochron iButton). Three registers per treatment were used. Vapor pressure deficit (VPD) was calculated from the recorded temperature and relative humidity, using the empirical equation $VPD = 6.108 \exp[(17.27T)/(T+237.3)](1-RH/100)$ (English *et al.* 1989). Evaporation rate was estimated by placing a petri dish filled with a known weight of destiled water in the fruit zone of the canopy. After 24 hrs, remainding water in the petri dish was weighted usin a micro-scale (Precisa XB 4200C serie 320 XB), and water loss during the period was calculated. Evaporative rate was expressed in terms of mm/day of water lost. Measurements of evaporative rate were made two times during the growing season 20 days after each leaf removal treatments were applied. Three petri dishes per treatment were used. All data are reported as the mean of the three observations. In 2010 after fruit set (between stage 29-31 -Eichorn and Lorentz 1977), bud fertility was estimated by counting the number of bunches per bud of two representative shoots per plant in each experimental plot.

2.2.6 Berry temperature

Two weeks before harvest from 10:00 am until sunset of a sunny day at 30-min intervals, berry temperature was measured in three external berries from three clusters per vine in every plot. These readings were averaged per plot and data are reported as the mean of plots values for each treatment. A handheld infrared thermometer (Oakton 35639-00 InfraPro1) was used.

2.2.7 Sampling Protocols

During the post-veraison period at three dates including harvest, randomly collected samples of 100 berries were taken from each treatment replicate. At each sampling date berry weight was measured using a micro-scale (Precisa XB 4200C serie 320 XB) and the average per treatment was determined. The same berry samples were subsequently used to determine soluble solids. All treatments were harvested at the same date. In order to define harvest time randomly collected samples of 100 berries per trealment were taken once a week after grapes achieved 20º Brix as well the percentage and severity of Botrytis bunch rot affections were considered. At harvest total number of clusters were counted and total fruit weight per vine was measured using a scale (ES50Kx1 Napco Precision Instruments Company Ltd.). A representative sample of approximately 15 Kg of fruit per treatment replicate was retained for winemaking. At 2009 harvest an additional 200-berry sample was taken and stored at -30°C for subsequent analysis of MP and phenolic-free GG compounds.

2.2.8 Incidence and severity of bunch rot and sunburn

Clusters with rot, sunburn, and/or pests were collected separately from those unaffected. The percentage of bunches infected with *Botrytis* and/or sour rot (incidence) as well as the percentage of each bunch that was infected (severity) was determined by visual inspection using a six-point scale (0, 5, 25, 50, 75 and 100%). In the same way, incidence and severity of sunburn as well as pest affections (mealybugs) were evaluated. The relationship between berry damages (sunburn and pests) and bunch rot infestation were also evaluated.

2.2.9 Grape, must and wine composition

At harvest, grapes retained for winemaking were crushed and juice total soluble solids (SS) were determined using a hand refractometer (Atago N10). Must and wine pH was measured with a pH meter (Horiba F-13 series) and tritratable acidity (TA) was determined by tritation (NaOH, 0.1 N) and expressed as sulfuric acid (w/w). The concentration of malic and tartaric acids were measured for reflectometric determination after enzymatic reactions used the automatic system RQflex® plus 10 Reflectoquant® (Merck, Germany). Samples were diluted depending on the expected acid content and proceeded according of the manufacturing instructions. Free amino-nitrogen (FAN) was analyzed by formol tritation according to Zoecklein *et al.* (1999).

Must "browning" analysis. For each replication after destemed and cruching, a 80 ml glass botle was full filled with a sample of homogenized must. After parafilm sealed and placed in a cool room at 5ºC, samples were filtered through a 0.45 µm membrane filter. Color was determined directly by spectrophotometry. A UV-Vis Shimadzu Co. recording spectrophotometer (UV-160A – Japan) was used at 420 nm wavelength, with a 10 mm pathlength cuvett.

3-isobutyl-2-methoxypyrazine (IBMP) Analyses. Extraction and Quantification. At harvest two replicates of 100 frozen berries were performed for each plot. The

Extraction and Quantification protocol used was the same as described previously by Ryona *et al.* (2008).

Phenolic-free-GG determination. To prepare the homogenate fruit extracts, 100-berries samples from each plot were homogenized and phenolic-free GG was determined as described previously by Zoecklein *et al.* (1999).

2.2.10 Winemaking and analysis.

Approximately 15 Kg of fruit per treatment replicate were retained for winemaking each year. Once destemmed and crushed they were treated with 50mg/L sulfur dioxide and given two hours of must skin and seed contact at 2 ºC. The lots were pressed and taken into small-scale wine lots. Must were subsequently inoculated with 0.25-g/L Saccharomyces cerivisiae ALG804. Following fermentation at 16 ºC, wines were cold stabilized, racked, SO2 – adjusted and immediately bottled. All treatment replicates were kept separated during fermentation.

2.2.11 Statistics.

Statistical analyses were conducted with SAS statistical software (SAS Institute, Cary, NC). Individual replicate data were averaged by treatment and treatment means were compared using Tuckey's test procedures at the 5% significance level.

2.3 RESULTS

2.3.1 Fruit yields

Leaf removal did not influence vine bud fertility. Shoot density, number of clusters per vine, average cluster weight and fruit weight per vine were similar in all treatments. On the other hand, significant differences in berry weight between severe defoliated vines and the control was observed at harvest (Table 2) Differences were not significant in previous sampling dates (data not shown). During both seasons, berry size at harvest was affected by kaolin applications. In 2008, these differences were significant just within VT treatment, while in 2009 berry size in EP+VT and VT treatments were significantly higher when kaolin was applied. Kaolin application had no effect on berry weight in partial defoliation treatments.

Table 2. Leaf removal and kaolin applications on Ravaz index, yield, berry weight, soluble solids and nitrogen concentration in grapes.

	Ravaz Index		Yield (Kg/pl)		Berry weight (g)		Brix		FAN (mg/L)	
Vintage 2008										
Treatment	K	Non K	K	Non K	K	Non K	K	Non K	K	Non K
EP	4.7	4.8	2.96	3.18	1.89 a[b]	1.89 a	23.6 a	23.8 a	124.9 b	123.8 b
ET	4.8	5.0	3.03	2.96	1.79 b	1.75 b	23.8 a	24.0 a	79.7 c	87.3 c
EP + VT	5.0	4.9	3.01	2.96	1.81 b	1.78 b	24.0 a	24.0 a	119.2 b	120.1 b
VT	4.6	4.8	3.22	2.99	1.87 a	1.78 b	23.6 a	23.9 a	122.7 b	125.2 b
Control		4.8		3.17		1.89 a		22.7 b		148.3 a
Vintage 2009										
	Ravaz Index		Yield (Kg/pl)		Berry weight (g)		Brix		FAN (mg/L)	
Treatment	K	Non K	K	Non K	K	Non K	K	Non K	K	Non K
EP	4.6	4.5	2.99	2.87	1.80 ab	1.79 ab	24.4 a	24.6 a	108.1 b	102.3 b
ET	4.5	4.8	2.83	2.81	1.78 ab	1.75 b	24.4 a	24.5 a	74.1 c	71.3 c
EP + VT	4.4	4.7	3.26	2.94	1.83 a	1.75 b	24.2 a	24.7 a	107.5 b	108.5 b
VT	4.7	4.6	3.21	2.90	1.81 a	1.76 b	23.6 b	24.1 ab	119.3 ab	117.6 ab
Control		4.5		3.16		1.82 a		23.6 b		129.4 a

[a] Abbreviations: EP: partial early leaf removal; ET: early total leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: not leaf removal; Kg/pl: Kilograms per plant. FAN: Free amino-nitrogen. [b] Means followed by different letters within the same variable, indicate significant differences at $p \leq 0.05$.

2.3.2 Canopy characteristics

Leaf removal did not affect vine size as measured by cane-pruning weight or neither the ratio Yield/ Pruning weight. On the other hand, leaf removal reduced the amount of leaf area per kilogram of fruit from 2.8 m2/kg observed in the control to 2.20 m2/kg in the most drastic treatments (ET). Data collected in the canopy showed that leaf removal had a slight influence on the total canopy leaf density, expressed as LI at harvest. Although twenty days after early leaf removal, significant differences between the control and the most severe defoliated treatment were observed, after post-veraison canopy densities were similar in all treatments. For all treatments this ratio was always under 0.56, which indicated an extremely shaded canopy. Based on pre-veraison and post-veraison measurements leaf removal clearly increased PAR (%) measured at the fruit zone. The percentage of full light received by clusters along the canopy increased in average more than three times after partial defoliation treatments and more than seven times after total leaf removal were applied. Estimated evaporative rate in the fruit zone of the canopy was also significantly affected by defoliation treatments. Since alterations in air temperature and percent relative humidity regime were not detected for data loggers (data non shown), average water loss per day in total and partial leaf removal treatments were four and three times higher than the control (Table 3).

Table 3. Leave removal on total leaf area per vine; canopy shaded index, PAR at the fruit zone and evaporation rate at the fruit zone.

	Vintage 2008							
	20 days after early leaf removal				20 days after veraison leaf removal			
Treatment[a]	LA (m^2)	LI	PAR (%)	ER (mm/d)	LA (m^2)	LI	PAR (%)	ER (mm/d)
EP	5.61 b[b]	0.54 bc	9.1 b	2.35 b	6.35 b	0.52	9.3 b	2.10 b
ET	5.05 c	0.58 a	20.3 a	4.75 a	6.01 c	0.55	19.1 a	4.15 a
EP + VT	5.43 b	0.55 bc	9.7 b	2.15 b	6.05 c	0.54	20.7 a	4.30 a
VT	6.19 a	0.50 c	3.1 c	0.91 c	6.11 c	0.53	21.3 a	4.75 a
Control	6.27 a	0.51 c	2.9 c	0.95 c	7.34 a	0.51	2.3 c	0.85 c
	Vintage 2009							
	20 days after early leaf removal				20 days after veraison leaf removal			
Treatment	LA (m^2)	LI	PAR (%)	EP (mm/d)	LA (m^2)	LI	PAR (%)	EP (mm/d)
EP	5.19 b	0.58 ab	9.0 b	2.10 b	5.97 b	0.53	7.2 b	2.15 b
ET	4.61 c	0.61 a	19.4 a	4.85 a	5.05 d	0.56	19.4 a	4.75 a
EP + VT	5.24 b	0.57 ab	8.1 b	2.30 b	5.51 c	0.55	21.2 a	4.90 a
VT	5.76 a	0.51 b	3.1 c	0.95 c	5.56 c	0.53	19.7 a	5.05 a
Control	5.82 a	0.53 b	2.7 c	1.05 c	6.78 a	0.51	2.4 c	1.10 c

[a] Abbreviations: EP: partial early leaf removal; ET: early total leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: not leaf removal; LA: Total leaf area; LI: Canopy leaf index; PAR: Photosynthetic active radiation; ER: Evaporation rate. [b] Means followed by different letters within the same variable, indicate significant differences at $p \leq 0.05$.

2.3.3 Fruit surface temperature

Evolution of berry temperature during an entire sunny day for each treatment and representative of measurement taken during the post-veraison period in two seasons are shown in Figure 1. Maximum berry temperature in every treatment was registered during late afternoon. At that time in treatments where berries were most exposed to direct sunlight (T) average temperature was 14.7°C ± 3.7°C warmer than maximum air temperature while berries sprayed with kaolin in the same defoliation treatment were only 7.5 ± 2.3°C warmer (7.2°C cooler than the same treatment without the application). A significant effect of kaolin applications on berry temperature in partial defoliation treatments (P) was also observed. In partial leaf removal treatment maximum berry temperature with and without the application was 7.1 ± 2.1 and 9.5 ± 1.8 warmer than the maximum air temperature respectively. Maximum berry temperature in the control treatment was 6.9°C ± 1.4°C warmer than air temperature. Note that in severe and partial defoliated treatments without kaolin application berries spend more than 6 and 4 hrs per day respectively over 35ºC while berries totally exposed reached temperatures above 40ºC.

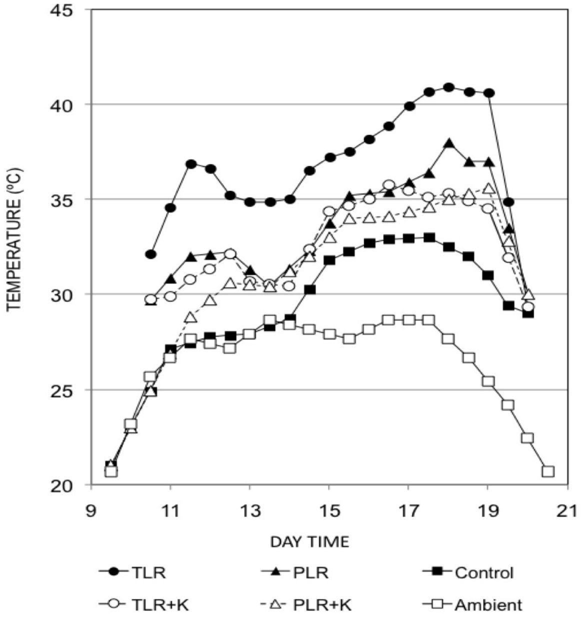

Figure 1: Berry temperature during an average pre-harvest sunny day. [a] Abbreviations: TLR: Total leaf removal; PLR: Partial leaf removal; K:

with kaolin application.

2.3.4 Bunch Rot Incidence and severity

Disease incidence and severity were significantly higher in control treatment than in all others. Bunch rot incidence and severity were significantly reduced by leaf removal treatments and total leaf removal was significantly more effective than partial defoliation. In 2009 leaf removal treatments were significantly more effective when they were applied early in the season. In general, no significant effects of Kaolin application were observed. Bunch rot incidence and severity were similar between defoliated treatments with and without the application (Table 4 and 5). No significant differences in latent infections were detected at veraison (data not shown).

Table 4. Effect of leaf removal and kaolin applications on incidence and severity of bunch diseases (vintage 2008).

	Incidence (percent diseased clusters)					
	Botrytis		Sour rot		Total disease	
Treatment[a]	K	Non K	K	Non K	K	Non K
EP	3.4 b[b]	2.4 bc	6.2	5.8 b	9.6 b	8.2 bc
ET	1.5 c	1.0 c	3.6	3.8 b	5.2 c	4.8 c
EP + VT	1.6 c	0.9 c	2.9	4.4 b	4.5 c	5.3 c
VT	2.0 bc	2.0 bc	4.1	4.9 b	6.2 c	7.0 bc
Control		10.2 a		21.2 a		31.4 a

	Severity (percent rot per cluster)					
	Botrytis		Sour rot		Total disease	
Treatment	K	Non K	K	Non K	K	Non K
EP	32.4 b	29.2 b	26.	24.9 b	28.5	26.2 b
ET	17.3 c	22.9 c	16.	21.1	16.9	21.5 bc
EP + VT	17.5 c	20.5 c	19.	16.9 c	18.7	17.7 c
VT	23.4	25.6	25.	28.1 b	24.6	27.4 b
Control		39.2 a		34.6 a		36.1 a

[a] Abbreviations: EP: partial early leaf removal; ET: early total leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: not leaf removal.

[b] Means followed by different letters within the same variable, indicate significant differences at $p \leq 0.05$.

Table 5. Effect of leaf removal and Kaolin applications on incidence and severity of bunch diseases (vintage 2009).

	Incidence (percent diseased clusters)					
	Botrytis		Sour rot		Total disease	
Treatment[a]	K	Non K	K	Non K	K	Non K
EP	1.4 c[b]	1.1 c	0.0		1.4	1.1 c
ET	0.2 d	0.2 d	0.0		0.2	0.2 d
EP+VT	0.2 d	0.3 d			0.2	0.3 d
VT	3.1 b	2.6 b			3.2	2.5 b
Control		12.6 a				12.9 a

	Severity (percent rot per cluster)					
	Botrytis		Sour rot		Total disease	
Treatment	K	Non K	K	Non K	K	Non K
EP	10.3 b	14.3 b	0.0		10.	14.3 b
ET	5.0 d	5.0 d			5.0	5.0 d
EP + VT	5.0 d	5.0 d			5.0	5.0 d
VT	7.5 c	7.9 c			7.5	7.9 c
Control		20.6 a		20.4 a		20.6 a

[a] Abbreviations: EP: partial early leaf removal; ET: early total leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: no leaf removal.

[b] Means followed by different letters within the same variable, indicate significant differences at $p \leq 0.05$.

2.3.5 Sunburn incidence and severity

All leaf removal treatments significantly increased cluster sunburn incidence. Although in 2009 no differences were observed within defoliation treatments, in 2008 cluster sunburn was significant high in severe thinned vines (ET, ET+VT and VT). Kaolin applications clearly reduced cluster sunburn incidence in defoliation treatments, being in 2008 similar to the control. The effect was significantly higher in severe treatments where affected clusters on treated plants were reduced in average more than 70% and 51% in 2008 and 2009 seasons respectively. No effect over clusters sunburn severity was observed between defoliation treatments. In 2008 Kaolin application reduced cluster sunburn severity while no significant differences were observed in 2009 (Table 5). In the same way severe leaf removal increased “browning” (must absorbance 420 nm). The effect was significantly greater when the treatment was applied early in the season. A significant reduction of browning was achieved through kaolin applications. Browning of totally exposed clusters with kaolin was not significantly different to those shaded in the control (Table 6). No relationship between rots and sunburn incidence and severity were found.

Table 6. Effect of leaf removal and Kaolin applications on clusters sunburn and must color.

	Vintage 2008

	Incidence (%)		Severity (%)		Abs /420	
Treat.[a]	K	Non K	K	Non K	K	Non K
EP	4.8 c[b]	8.7 b	17.7 b	20.7 a	0.167 c	0.167 c
ET	5.1 c	17.2 a	17.6 b	22.1 a	0.162	0.223 a
EP+VT	3.1 c	15.0 a	14.5 b	22.7 a	0.161	0.197 b
VT	2.4 c	14.2 a	14.6 b	21.8 a	0.161	0.200 b
Control		3.8 c		22.2 a		0.165 c

Vintage 2009

	Incidence (%)		Severity (%)		Abs/420	
Treat.	K	Non K	K	Non K	K	Non K
EP	17.9 b	29.4 a	9.5	9.5	0.244 c	0.240 c
ET	16.8 b	36.5 a	11.9	11.8	0.262 bc	0.378 a
EP+VT	14.9 b	30.2 a	10.7	10.6	0.247 c	0.299 b
VT	13.0 b	30.5 a	11.5	11.1	0.288 b	0.294 b
Control		8.4 c		10.7		0.262

[a] Abbreviations: Treat: Treatment; EP: partial early leaf removal; ET: early total leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: not leaf removal; Abs.: Absorbance.

[b] Means followed by different letters within the same variable, indicate significant differences at p ≤ 0.05.

2.3.6 Fruit and must composition

In the 2008 harvest all treatments with leaf removal had significantly higher fruit soluble solids concentrations in comparison with the control. In the 2009 harvest these differences were significant only when the treatment was applied early in the season. No significant effects in sugar concentration were observed between treatments with or without kaolin applications in any sampling date and year (Table 2). During both years differences in soluble solids were no significant until 15 days before harvest (data non shown).

In 2008, leaf removal treatments decreased TA and increased must pH compared with the control although these differences were not significant in 2009. During both seasons no significant differences in must TA and pH were observed between defoliation treatments. Although, in general treatments with kaolin showed similar must pH than the same treatment without the application, must pH in VT treatment at 2008 harvest was significantly lower when kaolin was applied (Table 7). Kaolin application had no effect on must TA.

Table 7. Leaf removal and kaolin application on titratable acidity, organic acids, potassium content and pH of musts and wines.

Vintage 2008

	TA		Tartaric (g/L)		Malic (g/L)		Potassium (mg/L)		pH must		pH wine	
Treatment	K	Non K	K	Non K	K	Non K	K	Non K	K	Non K	K	Non K
EP	5.3 b	5.0 b	3.93	3.68	3.28 c[b]	3.27 c	1257 bc	1204 bc	3.25 b	3.28 ab	3.37	3.39
ET	5.0 b	5.2 b	3.85	3.92	3.07 c	3.01 c	1114 c	1098 c	3.24 b	3.28 ab	3.40	3.41
EP + VT	4.9 b	5.1 b	3.93	4.02	3.24 c	3.12 c	1105 c	1099 c	3.27 ab	3.32 a	3.37	3.38
VT	5.1 b	5.1 b	3.93	3.80	3.95 b	3.74 b	1456 b	1470 b	3.25 b	3.33 a	3.40	3.41
Control		6.6 a		3.75		4.01 a		1631 a		3.20 c		3.38

Vintage 2009

	TA		Tartaric (g/L)		Malic (g/L)		Potassium (mg/L)		pH must		pH wine	
Treatment	K	Non K	K	Non K	K	Non K	K	Non K	K	Non K	K	Non K
EP	4.2	4.2	3.25	3.27	3.66 b	3.58 b	985 b	1004 b	3.27	3.29	3.31	3.37
ET	4.1	4.1	3.10	3.22	3.43 b	3.39 b	957 bc	893 c	3.27	3.28	3.33	3.34
EP + VT	4.1	4.1	3.15	3.30	3.53 b	3.45 b	933 bc	899 c	3.26	3.26	3.31	3.33
VT	4.2	4.2	3.13	3.25	4.02 a	3.67 b	1148 ab	1115 ab	3.27	3.29	3.33	3.35
Control		4.3		3.20		4.09 a		1198 a		3.26		3.32

[a] Abbreviations: EP: partial early leaf removal; ET: early total leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: not leaf removal; TA: Titratable acidity. [b] Means followed by different letters within the same variable, indicate significant differences at $p \leq 0.05$.

Even though Tartaric acid content was not affected by treatments, in both seasons significant differences in Malic acid content were observed between defoliation treatments and the control. In 2008, Malic acid levels were similar within early defoliation treatments and lower than the later leaf removal and the control. In 2009, Malic acid content in the control treatment was higher than defoliation treatments, however no significant differences between defoliation times were observed. Although in general no significant differences were observed between treatments with and without kaolin application, in 2009 Malate content in VT+K was significantly higher than the same treatment without the application and similar to the control.

Leaf removal showed a significant effect on the fruit potassium content. Both years significantly high values of this mineral were observed in control treatments. The reduction was more important when leaf thinning were severe (T) in 2009 and early in the season both seasons. Kaolin application showed no effects on potassium contents in grapes (Table 7).

FAN concentration in must was significantly reduced in all leaf removal treatments in 2008, while FAN content in VT treatment was similar to the control in 2009. During both seasons, this reduction was maximized when severe leaf removal was applied early in the season. Kaolin application showed no effects on FAN must concentration (Table 2).

IBMP concentration at harvest ranged from 1.24 to 5.05 pg/g. All leaf removal treatments significantly reduced IBMP compared to the control. IBMP concentration was strongly reduced by early defoliation; at the time that severe thinning (T) was significantly more effective than partial defoliations (P). With the only exception of VT treatment IBMP concentration was similar to the same defoliated treatments with and without kaolin application (Table 8).

Table 8. Effect of leaf removal and Kaolin applications on grapes aroma compounds (vintage 2009).

	IBMP (pg/g)		PFGG (µM/Kg)	
Treatment[a]	K	Non K	K	Non K
EP	3.06[b] b	2.98 b	541 a	481 ab
ET	1.24 d	1.08 d	532 a	541 a
EP + VT	1.81 c	1.58 cd	529 a	487 ab
VT	3.16 b	2.45 c	464 bc	421 c
Control		5.05 a		384 d

[a] Abbreviations: EP: partial early leaf removal; VT: total veraison leaf removal; K: with kaolin application; Non K: without kaolin application; Control: not leaf removal; IBMP: 3-isobutyl-2-methoxypyrazine. PFGG: Phenolic-free-GG.

[b] Means followed by different letters within the same variable, indicate significant differences at $p \leq 0.05$.

Phenol-free GG (PFGG) concentration in fruit at harvest ranged from 384 to 541 µM/Kg. Leaf removal increased the concentration of grape glycosides as estimated by the analysis of PFGG. Early thinning was significantly more effective than those made later during the season, while in general no significant differences between partial and total fruit exposure were observed. Kaolin application had no effect on PFGG content in grapes (Table 8).

2.4 DISCUSSION

2.4.1 Yield components and vine balance

Several defoliation studies reported no effect of leaf removal on berry weight (Zoecklein *et al.* 1998, Diago *et al.* 2010, Tardaguila *et al.* 2010), increases (Dookozlian 1990), while reductions like in the current study were also noted (Crippen and Morrison 1986, Reynolds and Wardle 1989). Although high berry temperatures early in the season (>30 ºC) caused by increased cluster exposure in leaf removal treatments, may have inhibited berry growth (Kabayashi *et al.* 1967), and a lower cell number within each berry has been reported (Petrie *et al.* 2000), in the current study differences between treatments were not significant in pre-harvest samplings. Significant differences observed at harvest were probably due to the high transpiration losses experienced during Stage III of berry growth as was previously suggested by Reynolds and Wardle (1989). The fact that, when kaolin was applied no significant differences between the control and the VT treatment in 2008, and between the control and any severe defoliation treatments in 2009 were observed, also supports the idea that higher transpiration rates due to a higher berry temperatures in well-exposed berries during Stage III (>35ºC) may be the principal reason for those differences observed at harvest. Even though defoliation treatments applied after fruit-set may not affect the number of berries per cluster (Tardaguila *et al.* 2010), those differences in berry weight were no later noted in average cluster weight or average fruit weight per vine. However, considering that differences observed in berry weight were always lower than 8% between treatments, natural vine variability and less bunch rot in these severe defoliated plants may explain the results (Table 2).

2.4.2 Bunch Rot Incidence and severity

There have been several studies that aimed at influencing canopy microclimate for bunch rot control (Savage and Sall 1982, Gubler *et al.* 1987, Zoecklein *et al.* 1992, Reynolds *et al.* 1996). In most cases disease reductions associated to cluster exposure were reported but the alteration in air temperature and humidity achieved have been relatively small (Carrosio *et al.* 2005). In concordance, even when no significant differences in frequency and duration of periods of water vapor deficit were detected, a strong effect on disease incidence was observed. On the other hand, differences in radiation (PAR %) observed and its related pattern of berry temperatures, reflect important changes in the dynamics of radiant energy balance through the day (figure 1). Therefore, percentage of full light received by clusters and wind movement within the cluster zone (Savage and Sall 1984, English *et al.* 1989) may likely explain the significant increases in evaporative rate (mm/day) observed and its associated disease control (Table 3). Besides these, differences in air humidity within clusters could be another explanation for the results (Savage and Sall 1984).

As in previous reports (Savage and Sall 1982), during this study no differences in latent infections were detected between treatments. It was expected based on the time where treatments were applied. Botrytis has a strong ability to infect immature grape berries, via senescing flower parts during the post-bloom period (McClellan and Hewitt 1973). Then infections may have occurred before the time that defoliation treatments were applied. However even if during our study those differences were not detected, in 2009 leaf removal treatments were significantly more effective when they were applied early in the season. According to Gubler *et al.* (1987) fungicide applications at or near to veraison on vines with heavy canopy growth like in our experiment, are inefficient because it is virtually impossible to penetrate the canopy with enough volume to adequately protect the target cluster. These results may include the benefit of improved fungicide coverage on clusters as an additional aspect of leaf removal (Chellemi and Morois 1992). On the other hand, the etiology of sour rot is much more complicated than that of botrytis bunch rot. Sour rot is associated with yeasts and acetic acid bacteria, but can also be incited by berry injury (Zoeklein *et al.* 1992). Damage to grape berries occurred during the late thinning could also help to explain the results.

2.4.3 Cluster sunburn and wine color

Temperatures 15°C higher than ambient in clusters exposed to direct sunlight like in this trial are normally reported (Spayd *et al.* 2002). The patterns of berry temperature changes observed not only had an impact on bunch rot. At the temperatures registered in this trial (above 40ºC in most severe treatments), evaporative rate increase, respiration rate drastically increase and injury or even death of berry tissue may occur (Uhlig 1998). Chlorophyll pigments are first affected and then yellow carotenoid pigments decline with the lesion becoming brown (Greer *et al.* 2003). A wine made from those berries typically presents an intense yellow color (coloured phenolics) (Greer y La Borde 2006), which is generally considered unacceptable for a fresh wine (Marais *et al.* 1992). In our trial severity of leaf removal showed a strong effect on berry temperature, cluster sunburn and must color (absorbance 420 nm). Although late leaf removal may increase the risk of sunburn in grape berries because they do not develop protective compounds to absorb harmful UV light (Spayd *et al.* 2002) no differences were detected between severe defoliation treatments. However contrary to expectations the significant higher absorbances values observed in ET compared with VT treatment suggest that an important tissue deterioration occurred from fruit set to veraison. Kaolin applications reduced these negative effects of over-exposure to sunlight, being values of all applied treatments comparable with the control (Table 6).

2.4.4 Fruit and must composition

Many researchers have reported that fruit exposure enhanced sugar concentration (Kliewer *et al.* 1988, Smith *et al.* 1988, Bledsoe *et al.* 1988) as was found in this work. Increased sugar observed in severe defoliations may be basically explained by lower water content of sun-exposed berries (Crippen and Morrison 1986), and/or a delay in sugar accumulation of excessively shaded fruit (Reynolds and Wardle 1989).

No effects on tartaric acid and reduction in malic acid associated with leaf removal in this study are consistent with many previous studies (Reynolds *et al.* 1995) while the greater effect of early thinning observed in 2008 has been also reported (Hunter *et al*. 2004). TA was also reduced by basal leaf removal, which is also consistent with previous work (Ruffner 1982, Kliewer *et al.* 1988, Reynolds *et al.* 1995). Decreases in malic acid concentration and TA were generally explained by temperature-driven enhanced malic acid degradation (Kliewer 1971, Lakso and Kliwer 1975, Ruffner 1982). However, even though in this study leaf thinning severity and Kaolin applications showed a strong effect on berry temperature, differences in malic acid concentration and TA between treatments were in general not significant. Besides the high dehydration-concentration processes in totally exposed fruit (T) during the pre-harvest period, another explanation for these results may be that degradation rates of malic acid via malate enzyme activity is not greatly enhanced above 30 ºC (Lakso and Kliwer 1975). Note that on an average pre-harvest day fruit temperature for all defoliation treatments was above 30ºC from 10:00 am to 7:00 pm hours whereas fruit from the control was below this temperature until 3 pm (Figure 1). In the same way Buttrose *et al.* (1971), working under controlled conditions, shows that an enhancement in the daily ambient temperature from 20 to 30 ºC previous to veraison, has a greater impact reducing malic acid synthesis than the same enhancement acid malic degradation during post-veraison period. That may explain those differences observed between ET and VT treatments in 2008 season.

Even thought in 2008 all treatments of leaf removal increased must pH compared with the control, wine pH was never significantly different. Even maceration time in our trial was relatively short (2 hrs.) this may be associated with a significantly high potassium accumulation observed in the control (Smart *et al.* 1990, Hunter *et al.* 2004). Although potassium accumulation appears to be a function of leaf vs. cluster shading (Rojas-Lara and Morrison 1989, Morrison and Noble 1990), reductions associated with basal leaf removal were also reported (Smith *et al*. 1988, Reynolds *et al.* 1995, Hunter *et al.* 2004).

There are differences in opinion concerning the acceptability of the typical bell pepper-like aroma of *Sauvignon Blanc*. Although an intense aroma may be unacceptable, in general is considered a positive quality parameter, as long as it is not one-sided or dominant (Marais 1994). The intensity of this “bell pepper” character of the Bordeaux variety wines like *Sauvignon blanc* is well correlated with 3-isobutyl-2-methoxypyrazine (IBMP) (Roujou de Boubee *et al.* 2000). Even this grassy/green pepper-like aroma in grapes and wine is normally maintained at high levels in vigorous-shaded canopies (Allen and Lacey 1993) the 5.05 pg/g IBMP concentration observed in the control can be considered acceptable. Through leaf removal, relatively low IBMP values were achieved [threshold value = 2 pg/g (Mega 1989)]. In concordance with Scheiner *et al.* (2010), concentrations were highly reduced by early defoliations, at the time that severe treatments (T) were significantly more effective than partial defoliations (P). Previous studies also reported that pre-veraison cluster exposure is more critical than post-veraison exposure in reducing IBMP accumulation (Marais *et al.* 1999, Sala *et al.* 2004, Ryona *et al.* 2008) since post-veraison exposure does not increase IBMP degradation on a percentage basis (Ryona *et al.* 2008). In this study through Kaolin applications on sun-exposed fruit, high differences in cluster temperature within defoliation treatments were achieved. However no significant differences in IBMP were detected. While the factors governing IBMP degradation are still not fully understood (Hashizume and Samuta 1999, Ryona *et al.* 2008), these results support the previous hypothesis that postveraison degree-day heat accumulation did not have a strong influence on final IBMP concentration (Scheiner *et al.* 2010).

Increases in bound secondary grape metabolites estimated by the analysis of Phenolic-free-GG, due to leaf removal like in this study, have been reported previously (Zoecklein *et al.* 1998). These increases represent, in part, an increase in the pool of potential aroma and flavor components (Zoecklein *et al.,* 1998) and may indicate an enhanced potential for high quality wines (Reynolds *et al.* 1991). Various macro and microclimatic factors may affect the development of free and bound flavor compounds in grapes. There is previous evidence that direct sunlight stimulus increase bound aroma compounds (Macaulay and Morris 1993, Reynolds *et al.* 1996, Zoecklein *et al.* 1998, Razungles *et al.* 2000), however no significant differences between partial and total exposed fruit were detected. Kaolin applications had no effect on PFGG contents either, even when high differences in cluster temperatures were achieved. Changes in berry composition apparently more closely related to the effect of light than temperature, probably explain these results (Noble 1990). Regarding leaf removal timing, our results showed that early thinning was significantly more effective than when it was done later in the season. A number of studies have focused on the effect of leaf removal and fruit exposure on glycoconjugate compounds (Zoecklein *et al.* 1998, Razungles *et al.* 2000), however the impact of defoliation timing has received little attention. Sunlight exposure before veraison has been reported to increase carotenoid molecules, precursors of norisoprenoids as well as norisoprenoids levels at harvest (Fariña *et al*. 2010, Marais *et al.* 1992). Considering that conjugated forms of norisoprenoids can represent an important percentage of total PFGG in Sauvignon grapes (Fariña *et al*. 2010). This may in part explain our results.

2.5 CONCLUSION

Leaf removal did not influence yield as well as bud fertility. Although berry weight of sun-exposed grapes was significantly reduced by severe defoliation treatments, those differences were not detected later in average cluster weight. Leaf removal had a determining impact over bunch rot affections as well as primarily and secondary compounds responsible for the greatest part of the final flavor of *Sauvignon blanc*. Thinning during the pre-veraison phase of the vine growth was more effective than later timing. The way in which it was applied was of the utmost importance to create a favorable canopy microclimate and to enhance berry composition. Even severe defoliations (T) were more effective than partial thinning controlling bunch rot affections, when it was applied early during the season (ET) also significantly reduced

FAN contents and increased yellow color of the must (absorbance 420 nm), which in most cases is considered undesirable. However, if under circumstances where a high disease pressure justifies severe treatments, our results suggest that an early partial defoliation complemented by total thinning at veraison (EP+VT) should be considered. In this situation, Kaolin application is a useful tool to avoid these negative effects of an excessive sun exposure. Finally, although IBMP berry levels found in control plants could be considered compatible with high quality *Sauvignon blanc* wines, and those levels found in leaf removed plants relatively low, it may well be that in average years with much water availability promoting vigorous growth and shaded canopies, higher levels above those observed do occur. In these case early leaf removal is highly recommended.

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3. FRUIT ZONE LEAVE REMOVAL AND KAOLIN APPLICATIONS INFLUENCES GRAPE BUNCH ROT INCIDENCE, GRAPE COMPOSITION AND WINE SENSORY ATTRIBUTES OF VITIS VINIFERA L. SAUVIGNON BLANC[2]

Abstract

In Uruguay as a result of favorable climatic conditions, fertile soils and viticulture practices, excessive vegetative growth and shaded canopies are normally observed. These dense canopies determine an unfavorable canopy microclimate leading to decreased grape and wine quality as well as increased bunch rot. Under such conditions disease cannot be managed effectively only with fungicides, so applying management practices become imperative. Leaf removal is beginning to receive for many growers consideration as an important cultural practice. It is normally applied after veraison and almost escclusively on red varieties because of the high sunburn susceptibility of white ones. In this context the aim of our study was to determine how fruit exposure influences bunch rot, fruit and wine composition of *Sauvignon Blanc* and at the same time if it is possible through kaolin application to reduce sunburn and other berry damages theoretically associated with cluster solar exposure. The effects of leaf removal and kaolin application were evaluated during 2009/10 season. Leaf removal significantly reduced bunch rots and had a critical impact on primary and secondary metabolites responsible for the final taste of wine. Although theoretically a low cluster exposure should contribute to the aromatic complexity of the variety, in our trial the sensory panel preferred wines from leaf removal treatment over than wines from the control (no leaf removal). Kaolin application significantly reduced berry temperature, sunburn and other berry damages associated with cluster solar exposure, being wines from leaf removal in combination with kaolin application were even better evaluated for the sensory panel. These higher scores obtained were more related to a high intensity of fruity and tropical

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[2] Este capítulo será enviado a la revista American Journal of Enologie and Viticulture para ser publicado como: Coniberti, A., Ferrari F., Gepp V., Boido E., Fariña L., Dellacassa E., Disegna E. Fruit Zone Leave Removal and Kaolin Applications Influences Grape Composition and Wine Sensory Attributes of Vitis Vinifera L. *Sauvignon Blanc*.

notes rather than the absence of green notes in consequence of leaf removal.

Key words: Leaf removal, Kaolin, Bunch rot, IBMP, Browning, *Sauvignon blanc*.

3.1 INTRODUCTION

In Uruguay as a result of favorable climatic conditions, fertile soils and viticulture practices (rootstocks, plant material free from viruses, soil management, trellis and vine spacing and so on) excessive vegetative growth and shaded canopies are normally observed. These dense canopies determine an unfavorable canopy microclimate leading to decreased grape and wine quality as well as increased rots incidence and severity. In Uruguay, crop loss due to bunch rot can be as high as 100% in a given year and in many cases it becomes the main factor determining harvest. Under such conditions disease cannot be managed effectively only with fungicides (Gubler *et al.* 1987), so applying management practices become imperative. Several studies have been focused on the effect of grapevine microclimate on bunch rot, fruit composition and wine quality. Even effectiveness of fruit zone leaf removal depends on initial fruit microclimate, cultivar and lifting timing and intensity; defoliation is in general extremely effective enhancing wind movement, cluster sunlight exposure and evaporative potential, reducing bunch rots consistently (Savage and Sall 1984, English *et al.* 1989, Reynolds *et al.* 1996). On the other hand, although, the intensity of the typical vegetable, grassy, and bell-pepper like aroma of *Sauvignon blanc* is affected by origin/climate (Allen and Lacey 1993), the impact of leaf removal in particular on *Sauvignon blanc* must composition and wine sensory attributes, is in general considered detrimental (Smith *et al*. 1988, Dry 2009). High temperature achieved as a result of excessive bunch exposure, may negatively affect secondary grape metabolites responsible for final wine sensory properties (Dry 2009). Kaolin (Surround, Engelhard Corp, Iselin, NJ, USA) is beginning to receive consideration as a potential alternative pest management product (Glenn *et al.* 1999, Puterka *et al.* 2000) and is listed by the Organic Materials Review Institute (OMRI) for use in organic production. The white film formed on the crop when kaolin is applied, protect fruit from high temperatures and sunburn (Glenn *et al.* 2002). In this context the aim of our study was to determine how fruit exposure influences bunch rot, fruit composition and wine quality and at the same time to evaluate if it is possible through kaolin application reduce the posible negative impact due to an excessive sun-exposure.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The experiment was conducted over the 2009/10 season in southern Uruguay (34º 44 S 56º 13 W). Uruguayan climate can be classified as temperate – humid without a notorious dry season (Köeppen 1931). Total rainfall in Southern Uruguay can rise up to 1100 mm in an average year, 650 mm of those during the growing season. Although yearly rainfall is even distributed, there is an historical tendency of slight increases in rainfall during early fall and spring. Weather data details can be accessed at http://www.inia.org.uy/gras/agroclima/cara_agro/index.html

3.2.2 Experimental vineyard

The experimental vineyard was a 12-years old *Vitis vinifera L. cv. Sauvignon Blanc* grafted onto SO4 rootstock. Vines were trained on vertical shoot positioning (VSP) system, where vines were spaced 1.2 m apart, in north-south rows 2.8 m wide. Bilateral cordon-trained plants were pruned to twelve two-bud spurs. The height of each cordon was 0.6 m and the top wire was located 1.8 m above ground. At approximately 30 cm shoot length all infertile shoots as well as shoots not-located on spurs were removed. During the season shoots were positioned (by hand) vertically above the spurs and topped 30 cm above the top wire. Standard pests and disease control was applied and included combinations of Captan, Iprodione, Ciproxinil + Fludioxinil and downy mildew fungicides. Vineyard was not irrigated and standard cover crop management was also applied.

3.2.3 <u>Experimental design</u>

In the experimental vineyard three internal rows were selected as “experimental rows”. At bloom 2009 in each experimental row, two blocks of 21 adjacent vines were selected on the basis of uniform vine size and canopy continuity giving a total of 6 experimental blocks. Each block consisted in seven consecutive groups of three vines. In order to avoid differences between vines, treatments were applied on each side of the same plant (Figure 1).

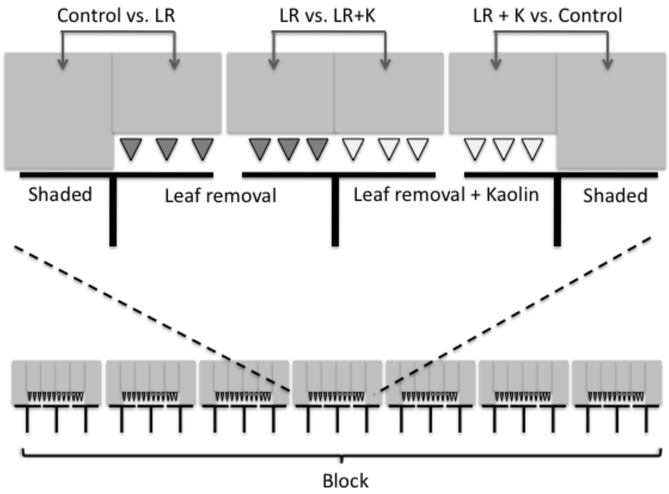

Figure 1 Experimental design; Leaf removal (LR); Kaolin application (K).

Hand defoliation treatment was applied as folows: Approximately three weeks after full bloom (stage 29 - Eichorn and Lorentz 1977), leaves and laterals opposite and below of the most distal cluster of each cane were removed. Care was taken to retain leaves, which shaded clusters from direct sunlight, theoretically giving a 50% of sun exposure in the fruit zone (PLR). Later at veraison (stage 35 - Eichorn and Lorentz 1977) the first defoliation was complemented, eliminating all leaves and laterals located below to the second node above the most distal cluster were removed, giving a 100% of fruit sun exposure (TLR). After the second defoliation treatment was performed, over the exposed side of seven of those 14 partial defoliated vines and over one side of total defoliated ones, kaolin was applied. Exposed clusters received two applications of kaolin (Surround -10% in water) from post-veraison to harvest period. The first application was made at 12 Brix and the other after a heavy rain. Differences between exposed and shaded clusters from partial defoliated vines (Leaf removal vs. none leaf removal side) and clusters from each side of total defoliated vines (Kaolin vs. non kaolin applied side) were analyzed separately as figure 1 shows. This experimental design was intended to minimize biological variability by comparing exposed vs. shaded clusters and treated vs. not kaolin-applied clusters within the same vines.

3.2.4 Kaolin applications

A backpack air blast power sprayer Hatsuta Industrial Co. Ltd. CN-73 4-Gallon, was employed. Applications were performed at early morning at a rate of 10% (w/v) kaolin water suspension (Surround WP, Teessenderlo Kerley, US). Grapes received two applications of kaolin during the post-veraison to harvest period. The first one was made at 12 Brix and the other after a heavy rain.

3.2.5 Canopy measurements

After defoliations were applied and prior to harvest, total leaf area (TLA) per vine of each treatment was estimated as follows: 1) leaf blade length was measured for all leaves of two representative shoots per vine side of every block, 2) the relationship between leaf blade length and leaf area was estimated using the ecuation first established (Coniberti *et al.* 2011 – Articulo 1) 4) finally total leaf area per shoot (TLA/shoot) was calculated and total leaf area per plant side (TLA) estimated multiplying the average leaf area of these shoots by the total number of shoots of each vine side. The exterior area of the canopy (ELA) at each side of two plants per block was also measured according to (Schneider 1989) and leaf index (LI) of those plants was calculated using the ecuation: LI = (1 - t/d) EA/TLA proposed by Schneider (1989) where 1 –t/d estimates the gaps in the canopy. Measurements were made at bloom and 20 days after defoliation treatments were applied. Photosynthetic active radiation (PAR) of full sunlight and that received by the bunch zone was measured using a Ceptometer (AccuPAR-LP80 Decagon Devices Inc). Measurements were taken two times during the growing season (after each leaf removal was made) between 10:00 am and 3:00 pm hours. Ambient measures were taken by positioning the Ceptometer at the bunch zone height outside the canopy. Three readings per replication were made at the fruit zone on both sides of each vine with the Ceptometer positioned parallel to the cordon and pointed upward. Those three interior readings were averaged and divided by a single reading taken outside the canopy to determine the percentage of PAR available at the fruit zone. Evaporation rate was estimated by placing a petri dish filled with a known weight of destiled water in the fruit zone of the canopy. After 24 hrs, remainding water in the petri dish was weighted usin a scale (Precisa XB 4200C serie 320 XB) and water loss during the period was calculated. Evaporative rate was expressed in terms of mm/day of water lost. Measurements of evaporative rate were made two times during the growing season, 20 days after each leaf removal treatments were applied. Three petri dishes per treatment were used and data are reported as the mean of the three observations. Next bloom (spring 2010) shoot density and number of clusters per shoot were evaluated.

3.2.6 Air and berry temperature

Between bloom and harvest air temperature (T) were registered at 30-min intervals using temperature and humidity data loggers (DS1923-F5# - Hygrochron iButton). From 10:00 am until sunset of a sunny day at 30-min intervals, berry temperature was measured in three external berries from three clusters per vine in every plot. These readings were averaged per plot and data are reported as the mean of plots values for each treatment. An infrared thermometer (Oakton 35639-00 InfraPro1) was used. Measurements were made two times during the growing season (20 days after each leaf removal treatments were applied).

3.2.7 Sampling Protocols

During the post-veraison period at four dates including harvest, randomly collected samples of 100 berries per block were taken from each treatment. Average berry weight at each sampling date was determined from these berry samples, which were subsequently used for soluble solids, titrable acidity and organic acids (tartaric and malic) and pH measurements. In order to define harvest time randomly collected samples of 100 berries were taken from each treatment once a week after grapes achieved 20º Brix as well the percentage and severity of Botrytis bunch rot affections were considered. All treatments were harvested at the same date and total number of clusters form each side of evaluated plants were counted and total fruit weight per vine was measured using a scale (ES50Kx1 Napco Precision Instruments Company Ltd.). An additional 200-berry sample was taken at harvest and stored at -25°C for subsequent analysis of phenolic-free GG compounds. A representative sample of approximately 10 Kg of fruit per treatment block was retained for winemaking.

3.2.8 Incidence and severity of bunch rot

All the clusters were collected at harvest and total fruit weight per vine side were recorded. Clusters with rot, sunburn, and/or pest damage were collected separately from those unaffected. The percentage of bunches infected with *Botrytis* sp. and/or sour rot (incidence) as well as the percentage of each bunch that was infected (severity) was determined by visual inspection using a six-point scale (0, 5, 25, 50, 75 and 100%). In the same way, incidence and severity of sunburn as well as mealy bug incidence were evaluated. The relationship between berry damages (sunburn and pests) and bunch rot affections were also evaluated.

3.2.9 Grape and must composition

At harvest, grapes retained for winemaking were crushed and juice total soluble solids (SS) were determined using a hand refractometer (Atago N10). Must and wine pH was measured with a pH meter (Horiba F-13 series) and tritratable acidity (TA) was determined by tritation (NaOH, 0.1 N) and expressed as sulfuric acid (w/w). The concentration of malic and tartaric acids were measured for reflectometric determination after enzymatic reactions used the automatic system RQflex® plus 10 Reflectoquant® (Merck, Germany). Samples were diluted depending on the expected acid content and proceeded according of the manufacturing instructions. Free amino-nitrogen (FAN) was analyzed by formol tritation according to Zoecklein *et al.* (1999).

Wine "browning" analysis. A sample of 50 ml of each experimental wine was filtered through a 0.45 µm membrane filter. Color was determined directly by spectrophotometry. A UV-Vis Shimadzu Co. (UV-160A/Japan) was used at 420 nm wavelength, with a 10 mm pathlength cuvett.

Phenolic-free-GG determination. 100-berries samples for each plot were used to prepare homogenized fruit extracts. The protocol used was the same as described previously by Zoecklein *et al.* (1999)

3.2.10 Winemaking and analysis

Approximately 10 Kg of fruit per treatment replicate were retained for winemaking. After destemmed and crushing they were treated with 50 mg/L sulfur dioxide and given two hours of must skin and seed contact at 2 ºC, then the lots were pressed and taken into small-scale wine lots. Must were subsequently inoculated with 0.25-g/L

Saccharomyces cerivisiae ALG804. Following fermentation at 16 ºC, wines were cold stabilized, racked, SO2 – adjusted and immediately bottled. All treatment replicates were kept separate during fermentation. For each wine, a 125 mL sample was taken for analysis of TA and pH by standard methods (OIV, 2009). All wines were stored at 11 ºC until a sensory analysis 9 months later. After bench testing the individual fermentations of wines from adjacent field blocks (block 1+2, block 3+4, and block 5+6) were blended into a three testing replicates per treatment.

3.2.11 Wine testing

Prior to descriptive analysis, wines were compared. At the time, terms were gathered as possible descriptors for flavor profiles. After discussion, nine sensory attributes (five aroma attributes, overall aroma intensity and three attributes by mouth) were select to be used by panelists. Two aroma descriptors were assumed more in keeping with the higher-order category green (Grassy; bell-pepper) and three with ripe/fruity/tropical (grapefruit/citrus; passionfruit; stone-fruit). Finally, a liking task was included in the study. Considering that professionals are trained to differentiate their own preferences from typicality wine style under consideration, high liking ratings would correspond with wines perceived to be high in varietal definition. Participants were asked: Please now rate the wine as to how good you think it is as an example of your concept of ‘‘*Sauvignon blanc*”. Each component was evaluated on a five-point scale (1 = low, 5 = high). A group of 12 enologists was assembled as a descriptive testing panel. Experts were drawn from different local wineries and were selected on the basis of having extensive experience with the product of interest, namely *Sauvignon blanc* wines. All of them had participated in quantitative sensory profiling before. Prior to participation and in keeping with ethical requirements, each person was provided information about the impending study and, after having answered any questions, completed and signed an informed consent form. The study was conducted in two one-hour sessions, separated by a 20-min break. To compare the effect of treatments three rounds of two wines from each experimental block were testing per session (Figure 1). Wines were 50 ml samples served at 15ºC, in standard, coded, ISO (1977) wine tasting glasses. Fresh water was available. Expectoration of all wine was a requirement of participation.

3.2.12 Statistics

Statistical analyses were conducted with SAS statistical software (SAS Institute, Cary, NC). Individual replicate data were averaged by treatment and treatment means were compared using Tuckey's test procedures at the 5% significance level.

3.3 RESULTS

3.3.1 Fruit yields

Average cluster weight and fruit weight per vine side were comparable at harvest between treatments. Significant differences in berry weight at harvest were observed just between leaf removal treatment (LR) and the control (Table 1). These differences were not significant in any previous sampling dates.

3.3.2 Canopy characteristics

LR reduced approximately 20% average leaf area per shoot. Leaf area to fruit weight ratio (LA/FW) was reduced from 2.65 m^2/kg in control shoots to 2.10 m^2/kg observed in LR treatment at harvest. Based on pre and post-veraison measurements leaf removal clearly increased PAR (%) measured at the fruit zone. The percentage of full light received by clusters along the canopy increased in average close to three times after partial defoliation treatment (PLR) and more than six times after total leaf removal (TLR). Estimated evaporative rate in the fruit zone of the canopy was also significantly affected by defoliation treatments. Average water loss after PLR and TLR treatments were two and five times higher than the control (Table 1).

Table 1. Characteristics of treatments canopies.

	Control	LR	Control	LR+K	LR	LR+K
20 days after early leaf removal:						
Leaf area/shoot (m^2)	0.49 *	0.40	0.50 *	0.41	0.42	0.41
Leaf area/surface area	0.51	0.54	0.52	0.54	0.51	0.50
PAR (%)	3.3 *	9.4	3.5 *	9.1	9.6	9.9
Evaporation (mm/day)	1.15 *	2.75	1.25 *	2.50	2.65	2.80
20 days after late leaf removal:						
Leaf area/shoot (m^2)	0.55 *	0.47	0.59 *	0.49	0.46	0.47
Leaf area/surface area	0.52	0.55	0.51	0.55	0.53	0.54
PAR (%)	3.1 *	17.6	2.9 *	18.9	19.7	20.1
Evaporation rate (mm/day)	0.85 *	4.50	0.95 *	5.10	5.05	4.85
Harvest:						
Berry weight (g)	1.91 *	1.85	1.91	1.90	1.81	1.85
Cluster weight (Kg)	0.169	0.162	0.171	0.169	0.161	0.166
Fruit weight /vine (Kg)	3.05	3.05	3.04	3.04	3.09	3.09
Shoot LA/FW (m^2/Kg)	2.65 *	2.21	2.61 *	2.13	2.17	2.10

[a] Abbreviations: LR: leaf removal; +K: Kaolin applied; LA: Leaf area; FW: Fruit weight. [b] *, Indicate statistical significance at the $p \leq 0.05$ between pairs of values.

Table 2. Effect of leaf removal and kaolin applications on incidence and severity of bunch diseases and sunburn of clusters.

	Control	LR	Control	LR+K	LR	LR+K
Botrytis cinerea:						
I (%)	61.1 *	29.3	69.5 *	24.8	28.3 *	21.1
S (%)	15.4 *	4.7	24.2 *	7.2	6.3	5.7
Sour rot:						
I (%)	14.6 *	2.8	27.3 *	7.7	4.4	5.1
S (%)	4.5 *	0.7	12.1 *	2.9	1.7	2.1
Total disease:						
I (%)	62.2 *	30.7	79.8 *	26.3	33.9 *	22.3
S (%)	15.5 *	5.1	29.7 *	7.2	8.3	6.1
Sun burn:						
I (%)	0.8 *	39.1	0.4 *	24.0	40.2 *	20.4
S (%)	1.1 *	12.5	0.4 *	9.9	13.4 *	8.4

[a] Abbreviations: LR: leaf removal; K: kaolin applied I: incidence; S: severity. [b] *, indicate statistical significance at $p \leq 0.05$ between pairs of values.

3.3.3 Fruit surface temperature

Evolution of berry temperature during an entire sunny day during pre and post-veraison period are shown in Figure 2. Maximum berry temperature in every treatment and period was registered during late afternoon. After partial leaf removal treatment maximum berry temperature was 4.3 ± 0.6 warmer than maximum air temperature. During post-veraison period after TLR exposed berries were 11.7°C ± 1.4°C warmer than maximum air temperature. Berry average temperature was significantly affected by kaolin applications, being sprayed berries 5.7°C cooler than those without the application. Shaded berries were in average 3.1 ± 0.6 and 3.8 ± 0.9 ºC warmer than air temperature during pre and post-veraison period respectively. Note that exposed berries during post-veraison period spent more than 6 hrs per day over 35ºC and reached temperatures above 40ºC during afternoon.

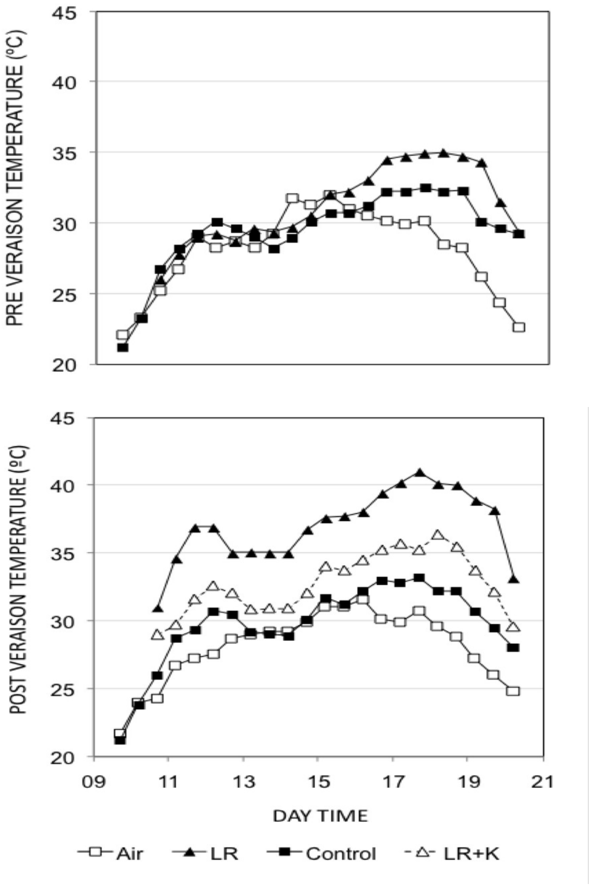

Figure 2: Berry and air temperature during an average pre-

veraison and post-veraison sunny day. [a] Abbreviations: LR: leaf removal; +K: with kaolin application.

3.3.4 Bunch Rot Incidence and severity

Disease incidence and severity were significantly higher in the control treatment, affecting more than 60% of the clusters and showing 15.5% of severity. LR reduced the number of clusters affected by *Botrytis cinerea* to less than half and the severity to less than a third. The effect of LR on sour rot was even higher, reducing incidence to less than a third and severity to less than a quarter. Kaolin application had a slight but significant effect on botrytis bunch rot. No effect on sour rot infections were detected (Table 2). No significant differences in latent infections were detected at veraison and although skin damage caused by sunburn may have led to invasion by secondary bunch-rotting fungi no relationship between rots and sunburn incidence or severity was found either (data non shown).

Table 3. Effect of treatments on must and wine composition.

	Control	LR	Control	LR+K	LR	LR+K
Must composition:						
° Brix	18.3 *	19.3	18.4 *	18.9	19.2 *	18.8
pH	3.27 *	3.33	3.27	3.28	3.32 *	3.28
TA (g/L)	6.13 *	5.64	6.28	5.92	5.51 *	5.92
Tartaric acid (g/L)	4.03	3.70	3.78	3.85	3.89	3.86
Malic acid (g/L)	4.75 *	3.55	5.15	4.75	3.33 *	4.67
Potassium (mg/L)	1431	1317	1473	1381	1269	1273
FAN (mg/L)	138.7	120.1	133.3	127.4	131.4	127.2
Phenol-free GG (μM/Kg)	261 *	369	289 *	385	375	359
Wine composition:						
pH	3.33 *	3.45	3.36	3.34	3.41 *	3.34
TA (g/L)	5.78	5.45	5.91	5.76	5.38	5.79
Abs/420	0.091 *	0.105	0.082	0.085	0.107 *	0.091

[a] Abbreviations: LR: leaf removal; +K: Kaolin applied, TA: Titratable acidity; FAN: Free-amino-nitrogen.
[b] *, Indicate statistical significance at the $p \leq 0.05$ between pairs of values.

3.3.5 Cluster sunburn incidence and severity

Leaf removal significantly increased cluster sunburn incidence and severity. Injured clusters were close to 40% and the affected berries were more than 12.5% (Table 2).

Leaf removal increased “browning” measured as wine absorbance at 420 nm. Kaolin application protected fruit from sunburn, halving the affected clusters compared to the LR treatment. Wine absorbance (abs/420) in LR+K treatment was significantly lower than the LR and similar to the control treatment (Table 3). Later during wine testing, experts detected no significant differences in wine color.

3.3.6 Must and wine composition

LR treatment had a significantly higher must soluble solids concentration than control treatment. At the same time, LR+K showed a significantly lower sugar concentration than LR but was higher than the control. These differences were not significant until 15 days before harvest. LR decreased must TA and increased must and wine pH. Although must TA and pH in LR+K treatment was significantly different compared with the LR treatment, wine TA was similar between treatments. No differences in must and wine pH and TA were detected between LR+K and the control. LR significantly reduced malic acid concentration while no differences between LR+K treatment and the control were detected. Tartaric acid, potassium and FAN accumulation at harvest were not affected by treatments. LR significantly increased the concentration of grape glycosides estimated at harvest by the analysis of Phenol-free GG (PFGG). Kaolin application had no effects over PFGG content in grapes (Table 3).

Table 4. Analysis of variance for intensity ratings of sensory attributes of wines made from shaded and exposed clusters with and without kaolin applications.

	***F* values**		
Olfactory descriptors:	Wine	Panelist	Wine x Panelist
Green (grassy/bell pepper)	33.6 *	3.58 **	1.78 *
Pomacee (pear, apple)	15.5	1.68	1.05
Citrus (grapefruit)	58.4 ***	3.44 ***	2.09
Tropical (Passion fruit/melon)	29.8 ***	4.02 ***	1.03
Overall aroma intensity	70.4 ***	1.54	1.53
Gustatory descriptors:			
Acidity	101.7 ***	2.47 **	1.41
Body	133.7 ***	3.59 ***	1.75

Persistence	78.2 ***	2.04 **	1.26
Color	0.17	1.76	0.71
Preference test	199.0 ***	1.79	1.22

*, **, *** Indicate statistical significance at the *p* <00.5, 0.01, 0.001 levels of confidence, respectively.

3.3.7 Wine sensory attributes

Highly significant differences were found among treatments in two (grapefruit and passion fruit) of the three fruity aroma evaluated terms while for green characters only slight differences were found between LR and the control. Descriptive analysis revealed that wines from LR treatments not only had distinctive sensory characteristics but also overall aroma intensity levels that exceeded control wines. Means of overall palatability ratings showed a similar enhancement but in this case LR+K treatment wines, were preferred by the experts (Table 4 and 5). High preference scores were strongly related with its high overall aroma intensity and wine body (R^2=0.67). No differences in wine color were detected during wine testing.

Table 5. Effect of treatments on average intensity rating for aroma and gustatory attributes.

Olfactory descriptors	**Control**	**LR**	**Control**	**LR+K**	**LR**	**LR+K**
Green (grassy/bell pepper)	1.6 *	1.3	1.6	1.5	1.2	1.4
Pomacee (pear, apple)	2.5	2.3	2.1	1.8	2.0	1.9
Citrus (grapefruit)	2.4 *	2.9	2.3 *	3.6	2.8 *	3.5
Tropical (Passion fruit/melon)	2.3 *	3.0	2.4 *	3.8	3.1 *	3.6
Overall aroma intensity	2.4 *	3.2	2.2 *	3.6	3.3	3.6
Gustatory descriptors						
Acidity	3.8 *	3.2	3.2	3.5	3,1	3.4
Body	2.2 *	3.0	2.2 *	3.6	3.1 *	3.5
Persistence	2.1 *	2.8	2.0 *	3.5	2.9 *	3.5
Preference	2.3 *	3.4	1.9 *	4.2	3.5 *	4.1

5-points intensity scale (1 = low, 5 = High); means followed by the same letter are not significantly different (*p* <0.05) between pairs of values.

3.4 DISCUSSION

3.4.1 Yield components and vine balance

Several studies have reported no effect of leaf removal on berry weight (Zoecklein *et al.* 1998, Diago *et al.* 2010, Tardaguila *et al.* 2010), others found increases (Dookozlian 1990), while reductions like in the current study have also been reported (Crippen and Morrison 1986, Reynolds and Wardle 1989). The fact that, when kaolin was applied no significant differences in berry size between the control and the LR+K treatment were observed, supports the idea that a higher transpiration rate due to a high berry temperature in exposed berries during Stage III (>40ºC) may be the main reason for the differences observed at harvest (Reynolds and Wardle 1989). Even if defoliation treatments applied after fruit-set may not affect the number of berries per cluster (Tardaguila *et al.* 2010), the differences in berry weight did not result in variation of cluster weight or fruit weight per shoot. Slightly differences observed in berry weight (always lower than 5%), natural cluster size variability and more dehydrated berries due to rotting in control treatments might explain these results.

3.4.2 Bunch rot incidence and severity

Several studies aimed at influencing canopy microclimate for bunch rot control (English *et al.* 1989, Reynolds *et al.* 1996, Disegna *et al.* 2005ab). As has been reported elsewhere, in this study bunch rot development was clearly reduced by leaf removal. Although influences of microclimate on pathogen or host plant behavior cannot be explained by just one factor; wind speed and photosynthetic active radiation (PAR) are the microclimate factors reported as most strongly affected by leaf removal (Savage and Sall 1984, English *et al.* 1989). Therefore, increases in PAR (%) received for exposed clusters in LR treatment and its related pattern of berry temperatures explain the significant increases in evaporative rate observed and its associated disease control. The benefit of improved fungicide coverage on clusters as an additional aspect of leaf removal should be taken into consideration (Chellemi and Morois 1992).

3.4.3 Cluster sunburn and wine color

Temperatures 11°C higher than ambient in direct sunlight exposed clusters like in this trial are normally reported (Spayd *et al.* 2002). When berry temperature goes above 40ºC (as registered after TLR) respiration rate drastically increases and injury or even death of berry tissue may occur (Uhlig 1998). Chlorophyll pigments are first affected and then yellow carotenoid pigments decline with the lesion becoming brown (Greer *et al.* 2003) and wines made from those berries typically present an intense yellow color (coloured phenolics) (Greer and La Borde 2006). However in spite of treatments showed a strong effect on berry temperature, cluster sunburn and wine color (abs/420) these changes were no later detected by the sensory panel (Table 4).

3.4.4 Must and wine composition

Many investigators have documented that fruit exposure enhances sugar concentration (Kliewer *et al.* 1988, Smith *et al.* 1988, Bledsoe *et al.* 1988) such as is reported here. Increases observed in LR treatment may basically be explained by lower water content in sun-exposed berries (Crippen and Morrison 1986), while a delay in sugar accumulation of excessively shaded fruit may probably explain differences observed between LR+K and the control (Reynolds and Wardle 1989).

No effect on tartaric acid and a reduction in malic acid associated with leaf removal in this study is consistent with many previous studies (Reynolds *et al.* 1995, Hunter *et al.* 2004). Associated with that, significantly low malic acid concentration, a low must TA and high must and wine pH were also observed which is also consistent with many other reports (Ruffner 1982, Kliewer *et al.* 1988, Reynolds *et al.* 1995). Decreases in malic acid concentration and TA are generally explained by temperature-driven enhanced malic acid degradation (Lakso and Kliwer 1975, Ruffner 1982). Note that in an average pre-harvest day fruit temperature in LR treatment was above 35ºC from 11:00 am to 7:00 pm hours whereas fruit from LR+K spent less than two hours a day above these temperatures. Fruit from the control treatment was never above 33ºC (Figure 1). No effect of kaolin application was detected in must TA and pH but wine pH was reduced by the application. Although differences observed have been associated with a higher potassium accumulation (Smart *et al.* 1990, Hunter *et al.* 2004), no significant differences in potassium and nitrate accumulation were detected.

Increases in bound secondary grape metabolites estimated by Phenolic-free-GG analysis, due to leaf removal like in this study, have been reported previously (Zoecklein *et al.* 1998) These increases represent, in part, an increase in the pool of potential aroma and flavor components (Zoecklein *et al.* 1998) and may indicate an enhanced potential for high quality wines (Reynolds *et al.* 1991). Various macro and microclimatic factors may affect the development of free and bound flavor compounds in grapes. There is previous evidence that sun exposure increases bound aroma compounds (Macaulay and Morris 1993, Reynolds *et al.* 1996, Zoecklein *et al.* 1998, Razungles *et al.* 2000). Results of PFGG analysis were not significantly different between LR and LR+K. The great reduction in berry temperature achieved through Kaolin application supports the hypothesis that changes in berry composition are more closely related to the effect of light than temperature (Morrison and Noble 1990).

3.4.5 Wine sensory attributes

In contrast with what is in generally reported (Arnold and Bledsoe 1990), in our trial leaf removal did not have a significant effect in reducing vegetable character of *Sauvignon blanc* wines. Fruity aroma terms and overall aroma intensity ratings of the leaf removal treatments significantly exceeded control wines. It is interesting that higher scores associated with greater typicity observed in LR+K treatment, were associated with riper notes and appear to be defined more in terms of abundance of tropical or fruity notes than in terms of an absence of green notes. These increases may be associated to a high pool of potential aroma and flavor components estimated by PFGG analysis (Reynolds *et al.* 1991, Zoecklein *et al.* 1998). Like what was previous reported for New Zealand *Sauvignon blanc* (Parr *et al.* 2007) in our study perceived olfactory components of wines had a major importance on final wine flavor, being strongly correlated with high liking ratings (Table 5).

3.5 CONCLUSION

Bunch rot development was clearly reduced by leaf removal. Increases in PAR (%) achieved and its related changes in the dynamics of radiant energy balance through out the day, was apparently the main factor affecting evaporative rate and its associated disease control. The high temperatures achieved, affected transpiration rate during Stage III and soluble solids concentration was increased probably as a consequence of berry dehydration. Nevertheless, cluster weight or fruit weight per shoot was not significantly affected by the treatments. Fruit exposure also had a determining impact on primarily and secondary compounds responsible for the greatest part of the final flavor of *Sauvignon blanc*. Associated with temperatures changes, fruit exposure decreased must malic acid concentration and TA; increased must and wine pH; while tartaric acid content was similar to shaded clusters. Berry temperature and its effect on fruit composition were in general attenuated by kaolin applications. However, no differences between exposed berries with and without Kaolin were detected by phenol-free GG analysis. It confirms previous hypothesis that changes in bound aroma compounds are more closely related to the effect of light than temperature.

Even though relatively low temperature and reduced sunlight exposure will theoretically contribute to the complexity of *Sauvignon blanc* aroma, in our study fruit exposure enhanced wine quality. High wine scores associated with greater typicality observed in LR and LR+K treatment, were associated with riper notes and appear to be defined more in terms of abundance of tropical or fruity notes than in terms of an absence of green notes.

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4. CONCLUSIONES GLOBALES

Los deshojados basales no afectaron significativamente la fertilidad de yemas ni la producción por planta. Sin embargo asociado a la deshidratación ocurrida durante la fase III de desarrollo del grano, el peso de bayas se ve significativamente afectado por los tratamientos severos (100% de exposición). La aplicación de caolinita reduce el fenómeno de deshidratación asociado a la insolación directa de bayas.

Los deshojados basales reducen la ocurrencia de podredumbres de racimo. Deshojados intensos (100% de exposición) son más efectivos en el control de *Botrytis cinerea* y otras podredumbres que aquellos realizados en forma parcial, donde la exposición de la fruta no es total (50%). El cambio de la dinámica del balance de energía de radiación durante el día a consecuencia de una mayor exposición directa de los racimos a los rayos solares, es probablemente el factor determinante de la mayor tasa de evaporación asociada al control de podredumbres. Al mismo tiempo los deshojados en post-cuajado (29 a 31- Eichorn and Lorentz 1977) son mas efectivos que los mismos realizados en envero (35 - Eichorn and Lorentz 1977), hecho probablemente asociado a una mayor susceptibilidad al daño en bayas durante el deshojado en envero y fundamentalmente a una pobre cobertura fitosanitaria a nivel de los racimos en canopias excesivamente sombreadas.

Los deshojados basales y fundamentalmente los realizados en forma severa (100% exposición) promueven el quemado y la oxidación fenólica de la uva. La caolinita es una herramienta de gran utilidad para reducir la temperatura y el quemado de racimos expuestos, evitando así aquellos efectos negativos asociados a la excesiva exposición solar de los racimos.

Los deshojados reducen significativamente el contenido de nitrógeno fácilmente asimilable del mosto (FAN). Tanto la intensidad como el momento en que es realizado tienen un impacto determinante sobre el contenido de este nutriente a cosecha. Bajo las condiciones de estudio, el deshojado severo realizado temprano en la temporada redujo los contenidos de FAN a niveles inferiores a los requeridos para la adecuada fermentación de mostos de uva.

Los tratamientos de deshojado afectan significativamente los metabolitos primarios y secundarios responsables del sabor final de los vinos *Sauvignon blanc*, siendo el impacto de esta práctica mayor cuando esta es realizada temprano en la temporada (29 a 31-Eichorn and Lorentz 1977).

Asociado a aumentos en la temperatura e iluminación, la exposición de los racimos reduce la acidez titulable y concentración de acido málico del mosto, a la vez que incrementa el pH del mosto y vino. La reducción de la temperatura observada en consecuencia de la aplicación de caolinita sobre racimos expuestos, permite reducir el impacto de la insolación directa sobre estos parámetros. Mostos y vinos provenientes de uvas de racimos expuestos tratados con caolinita presentan valores de acidez titulable, acido málico y pH similares a los provenientes de racimos a la sombra de la misma planta.

La concentración de compuestos aromáticos en uvas y mostos se ve igualmente afectada por los tratamientos de deshojado. Los compuestos glicosilados (PFGG) en uvas se ven incrementados por la exposición de los racimos y fundamentalmente en aquellos deshojados temprano. La gran diferencia de temperatura observadas en tratamientos con y sin la aplicación de caolinita y su reducido impacto sobre la acumulación de PFGG, sugieren que la iluminación y no la temperatura seria el factor determinante para su acumulación. La 3-isobutyl-2-methoxypyrazine (IBMP) en uvas se ven reducidas por la exposición de los racimos y fundamentalmente en aquellos deshojados temprano. Confirmando la hipótesis de que tanto los cambios en la síntesis y degradación de las pirazinas (IBMP), se encuentran mas íntimamente relacionados al efecto de la luz que el de la temperatura, no se observan diferencias significativas en la concentración de estos compuestos entre los tratamientos con y sin aplicación de caolinita.

Aunque en teoría una reducida exposición de la uva contribuiría a la complejidad aromática de la variedad, para las condiciones del estudio, los vinos de tratamientos de deshojado, fueron en la evaluación sensorial 2010 preferidos por sobre el control sin deshojar. Asociado seguramente a una menor temperatura y quemado de racimos, los vinos del tratamiento deshojado con aplicación de caolinita obtuvieron incluso mejores puntajes. Estas mayores puntuaciones observadas, se encuentran mas estrechamente ligadas a una mayor intensidad en notas frutadas o tropicales que a la ausencia de notas verdes.

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