Title: Girdling and Shading Effects on Inflorescence Necrosis and Rachis Composition of Pinot Noir Grapevine.

Abstract approved: ____________________________________ Porter Lombard

There are several grape disorders that reduce fruit set. One, millerandage, is from poor fertilization to produce "hen and chicken" or small seedless berries; while another, Coulure, is the failure of grape flowers to develop into berries. A third one, inflorescence necrosis (INec), is a disorder that should be in this category.

Inflorescence necrosis is a recently described disorder that can be an important cause of fruit set reduction in grapes. INec occurs at bloomtime and the affected tissue is characterized by brownish or black coloration. Only clusters are affected by INec. Flowers and pedicels are the only damaged tissue. Sometimes, the rachis can also be injured. In severe cases, clusters can be completely necrotic. The influence of girdling and shading on INec and fruit set was determined in mature field grown Pinot Noir grapevines in 1989. Shading with 60% shade cloth was imposed from one month before bloom through bloom.
Girdling was done one week before capfall. Shading increased the percentage of necrotic flowers by 2.3 to 2.7 times and reduced fruit set by 23% to 35% compared to exposed plants. Girdling increased fruit set 15% and 25% in two vineyard plots compared to ungirdled vines. The effect of shoot girdling and shoot density on INec was evaluated in 1990. Shoot density, which was adjusted before bloom, did not affect INec. Girdling did not influence the percentage of necrotic flowers in both years.

Free ammonium levels were measured in shoot xylem exudate, tendril, petiole, rachis, and flower tissue sampled at three growth stages: beginning bloom, full bloom, and shatter. In 1990, rachis tissue was also sampled for ammonium at harvest time. The highest ammonium level was found in the rachis while the flowers had the lowest. Rachis ammonium concentration was higher at beginning bloom and then declined afterward. Shade increased ammonium concentration 24% and 21% in the rachis at beginning bloom.

In 1989, soluble sugars and organic acids were determined in the rachis tissue. There was no significant effect from the girdling or shading treatments on total soluble sugars concentration. However, girdling increased rachis dry weight 21% and 33% at full bloom and shatter, respectively. On the contrary, shading reduced rachis dry weight 27% at full bloom and 31% at shatter stage. Glucose level was several times greater than fructose and glucose in the three sampling periods. Shading reduced 30% total
organic acids concentration at beginning bloom, and 20% at full bloom. Tartaric was the predominant acid. Shading reduced the concentration of α-ketoglutaric acid but no treatments had a significant effect on its rachis level during bloom.
Girdling and Shading Effects on Inflorescence Necrosis and Rachis Composition of Pinot Noir Grapevine

by

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Typed by  Antonio E. Ibacache
To my wife Viviana,

my daughter Viviana Paola,

and my son Toñito.
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Chapter 1

INTRODUCTION

Normal fruit set in grapes requires the following steps: pollination, pollen germination, fertilization, and the start of seed formation in the grape ovary (Winkler et al., 1974). Poor fruit set of vines can be a major contributor to low yields. Lack of set is often attributed to inadequate pollination and fertilization due to several possible factors. Among them: environmental conditions before and during bloom, the supply of stored carbohydrates, and carbohydrate competition among florets within the flower cluster as well as between the flowers and elongating shoots (Jordan et al., 1981). Another cause of flower loss is Inflorescence Necrosis.

Inflorescence necrosis (INec), also called Early Bunch Stem Necrosis (ESBN) (Jackson and Coombe, 1988a,b), is a recently described disorder which can severely reduce the yield in grapevines. This kind of necrosis has been reported by Jackson in New Zealand (1988a,b), Coombe in Australia (1988a,b), and Jordan in Oregon (1989). However, there is reason to think the problem is present in other grape growing areas, since the disorder can be confused
with other cause of fruit set reduction (e.g., Botrytis rot).

The cause of inflorescence necrosis is unknown. No pathogens have been found on the affected tissue and the disorder appears to have a physiological cause (Jackson and Coombe, 1988a). INec has been related with waterberry (syns., shanking, stiellahme, dessechement de la rafle, disseccamento del rachide, palo negro, bunch stem die-back, pedicel necrosis, stalk necrosis, (Christensen and Boggero, 1985), a disorder which occurs about harvest time (Jordan, 1989). Both develop symptoms of necrotic pedicels and rachis tissue. However, waterberry has received more research attention and the present hypothesis is that a toxic rachis ammonium concentration could be the cause (Christensen and Boggero, 1985)

Elevated ammonium levels in rachis tissue have also been related with inflorescence necrosis (Jordan, 1989). Lombard (pers.comm., 1988) found that shaded clusters had higher rachis ammonium concentration than rachis from exposed clusters. Jackson and Coombe (1988b), found the doubling of the incidence of the disorder in shaded rooted cuttings, compared with unshaded plants. The shading effect would suggest the role of photosynthetically produced carbohydrates for cluster development and ammonium metabolism. In fact, Given (1979) indicates that organic acid content (especially α-ketoglutaric acid) and a good
availability of carbohydrate reserves are necessary for ammonium assimilation.

Girdling or ringing is a commonly used method to improve fruit set in grapevines (Jensen et al., 1981, Brown et al., 1988). Girdling increases the available carbohydrates above the wound (Weaver and McCune, 1959) and improves fruit set because more assimilates are accumulated into the cluster just before fruit set (Hale and Weaver, 1962). On the other hand, there is evidence that, as a consequence of reduced photosynthesis rate, fruit set is negatively affected by shading (Patten and Proebsting, 1986, Marini and Sowers, 1990).

The objectives of this research were:
1) describe inflorescence necrosis symptoms in Pinot Noir grapevine growing in field conditions;
2) determine the influence of girdling and shading on inflorescence necrosis and fruit set;
3) determine ammonium concentration differences in xylem exudate, tendril, petiole, rachis, and flower tissue;
4) establish if ammonium, carbohydrate, and organic acid content in rachis is affected by girdling and shading treatments.
Inflorescence necrosis (INec) is a recently described disorder that can be an important cause of fruit set reduction in grapes. Although the disorder has been observed for many years, it has been described as poor set or infection from the fungus *Botrytis cinerea*. Recently, this problem has received more attention because of severe yield reduction. In 1988, some vineyards in Oregon, especially with the cultivar Pinot Noir, were severely affected with greater than 50% crop loss. Crop losses were attributed to INec (Jordan, 1989).

Inflorescence necrosis has been investigated very little, therefore, not much is known about its cause. However, several experiments have been carried out in the last years, especially in southern New Zealand and western Oregon.

Description

This disorder, affecting several grape cultivars, was
described in New Zealand and Australia (Jackson, 1988; Jackson and Coombe, 1988a,b). Also, it was reported throughout Pacific Northwest and California vineyards in the United States in the 1988 season (Jordan, 1989). However, this disorder may be causing fruit set reduction in other grape production regions.

Inflorescence necrosis appearance and development is similar to bunchstem necrosis (BSN), except the former occurs earlier in the season. Thus, the name of early bunchstem necrosis (EBSN) has been suggested by Jackson and Coombe (1988a,b). On the other hand, the name inflorescence necrosis (INec) was considered more appropriated than EBSN because it affects pedicels, flowers, rachis and it occurs at bloom time (Jordan, 1989).

**Symptoms**

Inflorescence necrosis has been described as occurring from before bloom until shortly after, but it is more common in the last week or two before flowering (Jackson and Coombe, 1988a).

Inflorescence necrosis differs from other disorders which reduce fruit set. Millerandage (also named "hen and chickens") is caused by poor flower fertilization (Winkler et al., 1974). In this case, clusters show many small
seedless berries besides the normal seeded berries. At shatter time, some unfertilized flowers and poorly developed berries fall green from the cluster. Conversely, when INec is present a lot of necrotic flowers and just set berries drop off. This characteristic distinguishes INec from other fruit set disorders.

There are other two disorders that reduce fruit set in grapevines. Filage is a cluster disorder where a section of the cluster is tendril like without flowers. It appears to be more common on vigorous vines (Jackson and Coombe, 1988b). Coulure is a term used when flowers that fail to develop into berries fall from the cluster few days after opening (Winkler et al., 1974).

Distribution

Fruit set reduction due to INec has occurred in some grape growing areas. In New Zealand has reduced the crop by 90% in muscat varieties (Jackson, 1988). In a survey carried out in North Willamette Valley (Oregon) vineyards in 1988, Jordan (1989) determined crop loss greater than 50% in Pinot Noir cultivar severely affected. The disorder was observed in all surveyed vineyards, although there were differences in INec incidence among them.

Using test plants, Jackson and Coombe (1988a) found
that cultivars differ in INec susceptibility. Also, they observed very different levels of necrosis on Pinot Noir in New Zealand. The same was observed by Jordan (1989) in Oregon. He determined that Pinot Noir in the Willamette Valley was more affected than other cultivars. Also, he found clonal differences among Pinot Noir in INec susceptibility.

Possible Causes

Research on this disorder is just beginning. No cause is known. Although the symptoms resemble fungal infection, pathologists have been unable to isolate any fungi from damaged tissue (Jackson and Coombe, 1988a,b). The disorder appears to have a physiological origin. Observations suggest that vineyards with low water content or low nutrition present more necrosis. Also, differences in INec incidence have been observed in different areas and in different seasons. It appears that the necrosis is worse in cooler regions and in cool wet seasons (Jackson and Coombe, 1988b). More INec is found in shaded parts of the vine. Riesling vines growing in four light regimes had all clusters necrotic when the amount of light exposure on clusters was 10% of normal (Jackson, 1988). Lombard (pers.comm., 1988), working in greenhouse found shaded
Cabernet Sauvignon vines had 31% INec compared to 1.5% in exposed vines.

Inflorescence necrosis has been related with waterberry, a disorder which occur later in the season. Both problems present similarities in their symptoms and it is thought that both have a similar cause. Waterberry is a well-known disorder in grapes, especially in some table grape cultivars. Waterberry defines the watery, and soft berries resulting from the interrupted flow of ripening constituents into the berries due to the stem necrosis (Christensen and Boggero, 1985). Winkler et al. (1974) indicate that affected berries may be confined to the tip of the rachis or they may be scattered throughout the cluster. Christensen and Boggero (1985) point out that its primary symptoms are necrotic spots which develop on pedicels and/or other parts of the cluster stem during the veraison period. Consequently, berry development is affected by flow cessation of carbohydrates and other constituents from necrotic tissue.

Working with the table grape cultivar Thompson Seedless, Christensen and Boggero (1985) found that the occurrence of waterberry symptoms is related to a higher cluster rachis level of nitrogen (approximately 1.5%) and NH₄⁻N (3000 ppm and above). They suggest that high NH4⁻N levels can be caused by enzymatic reduction of nitrogen to
amino radicals beyond the amounts required in amino acid and protein synthesis. The relationship between cluster necrosis and high rachis ammonium level has also been observed in cultivars for wine production. Cabernet Sauvignon rachis with high ammonium concentration (above 2.0 mg g\(^{-1}\) dw) had more necrosis than those with a lower concentration (Jordan, 1989). Ammonium concentration in the rachis from Cabernet Sauvignon clusters affected by INec, which were shaded, was three fold higher compared to rachis concentration from exposed and scarcely affected clusters (Lombard, pers.comm., 1988).

Other disorders affecting Thompson Seedless have also been associated to elevated ammonium levels. One of them is "false potassium" (FK) symptom in leaves. FK affected vines show potassium deficiency like symptoms on basal leaves before bloom (Christensen et al., 1990). The same authors indicate that this problem can induce flowers dropping at bloom time and may coincide with rachis necrosis. Symptom development was associated with high total nitrogen and ammonium nitrogen in the leaves. A later study suggests that one component of the nitrogen metabolism problem may be putrescine accumulation (Adams et al., 1990). The researchers point out that putrescine can be formed from either arginine or ornithine. The pathway from ornithine is related with meristematic or rapidly growing tissue. The
pathway giving rise to putrescine in potassium deficient plants generally starts with arginine. Bud necrosis in Thompson Seedless grapevine could be also related with ammonium accumulation (Perez and Kliewer, 1990). Bud necrosis is characterized by the presence of necrotic cells in the primary bud that cause a separation between the basal part of the bud and the apex, which later results in the death of the primary bud (Lavee et al., 1981). Perez and Kliewer (1990) state that shading increase bud necrosis, and some processes, such as ammonium accumulation may trigger the initiation of the disorder.

A close relationship between ammonium concentration and cluster necrosis (waterberry and INec) has been determined in grapes. High ammonium level appears as a critical factor. The toxic effect of ammonium on plant tissues has been demonstrated by Maynard et al. (1966) and Nevin and Lovatt (1987). However, the role of ammonium in grape disorders needs more specific investigation. Its elevated content in affected tissue could be a consequence, not a cause of the problem.
Ammonium in Plants

Ammonium Assimilation

Plants absorb most of their nitrogen in the nitrate and ammonium forms. However, grapevines take up most of the nitrogen as nitrate and in this manner it is transported to the leaves. There it is reduced to build proteins and other nitrogen compounds (Christensen et al., 1982). After absorption, nitrate is reduced to ammonium in roots, leaves or shoots (Joy, 1988; Bowman and Paul, 1988).

It is difficult to consider the molecule ammonia and the cation ammonium separately (Jordan, 1989). Ammonia reacts with water to give the ammonium ion. To avoid any confusion, only the name ammonium is used in this review, understanding that ammonium comprises both the molecule ammonia and the ion ammonium. A chemical equilibrium exists between aqueous ammonia and ammonium within tissue solutions, according to the following equation (Hageman, 1984):

\[ \text{H}_2\text{O} + \text{NH}_3_{(aq)} \rightarrow \text{NH}_4^+ + \text{OH}^- \]

Enzymes in Ammonium Assimilation

It seems that at low or normal intracellular ammonium
concentration, the pathway for its assimilation is the glutamine synthetase/glutamate synthase cycle (Givan, 1979; Fentem et al., 1983). The enzyme glutamine synthetase (GS) requires glutamate, ammonium, and ATP for its activity. GS acts along with glutamate synthase (GOGAT). Glutamate synthase mediates the conversion of α-ketoglutarate and glutamine to glutamate (Givan, 1979; Oaks, 1985). Equations 1 and 2 show the reactions including GS and GOGAT, respectively.

**Equation 1**

\[
\text{L-glutamate} + \text{NH}_4^+ + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + \text{P}_i
\]

**Equation 2**

\[
\text{Glutamine} + \alpha\text{-ketoglutarate} \rightarrow 2 \text{glutamate red ferredoxin}
\]

The enzyme glutamine synthetase is localized in the chloroplasts and cytosol in leaves and in the cytosol in roots. On the other hand, glutamate synthase is placed in chloroplasts in leaves or in plastids in roots (Oaks, 1985). GS is present in high amount and it has low \(K_m\) for ammonium. Thus, the equation 1 probably represents the main pathway for ammonium assimilation either in leaves or in roots (Oaks, 1985). Besides the GS/GOGAT cycle, there is another important pathway that might work at high concentration of ammonium. This reaction is catalyzed by the enzyme glutamate dehydrogenase (GDH) which has a high
\[ K_m \text{ for ammonium (Givan, 1979). Glutamate dehydrogenase transforms } \alpha\text{-ketoglutarate to glutamate in the presence of NADH or NAD(P)H and ammonium (equation 3).} \]

**Equation 3**

\[
\text{NH}_4^+ + \alpha\text{-ketoglutarate} + \text{NAD(P)H} \rightarrow \text{L-glutamate} + \text{NAD(P)}^+ 
\]

Because of its high \( K_m \) (above of 5 mM, Givan, 1979), this enzyme could not compete with glutamine synthetase for the available ammonium and probably, glutamate dehydrogenase works only under high ammonium concentration. However, trials using GS/GOGAT chemical inhibitors point out this cycle is still the main assimilatory route when an elevated ammonium level is present (Givan, 1979). Unlike GS, GDH is localized in the mitochondria in leaves and roots (Susuki, 1981). A higher ammonium content is found in mitochondria compared with chloroplasts (Yamaya, 1984). As a result, mitochondria could operate as an adequate source of ammonium for GDH activity.

Enzymes involved in ammonium assimilation have been detected in grapes. Roubelakis-Angelakis and Kliewer (1983a,b) found glutamate dehydrogenase and glutamine synthetase working in leaves and roots. However, the same authors (1983b) did not find glutamate synthase activity in roots. Rather than the lack of the enzyme, this could suggests some difficulty in extraction method (Jordan, 1989).
Experiments with barley plants show a close relationship between the availability of carbohydrates and the capacity of plants to use ammonium for growth (Givan, 1979). This supports the idea that an appropriate carbohydrate reserve is necessary to avoid the toxic effects of ammonium. Seedling plants are very sensitive to ammonium toxicity because of their low carbohydrate content and they are unable to assimilate ammonium rapidly to avoid its accumulation (Barker, 1980). Bowman (1988) indicates that ammonium assimilation uses stored sugars but it also depends on current photosynthate. Supplying both an energy source and carbon skeletons, carbohydrates play a key role in ammonium assimilation. When ammonium ions were utilized by root tissues, carbohydrates were the main respiratory substrates (Givan, 1979). Also, direct supply of α-ketoglutarate to higher plant tissues can greatly reduce their internal ammonium concentration (Matsumoto, 1971).

**Ammonium Toxicity**

Assimilation of ammonium is a very important process in nitrogen metabolism in plants. The cation may be taken up from the soil or may be internally generated from reduction of nitrate or from protein degradation (Barker, 1989). In the last case carbon skeletons are used as energy...
source in respiration (Givan, 1979).

The tolerance of plants for external supplies or for internal accumulation of ammonium is low. The cation can be toxic to plants even at 1 mg/kg on a dry weight basis in plant tissues (Maynard and Barker, 1969). However, plant species have different tolerance to ammonium accumulation (Barker, 1989). Changes in ammonium sensitivity among plants can be attributed to differences in the amount of ammonium that is detoxified by assimilation into amino acids and amides in the roots relative to the amount that is translocated to shoots (Maynard and Barker, 1969). Also, it seems that potassium can be involved in ammonium toxicity. Barker and Lachman (1986) reported that cultivars of tomato that are sensitive to ammonium toxicity accumulate much less potassium in their stems than do the strongly resistant mutant strains.

Although the toxicity of ammonium in plants is well known, the exact biochemical causes and the specific concentrations causing the injury are not always clear (Givan, 1979). High ammonium level may alter metabolism and conduct to serious physiological and morphological disorders including chlorosis, restricted growth, uncoupled photophosphorylation, inhibition of ATP formation, reduced CO$_2$ fixation within the chloroplast, inhibited reduction of NADP, reduced carboxylase enzyme activity, blocked starch
synthesis, lowered absorption of inorganic cations as calcium, potassium, and magnesium, inhibited electron transport system, and sometimes, death of the plant (Magalhaes and Wilcox, 1984; Vines and Wedding, 1960).

Sources of Ammonium

Reports frequently show that severe injury to plants results from ammonium nutrition in excess of the needs of the plants (Barker, 1989). Toxicity from ammonium fertilizers occurs when the cation remains in the root zone in large quantities and when ammonium rather than nitrate is the major form of inorganic nitrogen (Barker et al., 1966; Maynard et al., 1968). Conditions which lead to a predominance of ammonium are low soil pH, cool air temperatures, spring soil conditions which inhibit nitrification or when chemical nitrification inhibitors are added with ammoniacal fertilizers (Barker, 1980).

Ammonium supply to a given tissue can occur directly through the xylem. Presence of ammonium in grapevines xylem exudate has been reported by Andersen and Brodbeck (1989a,b,c) and Jordan (1989). However, the hypothesis that cluster ammonium level reflects the ammonium content in the xylem was not confirmed by Jordan (1989). Then, free ammonium in the tissue and ammonium concentration in the
xylem appeared to be independent. On the other hand, the amount of ammonium directly transported from root to shoots is somewhat low and most of the ion accumulating in shoots under severe exposure of a plant to toxic levels of soil ammonium results from ammonium internally generated (Givan, 1979).

Ammonium may be endogenously produced in large amounts during rapid nitrogen fixation or when proteins are being degraded (Givan, 1979). Durzan and Stewart (1983) report 30 reactions releasing ammonium in plant tissues. Among them, photorespiration is the major source in leaves of many plants (Miflin and Lea, 1980; Walker et al., 1984). But, because of their low photosynthetic activity, it is unlikely that photorespiration be an important contributor of ammonium in grape rachis and flowers (Jordan, 1989).

Ammonium level in plant tissue can be increased by some stresses (Rabe and Lovatt, 1986; Srivastava and Singh, 1987). Some examples are shading (Smart et al., 1988), temperature, pollution and infection (Srivastava and Singh, 1987), and nutrient deficiency (Rabe and Lovatt, 1986). Ough (1969) found variation in ammonium content in must of grapes due to climate changes, varieties, rootstocks, and areas. It appears that any stress could affect the availability of carbon substrates needed for ammonium assimilation. Besides, Srivastava and Singh (1987)
evidence that deamination activity is higher in stressed plants. As a consequence, plants under these stresses present greater ammonium levels compared to nonstressed plants.

**Ammonium Detoxification**

There are several ways in which plants can assimilate and dispose of high concentrations of ammonium. Plants may detoxify excessive ammonium by accelerating the usual pathway of nitrogen assimilation (Givan, 1979). The major route of ammonium assimilation in plants under nontoxic concentrations of the ion is by the glutamate synthase cycle (Barker, 1989). Glutamate synthase (GOGAT) activity depends on the presence of glutamine synthetase (GS) (Robert and Wong, 1986). The GS/GOGAT system is important when ammonium levels in tissues are low such as under normal conditions of nitrate reduction (Barker, 1989).

When ammonium is at high concentration in plant tissues, the glutamate dehydrogenase (GDH) reaction (equation 3) is important in ammonium detoxification (Givan, 1979). Also, Given (1979) indicates that the asparagine synthetase reaction (equations 4 and 5) may be another assimilatory way at very high ammonium levels.
Equation 4

Aspartate + glutamine + ATP $\rightarrow$ asparagine + AMP + PP$\textsubscript{i}$ + L-glutamate

Asparagine synthetase also can react with ammonium directly (equation 5), but it has a very high $K_m$.

Equation 5

Aspartate + NH$_4^+$ + ATP $\rightarrow$ asparagine + AMP + PP$_i$

Ammonium assimilation into amides within the roots appears to be a detoxification mechanism for plants to survive on high levels of ammonium nutrition (Barker et al., 1966). The assimilation into amides must be rapid to avoid the toxicity (Barker, 1980). This generalization applies also when ammonium is produced internally within plant tissues (Givan, 1979). For example, amides are accumulated during leaf senescence when soluble amino acids are deaminated and respired, and as a result ammonium is released (Givan, 1979). Ammonium assimilation may be limited by the availability of the various substrates requires as inputs for the synthesis of amides. Givan (1979) points out that these substrates are derived primarily from reserve carbohydrates.
Fruit Set in Grape Vines

In most grape varieties the setting of berries results from the sequence of pollination, fertilization, and seed development (Winkler et al., 1974). This is the mechanism which determines the set of seeded varieties. This kind of fruit set (normal set) results in maximum berry size for the variety when the conditions of production are favorable. Besides the normal set, Winkler et al. (1974) identify other two important types of set in grapes. They are:

Stimulative parthenocarpy: in this kind of set, although the pollen is of high germinability, there is no ovule development. As a result, the size of the berries is very small. It appears that the stimulus of pollination and the nutritional stimulus are required to have a satisfactory set. The cultivar Black Corinth is an example of stimulative parthenocarpy.

Stenospermocarpy: Thompson Seedless and Perlette are cultivars that present this type of set. The setting in these varieties follows the sequence pollination and fertilization but embryo abortion occurs later. Because some seed development takes place, berries are larger than those resulting from stimulative parthenocarpy.
Regulation of Fruit Set

Supply of organic nutrients, such as carbohydrates, to developing ovaries has been suggested as a controlling factor in fruit set (Mullins, 1967; Coombe, 1970; Winkler et al., 1974). However, Weaver et al. (1962) concluded that carbohydrates alone are the cause of fruit set, and they postulated that leaves produce factors in fruit setting. Mullins (1967), working with cuttings in which leaves, apices, and roots were removed, and with inflorescences that were cultured in vitro on a medium without the presence of exogenous growth substances, proved that fruit set is regulated by the supply of organic nutrients rather than by specific hormonal stimuli from organs external to the developing cluster. Skene (1969) and Coombe (1970) both comparing the effects of cycocel (CCC, chlormequat) and shoot tipping on fruit set, showed that set is increased by reducing shoot growth and competition between the developing leaves and berries for organic nutrients.

Means of Improving Fruit Set

There are some common treatments which can improve the percentage of set on Vitis vinifera. They are:
i) topping (removing 30 cm or more of the shoot tip) or
tipping (removing 7 cm or less of the succulent shoot tip) at blossom time (Winkler et al., 1974; Skene, 1969); ii) sprays of chlormequat (CCC, cycocel) before anthesis (Coombe, 1965, 1967, 1970; Brown et al., 1988); iii) application of gibberellin or 4-CPA (4-chlorophenoxyacetic acid), especially on seedless grapes (Considine and Coombe, 1972; Winkler et al., 1974); iv) girdling (Jensen et al., 1981; Brown et al., 1988).

Girdling and Fruit Set

Girdling, also named ringing or cincturing, is an old practice. It was introduced into Greece in 1983, as a mean of improving the set of Black Corinth (Winkler et al., 1974). Girdling consists of removing a complete ring of bark (3-6 mm) wide from the trunk or from an arm or a fruit cane. Girdling the trunk affects the entire vine, whereas girdling a cane influences only the part of the cane above the girdle. To produce the desired effects, it is essential that the ring of bark be completely removed. If a small section of the ring is left, there may be little or no response (Winkler et al., 1974).

Usually, vines are girdled to get the following three objectives: to improve the set of berries, to increase the size of the individual berries, and to accelerate
maturation. Each of these effects may be obtained by girdling at the proper time. The widest use of girdling is for increasing berry size in seedless varieties, when grown for table fruit. Girdling to advance maturation has been of minor importance.

According to Winkler et al. (1974), in cultivated varieties of grapes usually many flowers fail to set because of lack of pollination, lack of fertilization, or other causes. Unfertilized flowers drop off very soon in most varieties. Some varieties, such as Black Corinth, rarely produce anything but small, round seedless berries (shot berries) from some of the pollinated but unfertilized flowers. The number and size of seedless berries on Black Corinth vines or on vines of other seedless varieties, can be greatly improved by girdling (Weaver et al., 1962; Weaver, 1976).

The major effect of girdling on seeded grapes is promoting the retention of seedless berries which usually drop off the cluster. Berry set of normally seeded berries, on the other hand, is influenced very little by girdling (Winkler et al., 1974; Brown et al., 1988). However, an increment in the number of seeded berries, especially those with lower seed number, has also been pointed out (Coombe, 1959). The setting of shot berries is increased by girdling due to a reduction of berry drop which normally occurs
immediately after blooming. Therefore, girdling must be
done before normal berry drop takes place, that is, during
or just before bloom (Weaver, 1976; Brown et al., 1988).

Girdling and Carbohydrates Content

Because of girdling, the carbohydrate materials
(sugars and starch) and plant hormones produced in the
leaves accumulate in the parts above the wound, including
the clusters of blossoms or fruit, and influence their
development (Weaver and McCune, 1959; Weaver, 1976; Winkler
et al., 1974).

Hale and Weaver (1962) determined the direction of
translocation of $\text{C}^{14}$, following the assimilation of $\text{C}^{14}\text{O}_2$, by
using radioautographic techniques. They report that at
bloom time the cluster has small competitive power as a
sink, and the direction of the translocation is given by
the shoot tip and the parent vine. The cluster is unable to
withdraw photosynthate from the stream. Thus, girdling
improve the setting because it diverts more assimilates
into the cluster just prior to fruit set.

In Thompson Seedless, girdling increases the available
carbohydrates in shoots above the girdle and decreases them
in the root (Weaver and McCune, 1959; Roper and Williams,
1989). In this manner, the girdle avoids the translocation
of elaborated food materials from the shoots to the roots, and results in utilization of the stored starch in the roots. But, although girdling increases the level of carbohydrates in the shoots and reduces it in the roots, by the dormant season the level in girdled and ungirdled vines is about the same (Weaver and McCune, 1959). Roper and Williams (1989) found in Thompson Seedless that the leaves of girdled vines serve as carbohydrate storage organs for the clusters from shortly after being girdled until the girdle heals. They show that the accumulation of carbohydrates in clusters of girdled vines, which is greater than ungirdled vines, is similar to the amount of carbohydrates lost by the leaves of the girdled vines.

**Shading, Fruit Set and Carbohydrates**

There is strong evidence that fruit set in grapevines is regulated by supply of organic nutrients (Mullins, 1967; Skene, 1969; Coombe, 1970). Therefore, factors that reduce the production of carbohydrates at setting time (e.g., shading) could markedly affect fruit set.

Coombe (1970) determined a fruit set reduction when he shaded inflorescences of Muscat of Alexandria vines during the setting period. Also, on other perennial crops it is well documented that artificial or natural shade reduce the
percentage of fruit set in sweet cherry (Patten and Proebsting, 1986), peach and apple (Byers et al., 1984, 1985, 1990; Doud and Ferree, 1980; Jackson and Palmer, 1977; Schneider, 1978), and strawberry (Ferree and Stang, 1988).

Fruit abscission caused by shading suggests that natural fruit drop can be induced if photosynthesis is limited (Byers et al., 1984). Working with different grapevine cultivars, Perez and Kliwer (1982), and Smart et al. (1988), found that under reduced light conditions photosynthesis rate is decreased. Also the same was found in peach by Marini and Sowers (1990). Nobel (1983) indicates that a shaded leaf has a low rate of photosynthesis because the amount of radiation photosynthetically active reaching it is fairly small. Besides, at low light intensity the stomata generally are partially closed, which increases the stomatal resistance and further decreases photosynthesis. Increase in stomatal resistance due to shading has also been reported by Smart (1988), and Boardman (1977).

Results from a study with Redhaven peach indicate that shading has a negative influence on specific leaf weight (SLW) and net photosynthesis (Pn) of leaves (Marini and Sowers, 1990). Leaves under shade conditions present a reduced dry weight per leaf area, showing a reduced
capacity for photosynthesis and a reduced supply of carbohydrates (Kappel, 1989). Decrease in soluble solids concentration in artificially shaded pear (Kappel, 1989), and grapevines (Smart et al., 1988), suggests a reduction in the available carbohydrates for fruit due to shading.

**Canopy Management**

The canopy consists of leaves, shoot and fruit, arranged in some manner relative to the position of canes, cordon and trunk, dictated in turn by the training system (Smart, 1986). The term canopy management may be thought as achieving some desirable canopy configuration, in terms of surface area, volume, shoot leaf area, fruit exposure, shoot number, shoot orientation and even vine physiology (Smart, 1988; Archer and Strauss, 1989). Since excessive canopy shading is the common fault of modern canopy management, Smart (1986) emphasizes the use of a proper training system, shoot number control and vigour manipulation as techniques to reduce shading.

It has been observed that inflorescence necrosis incidence is increased by shading (Lombard, pers.comm., 1988; Jackson, 1988). Thus, the use of any practice to decrease shading could in turn reduce INec occurrence. Shoot density manipulation has been used as a partial
alternative to cluster thinning for crop control (Reynolds, 1989), and to increase light penetration into the canopy (Smart, 1988b). Low shoot densities (less than 10 shoots/m) produced open canopies and most leaves and fruit were well exposed to sunlight. On the contrary, high shoot densities (more than 30 shoots/m) caused that the majority of fruit and leaves were interior and shaded (Smart, 1988b).
Chapter 3

EFFECT OF GIRDLING AND SHADING ON INFLORESCENCE NECROSIS, FRUIT SET, AND TISSUE AMMONIUM LEVELS IN PINOT NOIR GRAPEVINES

Abstract

Inflorescence necrosis (INec), a disorder that causes large crop losses in plants of *Vitis vinifera* L., is described. INec occurs at bloomtime when flowers and pedicels are affected. Sometimes the rachis and, in severe cases, the whole cluster is necrotic. The influence of girdling, shading, and shoot density on INec and fruit set was determined in mature field grown Pinot Noir grapevines in 1989 and 1990. Free ammonium concentrations were established in shoot xylem exudate, tendril, petiole, rachis, and flower tissue sampled at three phenological stages: beginning bloom, full bloom, shatter, and harvest along with fruit set and INec. Shading with 60% shade cloth was imposed during a month before bloom. Trunks and cordons, and shoots were girdled one week before capfall and at first bloom, respectively. Shading increased the percentage of necrotic flowers by 2.3 and 2.7 times on two vineyard plots and reduced fruit set by 23% and 35% compared to exposed plants. The fruit set was 15% and 25%
higher in girdled than ungirdled vines. Girdling did not affect the percentage of necrotic flowers. Shoot exudate was insufficient to show the actual ammonium content. Among sampled tissues, the rachis had the highest ammonium levels and flowers the lowest. This difference suggests a different ammonium assimilation capacity. Rachis ammonium concentration was higher at beginning bloom and then declined in all treatments. Shade caused 24% and 21% increase in rachis ammonium concentration at beginning bloom and no effect after this stage. Shoot girdling above, and below and above the cluster increased the rachis ammonium level 1.3 and 1.6 times compared to control at shatter time. INec severity and rachis ammonium content were not affected by shoot density treatments. There was no significant effect of treatments on flower ammonium levels. Petioles from shaded vines had higher ammonium concentration than petioles from exposed vines. There was no clear trend in ammonium levels in tendrils during bloom or as affected by treatments. Reduction in percentage of fruit set and increase in necrotic flowers due to shading or to girdling above the cluster may be explained by reduced carbohydrates content. Increase in fruit set by girdling may be interpreted by photosynthate accumulation just prior to set to retain seedless berries.
Introduction

There are several physiological factors which occur before bloom that may cause yield reduction in grapes (Jackson, 1988). Poor inflorescence initiation, poor flower formation, early loss of inflorescences, filage, and millerandage are described as different phenomena reducing berry set (Jackson, 1988; Jackson and Coombe, 1988b). Lack of set is often due to inadequate pollination and fertilization (Winkler et al., 1974). Another cause of flower loss is Inflorescence Necrosis (Jordan, 1989), also called Early Bunchstem Necrosis (EBSN) (Jackson, 1988; Jackson and Coombe, 1988a,b).

Inflorescence necrosis has caused as much as 50% crop loss on Pinot Noir cultivar (Jordan, 1989), and 90% crop loss on some muscat varieties (Jackson, 1988). This problem has often been ascribed to botrytis or other fungal pathogens. However, pathologists were unable to find any fungi from damaged tissue (Jackson and Coombe, 1988a). Elevated ammonium concentration in rachis tissue has been related with inflorescence necrosis (Lombard, pers. comm., 1988; Jordan, 1989). Waterberry, another grape disorder that presents symptoms similar to INec, has also been associated with high rachis ammonium levels (Christensen and Boggero, 1985).

Ammonium accumulation in plant tissues could be a
consequence of low carbon substrates, which are essential for ammonium assimilation (Barker, 1980; Givan, 1975). Girdling is a well-known practice to increase berry set in grapes (Winkler et al., 1974). This occurs as a result of increased carbohydrates level (Mullins, 1967), or increased carbohydrates and growth regulators level in the cluster (Weaver et al., 1962). On the other hand, it has been indicated that shading reduces berry set in several fruit trees (Pattern and Proebsting, 1986; Ferree and Stang, 1988; Byers et al., 1990) as a consequence of a low supply of carbohydrates (Kappel, 1989).

The purpose of this research was to describe the occurrence and symptoms of inflorescence necrosis; and to determine the effect of girdling, shading, and shoot density on the disorder incidence, fruit set, and ammonium concentration in xylem fluid, tendril, petiole, rachis, and flower tissues.
Material and Methods

Plant Material and Experimental Design

Experiment 1: Effect of shade cloth and girdling (1989)

The *Vitis vinifera* L. plant materials used in this study were mature Pinot Noir vines planted 1.7 x 2.7 m grown at Woodhall III Vineyard, Alpine, Oregon and vines of the same cultivar planted 1.8 x 3.0 m grown at the Tyee Wine Cellars, Greenberry Road, South Corvallis, Oregon. Vines growing at Woodhall were pruned to two twelve-bud canes per vine. On the contrary, vines at Tyee were trained to a bilateral cordon and pruned to four or five three-bud spurs. In both locations plants were trained on a standard vertical trellis.

In 1989 forty vines at Woodhall and twenty at Tyee were selected for similar growth and size, and a factorial set of four treatments was imposed and distributed in a completely randomized design. Two factors were used, girdling and shading; both including two levels, with and without the presence of the factor. The four treatments are given in Table 3.1.

At Woodhall each treatment was imposed to ten plants. At Tyee girdling was done on one of the two cordons in each vine, in such a way that each of the twenty vines had a
girdled and an ungirdled cordon. Shading was imposed to ten whole vines. In this manner, each treatment at Tyee was made up of ten cordons instead of ten vines.

Girdling was performed on the trunk, 20 cm below the head of each treated vine (at Woodhall), and immediately before the first spur on each cordon (at Tyee). A 4.8 mm wide strip of bark was removed with a double blade knife. Girdling was done on 5 June, about one week before capfall. Shading with 60% shade cloth was imposed on 17 May until 10 August. At bloom time, the light intensity received at cluster level, either under the cloth and in exposed plants, was measured between 12:00 and 15:00. A Li-Cor 188B Quantum meter with a quantum sensor, placed within the canopy in line with the clusters, was used. The average photosynthetic photon flux density (PPFD) in $\mu E m^{-2} s^{-1}$ was 40.3 for exposed treatments and 21.6 for shaded vines on leaves and clusters in the fruiting area.

To determine fruit set and percentage of necrotic flowers, pollination bags were placed on forty clusters per treatment just before bloom. At shatter time, green and necrotic flowers and berries, either those remaining on the clusters and those dropped off were counted and recorded.

Shoot xylem exudate was extracted under suction (Jordan, 1989). Insufficient volume was obtained from the sample at beginning bloom time. Thus, results are only presented for the full bloom and shatter samples. However,
the amount of exudate obtained in these samples was inadequate and the results do not show the actual xylem ammonium content. Andersen and Brodbeck (1989a) indicate that vacuum or pressure chamber extraction yield very small volumes of fluid that may also contain compounds extracted from non-xylem tissue or non-functional xylem vessels. Also, at bud break time samples of xylem solution were collected from canes by cutting them with a knife. The fluid was collected in a test tube. In this case, vines did not bleed sufficient exudate for reliable analysis. Immediately following collection, the exudate was frozen at -18°C. Xylem fluid was thawed to room temperature prior to ammonium determination.

Plant tissue of tendrils, petioles, rachis, and flowers were sampled to determine ammonium concentration. Carbohydrate and organic acid content was also measured in rachis tissue. To obtain plant tissue for analysis, single and fruitful shoot samples were collected randomly early in the morning from each vine. Leaves were stripped from the sampled shoots before transport back to the laboratory. Rachis and flower tissue were separated from basal clusters. Plant material was collected at three stages: beginning bloom (clusters with 5% open flowers), full bloom (50-70% open flowers), and shatter. The sampling dates are presented in Table 2.
Experiment 2: Effect of girdling, shoot growth and shoot density (1990)

A mature Pinot Noir vineyard plot located at Tyee Cellars vineyard, South Corvallis, Oregon, trained in cordon onto a vertical trellising system was used in this study. Twenty eight representative vines were selected for a shoot density trial. Seven were used for each of the four treatments (Table 3.3).

Individual vines served as replicates (seven replications). The two extra canes with 12 buds each were left to reduce the vine vigour. They were located in the central part of the vine and trained upwards. Shoot thinning was done before bloom when shoot length was about 10 cm. Both fruiting and non fruiting shoots were removed.

In the same vineyard, twelve vines were used for a shoot girdling trial. Sixty uniform shoots per two vines were utilized for each of four treatments (Table 4.4).

Two vines were used within each replicate for a total of six replications. Girdling was performed around basal cluster at beginning bloom (about 2% open flowers). To do that, a girdling plier was used. In all treatments the leaf opposite to the basal cluster was removed at girdling time.

The plant spacing was 1.8 x 3.0 m. Vegetative growth in the plot could be described as very vigorous with a history of high inflorescence necrosis incidence. At
shatter time, the percentage of necrotic flowers on five clusters per treatment was determined in both trials. This was an estimate based on percent of cluster showing necrosis (severity) from black and brown colored pedicels and flowers plus in some cases including light green undeveloped elongated berries. Five basal clusters per treatment were removed in both experiments at bloom (40% open flowers), shatter, and harvest times for samples. Flowers and/or berries were clipped off, leaving the rachis for laboratory ammonium analysis. Radiant energy received at cluster level was measured in shoot density trial in the same manner described in Experiment 1.

Ammonium Analysis

In both experiments plant tissues were dried at 70°C in a forced air oven. Samples were ground to pass a 20 mesh screen in a rotary mill. Subsamples (0.1 g) of ground tissue were extracted for 1 h in 10 ml 2% (v:v) acetic acid in 16 mm test tubes. Samples were shaken by hand every ten minutes. After extracting the samples were filtered through an in-tube, serum filter (Plasma/Serum separator, Karlan Chem. Corp. California). Determination of the ammonium concentration was made on an ammonium analyzer (Wescan Ammonium Analyzer Model 360, Alltech Assoc., Inc./Wescan Instruments, San Jose, California).
The data were analyzed using analysis of variance for a factorial set of treatments distributed in a completely randomized design (Experiment 1), and a set of treatments arranged in a randomized block design (Experiment 2). Tukey's multiple range test was employed for means comparisons.
Inflorescence Necrosis: Description

Observations carried out for two seasons indicate that inflorescence necrosis affects only clusters. No other tissue showed similar symptoms. Flowers and pedicels were the only damaged tissue. Few or all flowers can be necrotic in a particular cluster (Fig. 3.1). Sometimes, the rachis can also be affected. When this occurs, necrosis begins from the apical section and continues toward the peduncle (Fig. 3.2). In severe cases, clusters can be completely necrotic (Fig. 3.3). Affected tissue is characterized by brownish or black coloration.

First symptoms were seen at beginning bloom or just before (Fig. 3.4). The number of necrotic flowers usually increased until full bloom. Fruit set was apparently the latest stage of inflorescence necrosis occurrence. After that, no new necrosis was seen. During bloomtime, necrotic flowers (with pedicels attached) fall very easily leaving an open cluster. However, some remain on the cluster for a long time and can still be seen at harvest time (Fig. 3.5). When the whole cluster is affected, necrosis was complete also by full bloom stage.

Another typical inflorescence necrosis symptom is the presence of numerous small undeveloped berries that remain
strongly attached to the cluster, without further development (Fig. 3.6). It appears they were slightly affected with necrosis at fruit setting or soon after. They differ from normal shot berries in size, shape, and color. Affected berries are smaller, more elongate, and have a lighter green color than normal shot berries. Sometimes, in severely damaged clusters only these small berries remain attached. When inflorescence necrosis occurs, vines may have some of the above described symptoms with a degree of severity.

The symptoms described in this paper are broader than that published by Jordan (1989), and Jackson and Coombe (1988a,b). The latter authors emphasized necrosis in sections of the bunches and early appearance of the disorder (one or two weeks before flowering). We consider flowers and pedicels as the mainly affected tissues, and only in severe cases the rachis also is damaged. In regards to the critical time, we observed necrosis taking place from the beginning of bloom (or just a day or so before) until full bloom.

Percentage of Necrotic Flowers

Experiment 1. There was no interaction between girdling and shading treatments on the number of necrotic flowers. Figure 3.7 shows that in both locations shading increased
the percentage of necrotic flowers. It was 2.3 and 2.7 times higher than that of exposed plants at Tyee Cellars and Woodhall, respectively.

Lombard (pers. comm., 1988) determined that 31% of inflorescence necrosis in shaded Cabernet Sauvignon vines grown in the greenhouse compared to 1.5% in exposed vines. Increasing effect of shading on necrotic flowers in greenhouse and field conditions has also been observed by Jackson (1988), and Jackson and Coombe (1988b). It seems possible that low carbohydrate status due to shading, may induce ammonium accumulation and leads to necrosis of flowers. Positive correlation between percentage of necrotic flowers and rachis ammonium concentration was found by Jordan (1989). Also, clusters may grow weakly because of shading, and in this manner they could be more sensitive to any toxic element. We found shading reduced the rachis dry weight (Fig. 4.4).

Girdling did not affect the percentage of necrotic flowers, although in both locations it was less than ungirdled plants (Fig. 3.7). We expected a reduction of necrotic flowers due to increase carbohydrates accumulation from girdling because of improved ammonium assimilation. But at bloom time when necrosis occurs, the cluster is a weak sink (Hale and Weaver, 1962). Besides, sugars accumulate preferentially in leaves after girdling and they are utilized later by the clusters (Roper and Williams,
Experiment 2. Treatments did not affect significantly the percentage of necrotic flowers in either shoot density or shoot girdling trials (Fig. 3.8, 3.9). The lack of effect may be attributed to the excessive shoot vigour, which in turn prevented a difference in radiant energy absorption at cluster level among treatments (Table A.1). Besides, the plot where the trial was established has had high inflorescence necrosis in last two seasons. This can be checked by the high percentage of necrotic flowers (severity) presented in Figures 3.8 and 3.9.

Fruit Set

Experiment 1. At Tyee Cellars vineyard the highest percentage of fruit set was found by girdling the exposed plants (Fig. 3.10). On the contrary, the lowest percentages were obtained by shading. Girdling increased fruit set 14.9% compared to control. Shading treatments reduced the set by 23% of the non girdled cordons. Girdling and shading did not show an interaction with fruit set at Woodhall III (Fig. 3.11). Berry set was 24.9% higher in girdled compared to ungirdled plants while shading reduced it by 35%.

Girdling is a technique to increase fruit set in grapes. Hale and Weaver (1962) found improved the set because more assimilates were accumulated into the cluster.
just prior to fruit set. Also, in normally seeded grapes, girdling mainly promotes the retention of seedless berries which usually drop off (Brown et al., 1988; Winkler et al., 1974).

Coombe (1970) suggests that set reduction in grapevines due to shading may be from limited supply of photosynthate to the inflorescence during and after bloom. Kappel (1989), and Nobel (1984) indicate that leaves under shade conditions present a reduced ability for photosynthesis and a reduced supply of carbohydrates. Shading has also been the cause of fruit set reduction in peach and apple (Byers et al., 1984, 1985, 1990; Jackson and Palmer, 1977; Schneider, 1978; Doud and Ferree, 1980), sweet cherry (Patten and Proebsting, 1986), and strawberry (Ferree and Stang, 1988). In our research, the high percentage of necrotic flowers caused by shading (Fig. 3.7), is also a contributing factor to set reduction.

Ammonium Concentration in Grapevine Tissues

Tendril

Experiment 1. Tendril is a long, slender, curled structure at some of the nodes of a shoot (Jordan et al., 1981). It can firmly attach the shoot to a support. Tendrils and flower clusters have a common origin (Winkler et al.,
1974). Detached tendrils show similar symptoms as necrotic rachis when exposed to ammonium solutions (Jordan, 1989).

A significant difference in ammonium concentration between shading and shading plus girdling treatments was obtained at full bloom and shatter at Tyee Cellars vineyard (Fig. 3.12). Shading plus girdling treatment had 39.6% and 53.6% more ammonium than shading alone at full bloom and shatter, respectively. There was no interaction at the Woodhall plot (Fig. 3.13). In this vineyard, tendrils from shaded plants had 45.4% more ammonium than exposed plants at beginning bloom. Also, girdling increased the ammonium concentration by 43.3% compared to ungirdled plants at shatter stage.

Detached tendrils are a useful model system to study ammonium toxicity and assimilation in tissue closely related to that of clusters (Jordan, 1989). However, in field conditions they do not present necrotic symptoms, despite the presence of many damaged clusters in the same plant. Data from this study do not indicate a relationship between tendril ammonium concentration and inflorescence necrosis incidence.

Petiole

Experiment 1. The petioles are normally used for analysis to determine fertilizer requirements in grapes, especially
those from leaves opposite the clusters toward the base of the shoot (Christensen et al., 1982).

Results from this research show no interaction between girdling and shading in both locations (Fig. 3.14, 3.15). The levels of ammonium in the petiole of the shaded plants at Tyee Cellars were 51.8%, 64.4%, and 100.0% higher at beginning bloom, full bloom, and shatter, respectively, compared to that of the exposed plants (Fig. 3.14). The same trend was obtained at Woodhall, where petiole ammonium concentration from shaded plants was increased by 45.6% at beginning bloom and 71.3% at shatter stage, compared to petioles from exposed plants (Fig. 3.15). There was no significant effect of girdling on petiole ammonium levels in both vineyards.

High correlation ($r = 0.66$) between inflorescence necrosis and petiole NH$_4^+$-N ppm from shaded plants, was found by Lombard (pers. comm., 1988) in greenhouse grown vines. However, it is doubtful that this tissue is involved directly with the necrosis, as only the necrotic rachis is symptomatic of the inflorescence necrosis and not the petiole.

Rachis

Experiment 1. Rachis ammonium concentration followed the same pattern in both locations (Fig. 3.16, 3.17). There was
no interaction between factors and only shading increased ammonium level at beginning bloom. At this stage, rachis from shaded vines had 24.4% and 21.0% more ammonium than rachis from exposed vines at Tyee Cellars and Woodhall, respectively. Higher ammonium concentration in shaded than exposed rachis, was also observed at full bloom and shatter stages in both vineyards, although the difference was not significant.

Artificial shade (76% shade) increased rachis ammonium level at bloom time from the exposed of 1.2 to the shaded of 4.0 mg NH$_4^+$/g DW in potted Cabernet Sauvignon vines grown in greenhouse (Lombard, pers. comm., 1988). Jordan (1989), working with field grown Cabernet Sauvignon vines, found that rachis from shaded vines at a similar stage had only about 25% higher ammonium concentration than rachis from exposed vines (about 1.5 mg NH$_4^+$/g DW). Results from this research show a higher rachis ammonium level at full bloom (about 2 fold) in shaded vines compared to both previous cited experiments. This could indicate a difference in cultivars susceptibility to ammonium accumulation. Jordan (1989), points out that Pinot Noir in the Willamette Valley (Oregon) was greatly affected by inflorescence necrosis in 1988, much more than most other cultivars.

Rachis was the tissue that had the highest concentration of ammonium compared to tendril (Fig. 3.12, 3.13), petiole (Fig.3.14, 3.15), and flowers (Fig. 3.20,
3.21). This result suggests that the rachis assimilates less, receives more, or produces more ammonium than the other tissues. Also, it has been demonstrated that rachis is a tissue with a high transpiration rate (Elboudwarej et al., 1990). In this manner, the cation (NH$_4^+$) could be concentrated in the rachis.

Maximum ammonium concentration was found at beginning bloom and declined from this stage to shatter (Fig. 3.16, 3.17). The first symptoms of inflorescence necrosis are seen at beginning bloom or just before. Thus, it coincides with the highest rachis ammonium level. Besides, the lowest dry weight and total organic acid concentration in rachis tissue occurred at beginning bloom in shaded plants (Fig. 4.4, 4.8). Therefore, at beginning of bloom the rachis was a very sensitive tissue and excessive ammonium could result in toxic concentration and cause necrosis. This condition occurs during the period when inflorescence necrosis takes place, from beginning bloom to fruit set.

Positive correlation between rachis ammonium concentration and inflorescence necrosis was obtained by Lombard (pers. comm., 1988) in vines growing in greenhouse, and Jordan (1989) in field grown vines. Christensen and Boggero (1985) found significant correlation between rachis ammonium level and the occurrence of waterberry. This grape disorder has similar symptoms as inflorescence necrosis but it occurs later in the season.
Experiment 2. The effect of shoot density treatments in 1990 on rachis ammonium concentration is given in Figure 3.18. There was no difference among treatments. Excessive shoot vigour may be the cause of lack of effect on INec. Maximum ammonium concentration (7.8 to 8.3 mg/g DW) in rachis occurred at bloom and declined from this stage to harvest (1.8 to 2.0 mg/g DW). The same trend was found by Jordan (1989); however, the values obtained in this study were four times higher at bloom and nine times higher at harvest. Different cultivars and the use of a plot with high INec incidence may be the difference.

Shoot girdling affected the rachis ammonium concentration (Fig. 3.19). Girdling above, and also below and above the cluster increased the rachis ammonium level 1.3 and 1.6 times compared to control at shatter time. At this time, the highest rachis ammonium concentration (9.7 mg/g DW) was achieved by girdling below and above the basal cluster. There was no effect of treatments at bloom and harvest stages. After berry set, developing fruit becomes a powerful sink and at the same time the shoot tip becomes a less powerful sink as the rate of shoot growth decreases (Hale and Weaver, 1962). The same authors indicate that after the set of the fruit, photosynthate moves apically into the cluster from leaves below the cluster, but photosynthate mainly moves basally from the tip. Thus, girdling done above, and below and above of the
cluster reduces its carbohydrate content. Increase in
rachis ammonium concentration at shatter stage by girdling
the cluster above, and below and above, suggests that
ammonium is accumulated due to a low carbohydrate
availability. Givan (1979) indicates that a good
availability of carbohydrates is necessary to improve
ammonium assimilation.

Flowers

Experiment 2. There was no effect of treatments on flower
ammonium concentration in both vineyards (Fig. 3.20, 3.21).
The data show lower ammonium levels (about 5 times) in
flowers compared to rachis (Fig. 3.16, 3.17) at beginning
bloom and full bloom stages. At shatter stage, when berries
are growing, ammonium concentration is greatly increased.
At the same time, rachis tissue has reduced ammonium
levels. This suggests berries as a stronger sink than
flowers, and ammonium is taken up from the rachis.
Difference between rachis and flowers indicate the two
tissues have different ammonium assimilation capacity.

The typical symptom of inflorescence necrosis has
many necrotic flowers, while only in severe cases the whole
cluster is damaged. Flowers appear as a very sensitive
tissue. Flower necrosis could be a consequence of high
rachis ammonium accumulation and consequently pedicel
necrosis.

**Ammonium Concentration in Xylem Exudate**

**Experiment 1.** Ammonium was not detected in samples taken at bud break time (data not shown). Maybe the insufficient quantity of fluid prevented ammonium detection. Andersen and Brodbeck (1988, 1989) found that ammonium was present in concentrations varying from 0.4 to up to 2.0 mM in xylem exudate collected by bud break stage.

The amount of ammonium in shoot xylem extract from samples of Tyee Cellars and Woodhall III vineyards is indicated in Tables A.2, and A.3, respectively. The values do not represent the actual amount of ammonium because of the inadequate quantity of exudate. However, it is possible to observe that shaded treatments had higher values, compared with exposed samples. Jordan (1989) found that shade caused three fold increase in xylem ammonium concentration by bloom time. He suggests that this response is based on the expectation that shade reduce carbon substrate and energy sources and so limited ammonium assimilation. As a consequence, elevated ammonium content in xylem exudate would result.
Table 3.1. Girdling and 60% shading treatments imposed on Pinot Noir vines at Tyee, Corvallis, and Woodhall III, Alpine, vineyards, 1989.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Girdling</th>
<th>Shading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(5 June, 1989)</td>
<td>(17 May-10 August, 1989)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Girdling</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Shading</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Shad-Gird</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 3.2. Tissue sampling dates of Pinot Noir vines under girdling and shading treatments, 1989.

<table>
<thead>
<tr>
<th>Sampling Stages</th>
<th>Woodhall III</th>
<th>Tyee Cellars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning Bloom</td>
<td>13 June</td>
<td>15 June</td>
</tr>
<tr>
<td>Full Bloom</td>
<td>18 June</td>
<td>20 June</td>
</tr>
<tr>
<td>Shatter</td>
<td>29 June</td>
<td>2 July</td>
</tr>
</tbody>
</table>
Table 3.3. Shoot density treatments imposed on Pinot Noir vines at Tyee vineyard, Corvallis, 1990.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10 shoots/m</td>
</tr>
<tr>
<td>10+C</td>
<td>10 shoots/m + 2 extra canes</td>
</tr>
<tr>
<td>24</td>
<td>24 shoots/m</td>
</tr>
<tr>
<td>24+C</td>
<td>24 shoots/m + 2 extra canes</td>
</tr>
</tbody>
</table>
Table 3.4. Shoot girdling treatments imposed on Pinot Noir vines at Tyee vineyard, Corvallis, 1990.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>no girdling</td>
</tr>
<tr>
<td>Below</td>
<td>girdled below cluster</td>
</tr>
<tr>
<td>Above</td>
<td>girdled above cluster</td>
</tr>
<tr>
<td>Bel-Abo</td>
<td>girdled below and above cluster</td>
</tr>
</tbody>
</table>
Fig. 3.1. Pinot Noir cluster at bloom affected by inflorescence necrosis (INec). Only flowers and pedicels are necrotic.
Fig. 3.2. Pinot Noir cluster at bloom affected by inflorescence necrosis (INec). The apical section of the rachis is necrotic.
Fig. 3.3. Pinot Noir cluster severely affected by inflorescence necrosis (INec).
Fig. 3.4. Pinot Noir cluster at beginning bloom affected by inflorescence necrosis (INec). Note only a few flowers and pedicels are necrotic.
Fig. 3.5. Pinot Noir cluster at harvest time affected by inflorescence necrosis (INec). Note the necrotic flowers and pedicels which are black or brown. Also, there are light green or white undeveloped and elongated small berries.
Fig. 3.6. Pinot Noir cluster at shatter affected by inflorescence necrosis (INec). Note the presence of numerous undeveloped small berries.
Fig. 3.7. (Exp. 1). Effect of girdling and 60% shading on percentage of Pinot Noir necrotic flowers, Woodhall III, Alpine, and Tyee, Corvallis, vineyards, Oregon. Error bars are standard errors of the means. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.8. (Exp.2). Effect of shoot density (10 and 24 shoots/m) and vigour reduction (with and without 2 extra canes) treatments on percentage of Pinot Noir necrotic flowers, Tyee vineyard, Corvallis. Error bars are standard error of the means. Each treatment represents 7 vines.
Fig. 3.9. (Exp.2). Effect of shoot girdling treatments (none, below, above, and below-above cluster) on percentage of Pinot Noir necrotic flowers, Tyee vineyard, Corvallis, Oregon. Error bars are standard error of the means. Each treatment represents 30 shoots.
Fig. 3.10. (Exp.1). Effect of girdling and 60% shading on Pinot Noir fruit set at Tyee vineyard, Corvallis, Oregon. Error bars are standard errors of the means. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.11. (Exp.1). Effect of girdling and 60% shading on Pinot Noir fruit set at Woodhall vineyard, Alpine, Oregon. Error bars are standard errors of the means. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.12.(Exp.1). Effect of girdling and 60% shading on Pinot Noir tendril ammonium concentration at three phenological stages at Tyee vineyard, Corvallis, Oregon. Error bars are standard errors of the means. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.13. (Exp.1). Effect of girdling (A) and 60% shading (B) on Pinot Noir tendril ammonium concentration at Woodhall vineyard, Alpine, Oregon. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.14. Exp.1. Effect of girdling (A) and 60% shading (B) on Pinot Noir petiole ammonium concentration at Tyee vineyard, Corvallis, Oregon. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.15. (Exp.1). Effect of girdling (A) and 60% shading (B) on Pinot Noir petiole ammonium concentration at Woodhall vineyard, Alpine, Oregon. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.16. (Exp.1). Effect of girdling (A) and 60% shading (B) on Pinot Noir rachis ammonium concentration at Tyee vineyard, Corvallis, Oregon. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.17. (Exp.1). Effect of girdling (A) and 60% shading (B) on Pinot Noir rachis ammonium concentration at Woodhall vineyard, Alpine, Oregon. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 10 vines.
Fig. 3.18. (Exp.2). Effect of shoot density (10 and 24 shoots/m) and vigour reduction (with and without 2 extra canes) treatments on Pinot Noir rachis ammonium concentration, Tyee vineyard, Corvallis, Oregon. Each treatment represents 10 vines.
Fig. 3.19. (Exp.2). Effect of shoot girdling treatments (none, below, above, and below-above cluster) on Pinot Noir rachis ammonium concentration, Tyee vineyard, Corvallis, Oregon. Mean separation by Tukey's multiple range test, 5% level. Each treatment represents 30 shoots.
Fig. 3.20. (Exp.1). Effect of girdling and 60% shading on Pinot Noir flower ammonium concentration at three phenological stages at Tyee vineyard, Corvallis, Oregon. Error bars are standard errors of the means. Each treatment represents 10 vines.
Fig. 3.21. (Exp.1). Effect of girdling and 60% shading on Pinot Noir flower ammonium concentration at three phenological stages at Woodhall vineyard, Alpine, Oregon. Error bars are standard errors of the means. Each treatment represents 10 vines.
Literature Cited


Chapter 4

EFFECT OF GIRDLING AND SHADING ON SOLUBLE SUGARS AND ORGANIC ACIDS CONTENT IN RACHIS OF PINOT NOIR GRAPEVINE

Abstract

Soluble sugars, and organic acids concentration was measured in rachis tissue from mature, field-grown *Vitis vinifera* L. (cv Pinot Noir) vines that had been girdled before anthesis, 60% shaded prior to and during anthesis in 1989, or both. There was no significant effect of treatments on total soluble sugars concentration. However, girdling increased rachis dry weight 21% and 33% at full bloom and shatter, respectively. On the other hand, shading reduced rachis dry weight 27% at full bloom and 31% at shatter stage. Glucose was the major sugar during the three sampling periods. Shading reduced 30% total organic acids concentration at beginning bloom and 20% at full bloom. Tartaric was the predominant acid. A low concentration of α-ketoglutarate was determined in rachis tissue. Shading reduced α-ketoglutaric levels (p= 0.11). Reduction in dry weight and total organic acids by shading could be explained by reduced photosynthesis rate in leaves under shaded conditions.
Introduction

Inflorescence necrosis (INec), a disorder that occurs at any time from early flowering to early fruit set (Jordan, 1989), is responsible for reducing fruit set in grapes in Australia and New Zealand (Jackson and Coombe, 1988a,b), and Oregon (Jordan, 1989). With increased severity of INec the yield can be greatly decreased. Although INec can affect many cultivars, Pinot Noir appears to be very susceptible.

At present time no cause is known. Pathogens can not be isolated from necrotic tissue. Current hypothesis for a possible cause point at nutritional imbalance, specifically elevated ammonium levels. High rachis ammonium concentration has been associated with affected clusters after shading greenhouse plants (Lombard, pers.comm., 1988; Jordan, 1989).

Growing plants need to assimilate nitrogen, but excessive concentration of ammonium ions is toxic to plants. The toxic effect of ammonium to higher plants has been demonstrated by Maynard and Barker (1969), Magalhaes and Wilcox (1984), and Barker and Lachman (1986). Givan (1979) indicates that most of the ammonium accumulating in shoots results from ammonium internally generated from proteolysis of leaf protein, followed by deamination of amino compounds.
To prevent its internal accumulation, ammonium must be assimilated rapidly. Barker (1980), and Givan (1979) point out that carbohydrates and ketoacids are essential substrates for ammonium assimilation. They indicate that ketoacids, such as α-ketoglutarate, are fundamental for the initial complexation of ammonium. Thus, plants rich in sugars are able to supply the necessary ketoacids for the assimilation of ammonium into amides and other amino acids.

Girdling or ringing is a common practice in grapevines, which increase carbohydrates content in the parts of the vine above the girdle (Weaver and McCune, 1959; Weaver, 1976; Winkler et al., 1974). On the contrary, it is well established that natural or artificial shade reduce carbohydrates content in plant tissues (Kappel, 1989; Smart et al., 1988).

The purpose of this study was to evaluate the effect of girdling and shading on soluble sugars and organic acids content in rachis of Pinot Noir grapevines at three early stages of growth.
Materials and Methods

Plant Material and Experimental Design

Plant material was obtained from mature vines cv Pinot Noir at Tyee Wine Cellars vineyard, South Corvallis, Oregon. Single, fruitful shoot samples were collected randomly early in the morning. Leaves were stripped from the sampled shoots before transport back to the laboratory. Rachis tissue (main axis and branches) was separated from basal clusters from each shoot. Plant tissue was collected at three different stages: beginning bloom, full bloom, and shatter. Details about treatments, sampling date, and experimental design are described earlier in Chapter 3 (Expt. 1). The values reported are the means of three replications per treatment. The data were analyzed using analysis of variance for a factorial set of treatments distributed in a completely randomized design. Tukey's multiple range test was employed for means comparisons.

Carbohydrate and Organic Acid Analysis

Rachis tissue (main axis and branches) was cut in small pieces with a razor blade. Samples of 0.5 g fresh weight were extracted for 30 min in 5 ml 80% ethanol at room temperature after maceration in a Brinkmann
Homogenizer PT 10-35. Solids were removed with a glass fiber filter. The filtrate was stored for four months at 0°C.

Soluble sugars and organic acids from a similar extract were isolated by the following ion-exchange procedure (Akhavan et al., 1980). A cation-exchange 1 cm diameter column (9 ml Dowex 50W, 200-400 mesh, H⁺) and an anion-exchange 1 cm diameter column (11 ml Dowex 1-X8, 200-400 mesh, CH₃COO⁻) were connected in series. The extract (5 ml) was applied and the columns washed with distilled water until a final volume of 25 ml containing the sugars was collected. The eluate was stored at -1.0°C. Organic acids were recovered by treating the 1-X8 column with 25 ml 4 N formic acid.

Soluble carbohydrates and organic acids were analyzed by high performance liquid chromatography (HPLC). For carbohydrate determination, samples of 5 ml were taken to dryness at 60°C in an Organomation Analytical Evaporator. The samples were resuspended in 0.5 ml distilled water and membrane filtered (0.2 μm) before injecting 20 μl into the HPLC. The HPLC equipment used in this study was a Beckman system (Beckman Instruments, Berkeley, California) consisting of a Model 110A pump load, Altex Model 500 Autosampler injector, and a Bio-Rad Refractive Index (RI) Monitor set at range 32. Output was recorded on a Shimadzu Chromatopac C-R3A digital integrator. Soluble sugars were
separated on an Alltech Amino Column 600CH, 300 mm x 4.1 mm. The column water jacket heater was maintained at 25°C and 75% acetonitrile plus 25% water was used as the eluent at a flow rate of 1.0 ml/min.

Organic acid samples were taken to dryness in a vacuum oven overnight. The samples were dissolved in 0.5 ml of 0.002 N sulfuric acid, membrane filtered (0.2 μm) and 20 μl injected into the Beckman HPLC. Prepared samples were analyzed using an Aminex HPX-87H organic acid analysis column (Bio Rad Laboratories). The column was operated at 65°C using 0.002 N sulfuric acid as the isocratic mobile phase. The flow rate was 0.6 ml/min. Acids were monitored with a Hitachi model UV detector at 210 nm. Organic acid chromatograms were obtained from a Beckman Recorder working at 25 cm/h. Quantitation, either sugars and organic acids, was accomplished by comparison to authentic external standards treated in a similar manner. Organic acids were quantitated through peak height determination.
Results and Discussion

Soluble Sugars Content

High performance liquid chromatography (HPLC) can be effectively used to quantify various soluble sugars in plant tissue (McBee and Maness, 1983). The present study was carried out to determine the presence of glucose, fructose, and sucrose in rachis tissue of grapevine. A chromatogram for the standard is shown in Figure 4.1. It was prepared to detect fructose, sorbitol, glucose, and sucrose. Figure 4.2 illustrates a typical chromatogram from a sample. The peaks for fructose, glucose, and sucrose coincide with those from the standard. The peak with retention time 13.58 minutes indicates the presence of other sugar. Kliewer (1966) identified verbascose, stachyose, raffinose, maltose, and galactose besides fructose, glucose, and sucrose in different grapevine tissues at three growth stages.

Soluble sugar concentration (the sum of glucose, fructose, and sucrose content from the HPLC analysis) in rachis tissue is illustrated in Figure 4.3. No significant difference was found among treatments for each sampling stage. However, girdling increased soluble sugar concentration. Girdling is known to increase total available carbohydrates (sugars plus starch) above the
girdle (Weaver and McCune, 1959; Hale and Weaver, 1962; Roper and Williams, 1989). Analysis of only three reducing sugars may be insufficient to entirely determine the effect on total sugars content. Starch appears as a high contributor to total carbohydrates content in grapevines (Amerine and Root, 1960; Weaver and McCune, 1959; Roper and Williams, 1989), while starch contribution to total nonstructural carbohydrates content in leaves from girdled vines varied from 33.5% to 44.3% (Roper and Williams, 1989).

Perhaps a better demonstration of the girdling effect on total carbohydrates accumulation can be detected in rachis dry weight. Girdling increased rachis dry weight 20.8% at full bloom and 32.9% at shatter stage (Fig. 4.4). No significant difference was found at beginning bloom. Since girdling was performed only eight days before beginning bloom, insufficient time occurred to cause an effect on cluster development at this stage.

Also, Roper and Williams (1989) determined that carbohydrates accumulated mainly in leaves after girdling. While in the leaves, they are stored until are preferentially utilized by the vine's reproductive organs. They found that 15 days after girdling were imposed, total carbohydrates content was higher in leaves from girdled than ungirdled vines. In the meantime, no difference among treatments was found in cluster and stem carbohydrate
content.

The rachis soluble sugar concentration at shatter time was less than at earlier stages except for the girdled-shaded treatment (Fig. 4.3). Hale and Weaver (1962) point out that flower clusters have limited power to compete with growing tip shoots and young leaves for photosynthates. Flower clusters continue to be weak sinks until berry set occurs (about ten days after full bloom). After that, the cluster becomes a powerful sink.

It has been demonstrated that natural or artificial shading reduces carbohydrates content in plant tissue (Kappel, 1989; Smart et al., 1988). However, no significant reduction was found in the rachis in this study with shading treatments (Fig. 4.3). Starch and other nonstructural carbohydrates can be important contributors to the total carbohydrates content. However, significant difference in dry weight was found between rachis from shaded and no shaded vines which may account for a dilution effect (Fig. 4.4). Shading reduced 31.8%, 27.1%, and 31.3% rachis dry weight at beginning bloom, full bloom, and shatter, respectively. Negative influence of shading on leaves dry weight has been indicated by Kappel (1989), and Marini and Sowers (1990). Leaves growing under shade conditions present a reduced dry weight, indicating a low photosynthetic capacity and a reduced supply of carbohydrates to other organs (Kappel, 1989).
The amounts of glucose, fructose, and sucrose in rachis tissue at three different stages are given in Figure 4.5. At all three stages glucose was present in higher amount than fructose and sucrose. The same trend was found in all treatments. Glucose concentration was four to seven times that of fructose, and three to four times that of sucrose. A similar pattern was found by Kliewer (1966) in different parts of the grapevine sampled at green stage. He also determined about equal amounts of glucose and fructose in peduncle at ripening stages. In the same tissue, sucrose increased during the season.

Free glucose and fructose in grapevines are believed to be produced by hydrolysis of sucrose (Winkler et al., 1974). Kliewer (1966) postulates that the presence of more glucose than fructose during the green stages could be a consequence of hydrolysis of starch reserves into glucose, conversion of fructose into glucose, or preferential metabolism of fructose.

**Organic Acids Content**

A standard chromatograph for organic acids (Bio-Rad Laboratories, 1979) obtained under similar conditions as used in this study is shown in Figure 4.6. This was the same acid separation achieved by Schneider et al. (1987) in a standard mixture solution of grape musts and wines. From
the latter work and the elution position of organic acids indicated in Table 4.1 (extracted from Bio-Rad Laboratories, 1979), the unknown peak in Figure 4.6 was identified as fumaric acid.

The HPLC chromatogram of a rachis tissue sample is shown in Figure 4.7. The retention times for the various acids are in Table 4.2. The position of α-ketoglutaric acid is corroborated by the information in Table 4.1. It appears that under the conditions used in this experiment, succinic and glutaric acids can not be separated (peak 6 in Fig. 4.6, and peak 8 in Fig. 4.7). The presence of both acids was demonstrated in various grapevine tissues by Kliewer (1966). Schneider et al. (1987) were unable to separate succinic acid from shikimic acid in grape juices and wines, despite the wide range of solvent strengths tested.

Oxalic, α-ketoglutaric, citric, tartaric, malic succinic (and maybe glutaric), fumaric, acetic, and traces of levulinic and propionic acids were identified in rachis tissue of grapevine. All of them, except acetic, levulinic, and propionic, were also identified in different parts of Vitis vinifera L. (cv Thompson Seedless) through paper chromatography (Kliewer, 1966).

The total organic acids concentration in rachis tissue at three stages of growth is shown in Figure 4.8. At beginning bloom and full bloom stages, organic acids level
in rachis from shaded vines was 30.3% and 19.8% lower than rachis from exposed plants, respectively. Reduction in acids concentration due to shading was also observed at shatter time, although there was no significant difference. Girdling had no significant effect on acid content, but at full bloom and shatter, girdling show higher values compared with ungirdled treatments.

Reduction in organic acids concentration under shading conditions can be associated to shortage of carbon skeleton substrates. Nobel (1983) points out that two factors are decreasing photosynthesis rate in shaded leaves; the first is the fairly small amount of radiation photosynthetically active radiation reaching them, and the second is an increase in stomatal resistance because the stomata generally are partially closed at low light intensity. Reduced capacity for photosynthesis in leaves under shaded conditions has also been demonstrated by Kappel (1989), and Marini and Sowers (1990).

Tartaric, malic, and citric were the major acids found in rachis tissue (Fig. 4.9). Tartaric was the most abundant acid except under shading at beginning bloom. Tartaric concentration was 1.7 to 3.3 times that of malic and 5.6 to 9.3 times that of citric. A similar relationship was obtained by Kliewer (1966) in peduncle and pedicel tissues sampled at green stage. Oxalic, acetic, and fumaric (Fig. 4.10), and α-ketoglutaric (Fig. 4.11), comprise another
group of acids present in less abundant amount in rachis tissue of grapevine. Oxalic was the predominant acid in this group. Kliewer (1966) indicates that there are at least twenty seven organic acids in grapevines, which suggests the presence of several metabolic cycles.

The concentration of α-ketoglutaric acid is shown in Figure 4.11. Its level is low in rachis tissue, compared with the other acids (Figs. 4.9, and 4.10). There was no significant effect of treatments on α-ketoglutaric concentration at any of the three stages. However, the values obtained in this experiment reveal a trend of lower α-ketoglutaric levels with shading treatments (p= 0.11). On the other hand, girdling showed a trend of increasing acid content with and without shading. Also, α-ketoglutaric could be assimilated in the control and girdling treatments during bloom (decrease of the acid concentration), while it was not in the shaded treatments. Givan (1979), Oaks (1985), and Bowman and Paul (1988) indicate that ammonium assimilation requires a good availability of carbohydrates. They supply both an energy source and carbon skeletons. The lack of α-ketoglutaric assimilation in shaded treatments could suggest a low availability of energy source. This ketoacid is essential for ammonium assimilation into amides and other amino compounds in plant tissues (Barker, 1980; Givan, 1979; Oaks, 1985). Thus, its presence can reduce ammonium accumulation and in this way avoid toxic effects.
Organic acids concentration and rachis dry weight were decreased by shading. In the same way, inflorescence necrosis (INec) occurrence and rachis ammonium concentration were increased by shaded treatments (see chapter 3). This suggests that carbohydrates are playing a key role in ammonium assimilation. Low availability of soluble sugars due to shading could cause ammonium accumulation, and in turn toxicity.
Table 4.1. Elution position of organic acids.

<table>
<thead>
<tr>
<th>Acid</th>
<th>K'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyceric</td>
<td>0.040</td>
</tr>
<tr>
<td>Oxalic</td>
<td>0.067</td>
</tr>
<tr>
<td>Maleic</td>
<td>0.280</td>
</tr>
<tr>
<td>α-Ketoglutaric</td>
<td>0.360</td>
</tr>
<tr>
<td>Citric</td>
<td>0.467</td>
</tr>
<tr>
<td>Tartaric</td>
<td>0.547</td>
</tr>
<tr>
<td>Malic</td>
<td>0.813</td>
</tr>
<tr>
<td>Succinic</td>
<td>1.240</td>
</tr>
<tr>
<td>Fumaric</td>
<td>1.533</td>
</tr>
<tr>
<td>Acetic</td>
<td>1.800</td>
</tr>
<tr>
<td>Levulinic</td>
<td>2.093</td>
</tr>
<tr>
<td>Propionic</td>
<td>2.280</td>
</tr>
</tbody>
</table>

The conditions were: eluent, 0.0045 N H$_2$SO$_4$; flow rate, 0.8 ml/min; column, Aminex HPX-87; column temperature, 50°C; detector, UV at 210 nm.
Table 4.2. HPLC retention time (RT) of organic acids from Pinot Noir rachis tissue.

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>RT (min)</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8</td>
<td>Oxalic (main axis), plus Glyceric and Maleic</td>
</tr>
<tr>
<td>2</td>
<td>8.2</td>
<td>α-Ketoglutaric</td>
</tr>
<tr>
<td>3</td>
<td>8.6</td>
<td>Citric</td>
</tr>
<tr>
<td>4</td>
<td>9.2</td>
<td>Tartaric</td>
</tr>
<tr>
<td>5</td>
<td>10.8</td>
<td>Malic</td>
</tr>
<tr>
<td>6</td>
<td>11.6</td>
<td>Unknown, possibly Galacturonic</td>
</tr>
<tr>
<td>7</td>
<td>12.9</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>13.7</td>
<td>Succinic, plus Glutaric</td>
</tr>
<tr>
<td>9</td>
<td>15.6</td>
<td>Fumaric</td>
</tr>
<tr>
<td>10</td>
<td>17.3</td>
<td>Acetic</td>
</tr>
<tr>
<td>11</td>
<td>20.2</td>
<td>Levulinic</td>
</tr>
<tr>
<td>12</td>
<td>20.8</td>
<td>Propionic</td>
</tr>
</tbody>
</table>

The conditions were: eluent, 0.002 N H₂SO₄; flow rate, 0.8 ml/min; column, Aminex HPX-87; column temperature, 65°C; detector, UV at 210 nm.
Fig. 4.1. Chromatogram for standard solution of fructose, sorbitol, glucose, and sucrose. Concentration is given in $\mu g \times 20 \, \mu l$ of solution.
Fig. 4.2. Chromatogram showing quantitative amounts of fructose, glucose, and sucrose in rachis tissue of grapevine. Concentration is given in \( \mu g \times 20 \mu l \) of sample solution.
Fig. 4.3. Effect of girdling and 60% shading on total soluble sugars concentration in rachis tissue of Pinot Noir grapevines at three stages of growth, Tyee vineyard, Corvallis, Oregon. Total sugars is the sum of glucose + fructose + sucrose from HPLC analysis.
Fig. 4.4. Effect of girdling (A) and 60% shading (B) on Pinot Noir rachis dry weight at three stages of growth, Tyee vineyard, Corvallis, Oregon. Each value represents the mean of 3 observations. Mean separation by Tukey's multiple range test, 5% level.
Fig. 4.5. Effect of girdling and 60% shading on glucose, fructose, and sucrose concentration in rachis tissue of Pinot Noir grapevine at beginning bloom, full bloom, and shatter stages, Tyee vineyard, Corvallis, Oregon.
Peaks - (1) Oxalic, (2) maleic, (3) citric, (4) tartaric, (5) malic, (6) succinic + glutaric, (7) acetic, (8) levulinic and (9) propionic.

Fig. 4.6. Standard chromatogram for organic acids. Column, Aminex HPX-87. Eluent, 0.0026 N H₂SO₄. Column temperature, 65°C. Flow rate, 0.8 ml/min. Detector, UV at 210 nm (extracted from Bio-Rad Laboratories, 1979).
Fig. 4.7. Organic acids chromatogram of rachis tissue: 1 = oxalic (plus glyceric and maleic), 2 = α-ketoglutaric, 3 = citric, 4 = tartaric, 5 = malic, 6 = unknown, 7 = unknown, 8 = succinic (plus glutaric), 9 = fumaric, 10 = acetic, 11 = levulinic, 12 = propionic.
Fig. 4.8. Effect of girdling and 60% shading on total organic acid concentration in Pinot Noir rachis at three stages of growth, Tyee vineyard, Corvallis, Oregon. Each bar represents the mean of 3 observations. Mean separation by Tukey's multiple range test, 5% level.
Fig. 4.9. Effect of girdling and 60% shading on tartaric, malic, and citric acids concentration in rachis tissue of Pinot Noir grapevine at three stages of growth, Tyee vineyard, Corvallis, Oregon.
Fig. 4.10. Effect of girdling and 60% shading on oxalic, acetic, and fumaric acids concentration in rachis tissue of Pinot Noir grapevines at three stages of growth, Tyee vineyard, Corvallis, Oregon.
Fig. 4.11. Effect of girdling and 60% shading on α-ketoglutaric acid concentration in rachis tissue of Pinot Noir grapevine at three stages of growth, Tyee vineyard, Corvallis, Oregon. Each point represents 3 replications. There was no significant difference among treatments (p < 0.05).


**Table A.1.** Effect of shoot density on photosynthetic photon flux density (PPFD), received on the clusters at bloomtime in the canopy of Pinot Noir vines from 11 AM to 2 PM, 29 June. Tyee vineyard, Corvallis, Oregon, 1990.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PPFD (µE m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 shoots/m</td>
<td>42.6</td>
</tr>
<tr>
<td>10 shoots/m + 2 extra canes</td>
<td>41.0</td>
</tr>
<tr>
<td>24 shoots/m</td>
<td>37.7</td>
</tr>
<tr>
<td>24 shoots/m + 2 extra canes</td>
<td>39.0</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table A.2. Effect of girdling and 60% shading on ammonium concentration (ppm) in xylem exudate from Pinot Noir grapevines at Tyee vineyard, Corvallis, Oregon, 1989.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ppm</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.22</td>
<td>0.05</td>
</tr>
<tr>
<td>Girdling</td>
<td>0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>Shading</td>
<td>1.04</td>
<td>0.17</td>
</tr>
<tr>
<td>Shad-Gird</td>
<td>0.79</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ppm</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.20</td>
<td>0.04</td>
</tr>
<tr>
<td>Girdling</td>
<td>0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Shading</td>
<td>0.75</td>
<td>0.12</td>
</tr>
<tr>
<td>Shad-Gird</td>
<td>0.71</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Table A.3. Effect of girdling and 60% shading on ammonium concentration (ppm) in xylem exudate from Pinot Noir grapevines at Woodhall vineyard, Alpine, Oregon, 1989.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ppm</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.26</td>
<td>0.01</td>
</tr>
<tr>
<td>Girdling</td>
<td>0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Shading</td>
<td>1.15</td>
<td>0.58</td>
</tr>
<tr>
<td>Shad-Gird</td>
<td>1.00</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Full Bloom (18 June, 1989) (ppm NH₄⁺-N Fresh Weight)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ppm</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>Girdling</td>
<td>0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>Shading</td>
<td>0.96</td>
<td>0.50</td>
</tr>
<tr>
<td>Shad-Gird</td>
<td>0.83</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Shatter (29 June, 1989)