THE IMPACT OF SOURCE AVAILABILITY ON CLUSTER MORPHOLOGY OF PINOT NOIR GRAPEVINES

By

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ABSTRACT

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Pinot noir has tight clusters and thin-skinned berries that are extremely prone to bunch rot diseases in the cool and wet Michigan climate. It was hypothesized that leaves removed at full bloom will significantly reduce fruit set and berry number and will result in a significant decrease of cluster compactness and bunch rot severity at harvest. The experiment was arranged as a randomized complete block design with leaf removal (LR) as a categorical factor with five levels of defoliation and conducted in two years. Removal of the leaves from 8 or 10 nodes (LR-8, LR-10) resulted in a decreased number of berries per cluster and percentage of fruit set. Rot severity was reduced with LR-4 and LR-6 in 2011. In 2012, previous year defoliation reduced the size of inflorescences and their number per vine. During fruit set, sources for a cluster development were provided from the same shoot while long distance translocation of sources did not affect fruit set. Compensation in higher leaf photosynthetic rate occurred only in LR-10 and it was not sufficient to increase fruit set.

To my father.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 LITERATURE REVIEW

1.1 Grape Production in Michigan

With 69,170,000 t produced in 2012, grapes are an important component of global fruit production (OIV, 2012). Of the total amount of grapes produced in 2011, 32% was used for fresh consumption, 2% for raisins and 66% for wine production. USA ranks 10th as a wine producer in the world, generating 18,740,000 hectoliters (OIV, 2012), with California being the most important state producer (3,871,000 t of grapes crushed for wine), followed by Washington (142,000 t), New York (54,000 t), Oregon (34,500 t), Pennsylvania (11,300 t) and Michigan (6,400 t) (USDA, 2012a).

Due to challenging environmental conditions for grape production (e.g. winter cold, spring frost, short growing season, and low heat accumulation), the majority of the cultivars grown in Michigan belong to *Vitis labrusca* L. and mixed-species hybrids, which are cold hardy and disease resistant and are mainly used for juice grape production and only partially for wine production. In fact, in the early 1970's in Michigan 95% of the wines produced were made from Concord, Niagara, or Delaware grape cultivars (Sabbatini, 2012). In 2011 less than 5% of Michigan wine is made from these cultivars. This historical change in less than 40 years was driven by several factors mainly related to market demand and Michigan State University and private cultivar trials. In 2011, there were 4,897 and 1,072 ha planted to juice and wine grape varieties, respectively (USDA, 2012b). European varieties (*Vitis vinifera* L.) are the major grape

varieties used now for wine production, and the most important in the state today are Riesling, Pinot noir, Chardonnay, Pinot gris, and Cabernet franc (USDA, 2012b).

Pinot noir is the most important red variety for the Michigan industry. It is an early ripening and cold-hardy *Vitis vinifera* L. and thus an attractive cultivar choice for cool climate vine growing regions (Reisch et al., 1993). For this reason, it is the most commonly planted red cultivar in Michigan with 95 ha in production (USDA, 2012 b). However, this cultivar has several viticultural challenges such as small and tight clusters with thin-skinned berries, which in the interaction with the cool and wet Michigan climate makes it extremely prone to bunch rot diseases. Therefore, to avoid great loss of yield due to cluster fungal infection, Michigan growers are often forced to harvest grapes before they reach fruit technological maturity and that, of course, negatively impacts quality.

1.2 Botrytis Bunch Rot and Disease Management Strategies

1.2.1 Botrytis Bunch Rot (*Botrytis cinerea*)

Botrytis cinerea is a necrotrophic plant pathogen, which attacks 200 crop species worldwide (Williamson et al., 2007). This pathogen thrives in the cool and wet Michigan climate and has the potential to significantly reduce both yield and fruit/wine quality by causing Botrytis bunch rot.

In the spring, fungal infection may occur on grapevine buds and shoots turning them brown and making v-shaped or irregular brown areas on the leaves (Isaacs et al., 2003). During flowering, the fungus penetrates the flowers through the two possible infection sites: a receptacle area located at the base of the stamen (Keller et al., 2003), and the style (McClellan and Hewitt, 1973). Infection is followed by quiescence during which the pathogen is present but disease symptoms do not occur (McClellan and Hewitt, 1973) due to the physical and chemical defenses of the host plant (Keller et al., 2003).

When fruit ripening commences, natural host defenses decline (Holz et al., 2003) allowing the pathogen to penetrate into the tissues. Airborne conidia may penetrate the berries directly or through wounds caused by insects, hail, wind or micro-cracks on the cuticle induced by berry swelling (Williamson et al., 2007). Mycelia growing on aborted flowers and calyptras (flower caps) may also cause late-season infection (Nair and Parker, 1985). The infected berry splits, turns brown and then shrivels, while the pathogens rapidly spreads to adjacent berries in tight clusters where moisture is trapped. These tight, damp clusters are an attractive growing environment causing rapid growth of the pathogen and the resulting yield loss and deteriorating fruit quality (Keller, 2010).

1.2.2 Management Strategies for Botrytis Bunch Rot

Chemical control for *Botrytis* bunch rot is a necessary vineyard management strategy, which usually targets critical phenological periods (e.g., bloom, bunch closure, veraison and pre-harvest). Prior to 1990, all fungicides used for Botrytis control can be grouped into three classes: the anilinopyrimidines, phenylpyrroles, and hydroxyanilides and their mode of action are either to inhibit fungal respiration, osmoregulation, microtubule assembly, or sterol biosynthesis (Rosslenbroich and Stuebler, 2000). In commercial vineyards, fungicides of different chemical groups should be always rotated to avoid the build-up of genetic resistance in the fungi.

A more sustainable approach for *Botrytis* control with biological agents has been developed recently in response to the development of new fungicide-resistant strains of *B. cinerea*. This class of alternative biological control agents are microbial antagonists and naturally occurring chemicals that can induce resistance (Elmer and Reglinski, 2006). Although highly efficient under low-to-moderate disease pressure conditions, they are rarely used in commercial production because of the inconsistent disease control in field conditions (Elmer and Reglinski, 2006).

Conditions that favor *Botrytis* development are moisture and high humidity (90%), reduced light and moderate temperature between 15 and 20°C (Verfoeff et al., 1988). Therefore, management strategies that can improve canopy microclimate through choice of trellis system, shoot positioning, shoot thinning and leaf removal also suppress the pathogen development (Savage and Sall, 1984; Gubler et al., 1987; English et al., 1989; Zoecklein et al., 1992). Since the pathogen is saprophytic and overwinters in vine pruning debris (Thomas, 1983)

and other necrotic tissues (Fowler et al., 1999), removing the old clusters and dead wood would reduce the amount of spring inoculum.

Grape varieties with tight clusters are especially prone to botrytis bunch rot. Increased cluster compactness results in greater contact area between berries, leading to water retention in the clusters and delayed berry drying after rain. The resulting high humidity and wetness favor *Botrytis* germination and infection. Dry and Thomas (2003) pointed out that grape cluster compactness is highly correlated with susceptibility to bunch rot in several wine grape cultivars. According to Marois et al. (1986), the contact skin area between berries has a thinner cuticular membrane and less epicuticular wax, making the area more susceptible to *Botrytis* infection compared to non-contact areas.

Therefore, the reduction of cluster compactness can reduce bunch rot incidence by improving within-cluster microclimate conditions. Currently, there are two approaches to achieve this objective: the use of gibberellic acid and early leaf removal. Gibberellic acid (GA) has been used in the pre-bloom period and inducing rachis elongation on numerous wine and table grape varieties (Hed et al., 2011) or the bloom period via reduction in the number of set berries due to the pollenicide effect of GA (Weaver and McCune, 1960). However, the application timing and dosage have to be strictly controlled in order to achieve a successful result. Inappropriate GA application increases the proportion of berries without seeds known as shot berries and negatively influences bud fruitfulness by promoting tendril formation and inhibiting branching during the process of inflorescence initiation and differentiation (Keller, 2010).

Early leaf removal is a novel approach for altering cluster architecture and, thereby,

manipulating cluster size and fruit composition. This will be discussed in more detail below.

1.3 Early Leaf Removal

Fruiting zone (the region of the canopy where fruits are located) leaf removal from berry set until veraison has been a common management practice applied in cool and humid growing areas to improve air circulation and spray penetration and, thus, reduce bunch rot incidence (Zoecklein et al., 1992). This practice also improves bud fruitfulness (capability of a compound bud to develop inflorescences; May et al., 1969; Sommer et al., 2000) and fruit composition by exposing the buds and clusters to more sunlight. The effects of leaf removal on sugar, skin anthocyanins and phenolics, titratable acidity, malate, potassium, and herbaceous characteristics of some wine grape varieties were reviewed by Jackson and Lombard (1993).

Recently, leaf removal applied at pre-bloom and bloom proved to be a successful technique for changing cluster morphology and for reducing the crop level by affecting the source-to-sink ratio of a vine. The first attempt to use early leaf removal in controlling the yield of highly productive varieties with tight clusters was described by Poni et al. (2005). The authors reported that pre- and post-bloom defoliation of eight basal nodes reduced fruit set in field-grown Barbera and Trebbiano and, consequently, reduced cluster size due to the reduced number of berries. Also, sugar concentration in fruit of defoliated vines was found to increase as a result of the lower yield per shoot and increased leaf-to-fruit ratio compared to the control.

If mechanized, early leaf removal could potentially be a desirable substitute for labor-intensive cluster thinning. Intrieri et al. (2008) evaluated the effectiveness of mechanical vs. manual early leaf removal applied pre- and post-bloom on Sangiovese vines. Although mechanical defoliation removed 48% of the total leaf area removed with manual defoliation of

six basal nodes, the total remaining leaf area among treated vines did not vary significantly. Both methods of leaf removal reduced fruit set, yield per shoot, cluster weight, number of berries per cluster and cluster compactness, while soluble solid concentration and anthocyanins on a fresh-weight basis were increased compared to the foliated control. The study confirmed that mechanical early defoliation could regulate yield in a timely and cost-effective manner. However, other researchers reported early defoliation had a limited effect on overall Sangiovese berry composition (Kotseridis et al., 2012). The positive influence of mechanical early defoliation on yield reduction, grape composition and bunch rot control were confirmed on Graciano and Carignan grapevines (Tardaguila et al., 2010). Moreover, when compared with mechanical cluster thinning, as another method for yield control, early defoliation provided more consistent effects on Tempranillo grapevines (Tardaguila et al., 2012). Additional evaluation of early leaf removal on berry growth and morphology was performed in two varieties: Barbera and Lambrusco (Poni et al., 2009). Besides reducing fruit set and yield per shoot, pre-bloom defoliation of the first six basal leaves resulted in improved soluble solids and anthocyanins due to the increased leaf-to-fruit ratio and relative skin weight. The authors found that absolute skin and seed weight were correlated to berry weight, but relative skin weight was not related to berry size. The study suggested that early cluster exposure to light and/or temperature had a positive effect on skin growth and overcame the influence of defoliation as a source limitation, which usually reduces berry development. The positive effect of early berry exposure to sun on skin development was also reported for Graciano and Carignan grapevines (Tardaguila et al., 2010).

The benefits of early leaf removal were reported for several other cultivars: Semillon (Lohitnavy et al., 2010), Merlot, Cabernet Sauvignon (Kotseridis et al., 2012), and Ciliegiolo (Palliotti et al., 2012).

Pinot noir is especially known for a low anthocyanin profile (Mazza et al., 1999) and several investigations were done about the effect of leaf removal timing on polyphenol occurrence in the grape berry during maturation (Lemut S. et al., 2011; Lee and Skinkis, 2013) and the fate of color-related phenolics during the vinification process (Lemut S. et al., 2013). In those studies, early leaf removal promoted the synthesis of anthocyanins and flavonols in the grape berry and increased their content in wine.

Recently, a three-year study of defoliation timing on Pinot noir was conducted at Michigan State University (Sabbatini, unpublished data) with the objective to examine and compare the effect of pre-bloom, bloom and post-bloom removal of six basal leaves on fruit set and cluster compactness. The results suggest that pre-bloom and bloom defoliation were more effective in reducing fruit set and cluster compactness. However, even pre-bloom and bloom removal of six leaves seemed to be insufficient to induce a source limitation stress that would trigger a significant fruit set reduction every year. As a result, our study was designed to focus on the further manipulation of the level of defoliation intensity and then measure its specific effects on fruit set, cluster morphology and bunch rot incidence with emphasis on the beneficial effects of increased cluster sun exposure for anthocyanin and phenolic accumulation.

1.4 Flowering and Fruit Set in Grapes

1.4.1 Physiological Processes Preceding Flowering

Flowering and fruit set are the important phenological phases in the grapevine seasonal cycle that influence yield and fruit quality. Flower formation extends over two seasons and comprises the processes of inflorescence induction ("process by which the presence of one tissue influences the development of others"; Induction, 2013), initiation (formation of uncommitted primordium), and differentiation. Scarce information has been available about flower induction in grape. Gibberellin and cytokinin are cited as two major internal regulators of flowering (Vasconcelos, 2009). Some authors reported 18 and 20 days as a time span between induction and initiation (Buttrose, 1969; Lavee et al., 1967). During flower initiation the shoot apical meristem produces a structure called anlagen or uncommitted primordium. The fate of uncommitted primordium depends on environmental factors and the developmental stage in which the anlagen is formed (Boss et al., 2003). If formed within a compound or latent bud, anlagen will develop into inflorescence primordium, which will differentiate into inflorescence and flower parts and this process usually requires 9 to 12 months after the initiation phase occurs. However, on a rapidly growing shoot, anlagen differentiates into a tendril during the current season. The hypothesis that inflorescences and tendrils have the same origin was confirmed with molecular analyses of genes VFUL-L and VAP1 that were expressed throughout tendril and inflorescence development (Calonje et al., 2004).

In the early spring, the apical meristem on a growing shoot in a compound bud produces 3 to 5 leaf primordia and the first of three potential inflorescence primordia (Srinivasan and Mullins, 1976). In the current season, inflorescence primordia start to

differentiate into three parts: a bract primordium, abaxial, and adaxial lobes 5 to 7 weeks after bud burst, which coincides with the period of approximately 2 weeks before bloom or with the developmental stage when shoots have 11 to 22 leaves developed (Vasconcelos et al., 2009). The lobes produce branch initials by the beginning of winter dormancy and subsequent development ceases until bud swell (May 2004). For instance, a characteristic wing of a Pinot noir cluster is a product of the first anlagen's division, but under yet undefined circumstances, this branch may degenerate and abscise (Vasconcelos et al., 2009). Contrary to the statement of May (2004), Pinot noir inflorescences in cool climates do not enter endodormancy (Jones et al. 2009), which suggests that winter conditions may influence inflorescence size and thereby potential yield.

During inflorescence initiation and differentiation, cumulative radiation and heat experienced by the buds, as well as the availability of assimilates, water, and the nutrient status of the vine play important roles promoting inflorescence formation and consequently increasing bud fruitfulness for the following season (Buttrose, 1969; May et al., 1969; Candolfi-Vasconcelos and Koblet, 1990; Sommer et al., 2000). The major carbohydrate assimilate contributors to a bud are the leaves located on the same side of the shoot (Hale and Weaver, 1962). Compared to developing shoot tips, flowers and clusters, buds are a very weak sink and any restriction of carbohydrate assimilates during bloom has a negative impact on the number of inflorescences per bud (Candolfi-Vasconcelos and Koblet, 1990). Light impacts bud fertility by moderating the photosynthesis level and, thereby, assimilate availability, but it also has a direct influence (Vasconcelos et al., 2009). Temperature impacts the formation of the inflorescence

primordia, the number of flowers per inflorescence, and the number of inflorescences per shoot (Buttrose, 1969; Petrie and Clingeleffer, 2005).

In the spring of the following season, additional branching of overwintering meristems resumes, and the conical formation of the inflorescence become recognizable during bud swelling. The structure of the grape inflorescence is called a thyrse, which is “a conical panicle characterized by multiple branching without terminal flower” (May, 2004). It contains an inner and an outer arm. The inner arm is the main part of the structure that further includes lower order branches. The outer arm is also called a wing or shoulder and it may contain few or many branches. It may develop as a tendril or be absent. The rachis is the central stem around which the branches are arranged. Branches are attached to the rachis in pairs close to each other oriented in the opposite direction and making an angle of 90° with the next pair.

With the formation of final branches, over a time course of 2 weeks, initiation of flower parts occurs and flower meristems differentiate into flower organs in the following order: calyx, petals, stigma and carpels (Pratt, 1971). Along the branches, flowers are mainly organized as triads or dichasium in which one king or central flower is surrounded by two other flowers (May 2004). The size and developmental stage of each flower vary depending on its position within the inflorescence (May, 2000). Furthermore, flower position may play an important role in fruit set and berry fate (May, 2004). Therefore, the terminal position on the smaller branch may certainly ensure berry setting and provide better berry maturation. However, due to the restricted size of the vascular connection between the terminal berry and the rachis, the terminal position may produce berries with less sugar, color and flavor (May, 2004).

1.4.2 Flowering

Grape flowers are all morphologically hermaphroditic, but physiologically they can belong to one of three groups: functionally hermaphroditic, functionally pistillate and functionally staminate. The main parts of each flower are: five sepals, which make up the calyx; five petals, which are joined together into the calyptra and connected to the calyx; five stamens and the pistil (Figure 1.1). With filament elongation, the calyptra separates from the calyx and falls off, releasing the stamens and marking bloom. Cross-pollination occurs in grapes, but self-pollination is more prevalent and may occur before the calyptras fall (May, 2004). If flowers are pollinated but not fertilized they develop small live green ovaries (Friend and Trought, 2007). Without fertilized ovules, the flower may abort. Excessive abortion of flowers and ovaries is known as *coulure* and can occur for up to 4 weeks after anthesis (Keller, 2010). After fertilization, a maximum of four seeds could be present in every berry since each carpel contains two ovules (Vasconcelos et al., 2009).

Under favorable weather conditions, all flowers in a particular inflorescence open within 5-7 days (Keller, 2010). Figure 1.2 presents a Pinot noir inflorescence at the beginning of bloom. Cold and wet conditions prolong flower opening, while a temperature of 25 to 35°C provides the most intensive flowering (Vasconcelos et al., 2009). Within the inflorescence flowers at the base, and the largest ones open the first. Also, the inflorescence size, its position on the shoot and shoot location in the canopy influence the timing of flower opening (Vasconcelos et al., 2009).

1.4.3 Fruit Set and Factors that Affect It

According to May (2004), fruit set represents the "morphological and physiological change from ovary to berry". Fruit set is calculated as the ratio between the number of set berries and the number of flowers per inflorescence. The inflorescence size influences the percentage of fruit set; as size increases, the ratio declines, because clusters tend to hold more or less the same number of berries (Vasconcelos and Castagnoli, 2000). Average fruit set for grapevines is about 50%, but subsequent coulure due to physiological or environmental conditions can reduce fruit set values below 30% (May, 2004).

Among the factors that affect fruit set, carbohydrate availability plays an essential role. Firstly, carbohydrate status of the vine in the year preceding flowering (over-wintering reserves) directly influences inflorescence initiation and differentiation, conditioning the number of inflorescences and the number of flowers per inflorescence (Bennett et al., 2005). Reserves, which are mobilized during the early spring, are used to support not only the development of inflorescences but also shoot growth and thereby impact photosynthesis and assimilate status of a vine in the current year (Zapata et al., 2004). Flowering coincides with a very delicate period in the vine seasonal cycle when the depletion of reserves and the transition from heterotrophic to autotrophic allocation of nutrients occurs. This transition makes the process of fruit set additionally vulnerable to adverse environmental conditions (Zapata et al., 2004). For Pinot noir, the transition occurs at early bloom after which vine sinks become dominantly supported by leaf assimilates. For instance, the transition in Merlot occurs later (at pea berry size), making the variety dependent on root reserves longer and more prone to

flower abscission under challenging climatic conditions compared to Pinot noir (Zapata et al., 2004).

When reserves are depleted, permanent vine organs switch from source to sink and compete with inflorescences and growing shoots for photoassimilates. The strength of inflorescences as sinks and the amount of assimilates they import from neighboring leaves depends on inflorescence i.e. cluster developmental stage (Hale and Weaver, 1962). During the early intensive growth, the inflorescence is supplied with assimilates by proximal leaves (Hale and Weaver, 1962), especially by those on the inflorescence/cluster side of the shoot (Motomura, 1990). Ten to 14 days before bloom, the inflorescence growth rate declines and this corresponds to the decreasing sink strength of the inflorescence that will last until berry set (Hale and Weaver, 1962).

During the intensive growth, inflorescences import carbohydrates, and also generate new assimilated carbon for themself and are able to distribute a significant portion of it to the growing leaves (Vaillant-Gaveau et al., 2011). However, parallel to the decrease in growth rate, the chlorophyll content declines as well resulting in a substantial drop of inflorescence photosynthesis, which becomes practically negligible at fruit set (Lebon et al., 2005). After this stage, the inflorescence/cluster is mainly an assimilate importer. If the assimilate import is restricted at bloom by defoliation, poor fruit set and the abortion of flowers are inevitable (Coombe, 1959; Candolfi-Vasconcelos and Koblet, 1990). A high rate of berry drop might occur during the three weeks after bloom if the supply of organic nutrients to the inflorescences is reduced (Candolfi-Vasconcelos and Koblet, 1990).

Unlike other cultivars, Pinot noir can set more berries under favorable conditions, up to 65%, resulting in high cluster compactness (Lebon et al., 2004). The presence of starch in Pinot noir ovules and male sporogenous tissues may enable this cultivar to overcome environmental stress and achieve higher fruit set under challenging conditions (reduced photoassimilate availability). For coulure-susceptible varieties, viticultural methods could be applied to increase carbohydrate availability and subsequent fruit set such as trunk and shoot girdling, shoot topping, and the application of growth retardant (maleic hydrazide; Coombe, 1959; Caspari et al., 1998). For the purpose of improved fruit set, delayed pruning and the removal of excessive inflorescences are also suggested (May, 2004). Besides carbohydrate availability, environmental factors and nutrient supply could influence fruit set as well. Temperatures above 35°C (Kliewer, 1977) or below 15 to 17°C (May 2004) reduce fruit set by reducing pollination and fertilization.

Unlike other species, grapevines are relatively insensitive to the ratio of red to far-red light with almost no effect on inflorescence initiation (Morgan et al, 1985). No effect of direct light on fruit set was observed (May, 2000). Precipitation during bloom may impair fruit set by hindering cap-fall and preventing fertilization (May, 2004).

1.5 Objectives

The objective of the research was to investigate the possibility of modifying the cluster morphology of Pinot noir, reducing cluster compactness and, consequently, bunch rot severity at harvest. This study encompassed several experiments in order to answer the questions related to carbohydrate availability at bloom and its influence on Pinot noir fruit set. Firstly, we studied the intensity of early leaf removal at bloom that would result in a significant decrease of both fruit set and cluster compactness. Additionally, we monitored partitioning and distribution of assimilates produced by retained leaves along partially defoliated shoots and determined its contribution to the cluster at fruit set. Secondly, we investigated the influence of the long-term application of early leaf removal on Pinot noir's performance in the cool-cold growing climate in Michigan. Our final experiment was designed to investigate the possible translocation of assimilates from permanent vine structure components and foliated shoots to the cluster on defoliated shoots and its effect on fruit set.

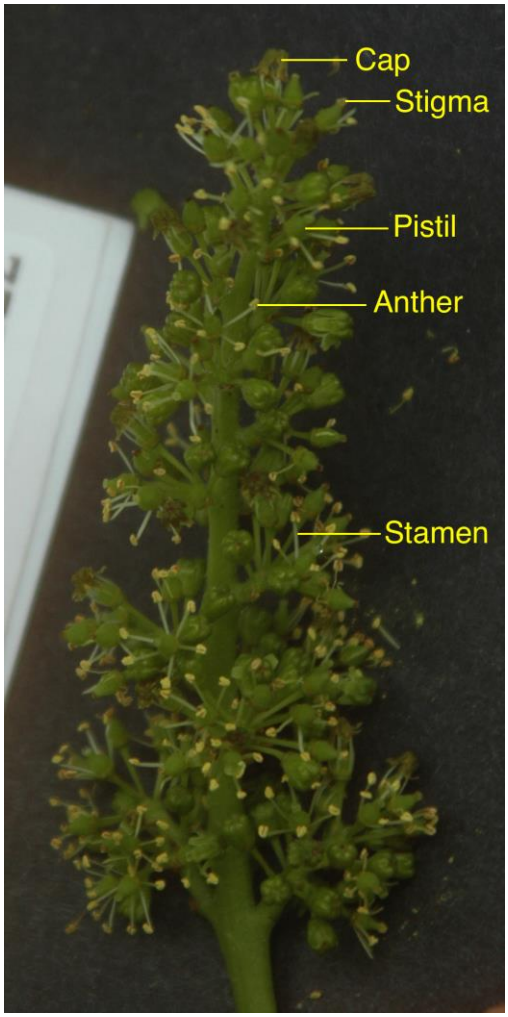

Figure 1.1 Flower parts: pistil, stigma, stamen, anther and cap. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Figure 1.2 Pinot noir inflorescence at the beginning of bloom. In this stage, a single flowers start shedding their caps (calyptras).

2 IMPACT OF EARLY DEFOLIATION ON FRUIT SET, CLUSTER MORPHOLOGY, BUNCH ROT AND FRUIT QUALITY OF PINOT NOIR CLONE 777 IN 2011

2.1 Introduction

Pinot noir is an early ripening and cold hardy red *Vitis vinifera* L. cultivar and thus an attractive choice for cool-climate vine growing areas (Reisch et al. 1993). For this reason, it is the most widely planted red cultivar in Michigan with 95 ha under cultivation (Michigan Department of Agriculture & Rural Development, 2011). However, the tight cluster morphology of Pinot noir and local weather conditions, e.g., rain, high humidity, favorable to bunch rot infections make growing this favorite in Michigan a real challenge for grape growers. Consequently, early harvest is often required before the ripening grapes reach technological maturity. More importantly, under favorable conditions for fungal infection, bunch rot rapidly spreads through a cluster causing significant yield reduction and compromising overall fruit quality.

In several scientific articles, early defoliation has been confirmed as an efficient tool to reduce cluster compactness, to reduce the spreading of bunch rot from infected to healthy berries, and to improve fruit quality and control of crop load in other varieties (Poni et al. 2006; Intrieri et al. 2008; Poni et al. 2008; Lohitnavy et al. 2010; Sabbatini and Howell 2010; Tardaguila et al. 2010; Tardaguila et al. 2012).

In a recent study on Pinot noir vines grown in Michigan, the effect of defoliation timing on fruit set and cluster compactness was tested in three consecutive years (Sabbatini, unpublished data). Compared to post-bloom defoliation of six basal leaves, pre-bloom and bloom defoliation were more effective in reducing fruit set and cluster compactness. However, even with pre-bloom and bloom treatment application, six leaves seemed insufficient to induce a source limitation stress that would trigger a significant fruit set reduction every year. This study raised a question: what level of leaf removal would result in a significant decrease of both fruit set and cluster compactness? In an attempt to answer the question, this work investigates the influence of early defoliation. We hypothesize that leaves removed at full bloom will significantly reduce fruit set, will do so to a degree correlated to the number of leaves, and will result in a significant decrease in cluster compactness.

2.2 Material and Methods

2.2.1 Plant Material and Experimental Design

The research was carried out in a 6-yr old vineyard of *V. vinifera*, cv. Pinot noir (clone 777 grafted on C3309 rootstock) during 2011. The vineyard was located at the Southwest Michigan Research and Extension Center (SWMREC; lat. 40°09′ N; long. 86°36′ W; elevation 220 m) near Benton Harbor, Michigan. Vines were planted in a Spinks loamy fine soil (U.S. Department of Agriculture, Soil Conservation Service, 1957), with a spacing of 1.8 m between vines and 3.0 m between rows, and trained to a vertical shoot positioning system (VSP). Vines were spur-pruned during the winter, leaving approximately sixty buds per vine. No additional shoot or cluster thinning was performed before treatment application. Recommended crop protection practices were followed, and the pest management program was based on scouting, experience and weather conditions. No sprays were applied during bloom time to avoid potential mechanical damage to flowers by the sprayer. A combination of fungicides and insecticides used for control were rotated to avoid resistance. Pertinent temperature data were recorded during the experiment by an automated weather station from the Michigan Automated Weather Network (MAWN) located 120 m from the experimental vineyard. Total monthly precipitation, daily precipitation, daily minimum, maximum, and average temperature and Growing Degree Days (GDD) calculated with the Baskerville-Emin method using a base temperature of 10°C (Baskerville and Emin 1969). When SWMREC's meteorology station was not operational, weather data were obtained from the nearest meteorology station in Scottdale, Michigan. No irrigation was used and standard summer vineyard practices were

applied. Shoots were trimmed with pruners (on July 25 on Day 206 of the year (DOY) when they reached 30 cm above the highest pair of catching wires.

The experiment was arranged in a randomized complete block design with one categorical factor, leaf removal (LR) with five levels of defoliation (Figures 2.1 and 2.2): no leaves removed (LR-0); leaves removed from 4 basal nodes (LR-4); leaves removed from 6 basal nodes (LR-6); leaves removed from 8 basal nodes (LR-8); and, leaves removed from 10 basal nodes (LR-10). Approximately 3 weeks before bloom, vines were organized in six blocks by the number of inflorescences, tagged, and then each treatment was randomly assigned to six vines. Additionally, a subsample of four shoots per vine was randomly chosen and tagged for detailed measurements of shoot length, degree of fruit set, cluster parameters, and fruit chemistry. Treatments were applied at full bloom, developmental stage 23 after Eichhorn and Lorenz (1977).

The timing of budburst, bloom, pea-size berries, and harvest were also recorded (see Table 2.1). The number of inflorescences and shoots per vine before treatment application were presented in Table 2.2.

2.2.2 Estimation of Leaf Area

Shoot length was measured weekly from two weeks before bloom up to one month after bloom. A sample of ten shoots, collected weekly from guard vines, was used for estimation of the total leaf area (LA) per shoot. Leaves removed using each defoliation level were collected in ziplock bags and transported to the campus laboratory. In the laboratory, total LA per shoot was determined by measuring the single LA with a leaf area meter (LI-

3050AHS, Lambda Instruments Corporation, Nebraska) and adding them up. A linear relationship between the LA per shoot (y) and shoot length (x): $y = 19.1x - 352.6$, $R^2 = 0.91$, was used for estimation of total LA per shoot (Figure 2.3). After defoliation, LA removed per shoot was measured and subtracted from total LA in a final calculation for the retained LA.

2.2.3 Estimation of Fruit Set

Basal cluster on each tagged shoot (n=120) were photographed in the field at developmental stages 20 (onset of bloom) and 31 (berries pea size), after Eichhorn and Lorenz (1977). Samples of twenty clusters at developmental stage 20 and twenty clusters at stage 31 from the guard vines were photographed in the field against a dark background and then separately collected in ziplock bags and transported to the laboratory. Using the same methodology described by Poni et al. (2006), the actual number of florets and berries were destructively counted. The number of florets and berries visible in the photos were counted using Microsoft Office Paint (Windows XP). The linear relationships between the actual number of florets (y) and the counted florets (x): $y = 2.03x$, $R^2 = 0.86$ (Figure 2.4); and actual number of berries (y) and counted berries (x) in the photos: $y = 1.50x$, $R^2 = 0.85$ (Figure 2.5) were used to estimate the initial number of florets and set berries of each basal cluster per tagged shoot.

The percentage of fruit set was expressed in two ways: the percentage of fruit set at developmental stage 31 (FS-31) and the percentage of fruit set at developmental stage 38, harvest (FS-38). FS-31 was calculated as the ratio between the estimated number of set berries

three weeks after bloom and the estimated number of florets. FS-38 was calculated as a ratio between the number of berries at harvest and the estimated number of florets.

2.2.4 Cluster Parameters and Morphology

After harvest, basal clusters from tagged shoots were collected and weighed. Berries were separated from the rachis and then total berry number, total berry weight, and rachis weight were recorded. Rachis length was calculated as the sum of the central axis length (inner arm), lateral wing or shoulder length (outer arm), and secondary branch length (if they were longer than 5 mm). The number of secondary branches on the inner and outer arms were also recorded. Cluster compactness was expressed in two different ways: as the ratio between the number of berries and rachis length (compactness index, CI) and as the ratio between the number of branches and rachis length.

2.2.5 Fruit Chemistry and Color Analysis

Basic fruit chemistry and color analysis were analyzed as described in Iland et al. (2004). Approximately 20 mL of juice was collected for soluble solids (^{o}Brix) analysis with an Atago PAL-1 refractometer (Kirkland, WA) and pH measurement with a Thermo Scientific Orion 370 pH meter (Beverly, MA). For total acidity (TA) determination, 10 mL of juice was titrated against a standardized 0.1*N* sodium hydroxide solution in an automatic titrator coupled to an auto sampler and control unit (Titroline 96, Schott, Germany) and expressed as g/L of tartaric acid equivalents. The remaining portions of the berries were briefly frozen and subsequently cold-ground with a tissue grinder (Model PT 10/35, Brinkmann Instruments Co, Switzerland).

Approximately 1 g of each sample was transferred to a 15 mL centrifuge tube. Anthocyanins and total phenolics of the berries were extracted in a 50% ethanol solution, pH 2, for 1 hr and then centrifuged at 20,000 rpm. One mL of supernatant was diluted into 10 mL of 1*M* HCl and stabilized for 3 hr. The absorbance of extracts were read at 520 and 280 nm on a spectrophotometer (UV-1800, Shimadzu, Japan) and expressed as mg of anthocyanins per gram of berry weight and absorbance units of phenolics per gram of fresh berry weight.

2.2.6 Yield Components

At harvest, yield per vine and the total number of clusters per vine were recorded. Harvest cluster rot was calculated and recorded as rot incidence (percentage of affected clusters per vine), where every cluster was considered to be affected if it was visually judged to have more than 2-3% of visible rot. Rot severity was calculated as a percentage of affected berries per tagged cluster. During winter pruning post-harvest, pruning weight per vine was recorded. Crop load, expressed as the Ravaz Index (RI, Ravaz, 1911), was calculated as a ratio between yield per vine and vine pruning weight.

2.2.7 Cluster Drying Speed

One week before harvest, samples of ten random clusters from LR-0 vines and 10 clusters from LR-10 vines were harvested and collected in ziplock bags. Clusters were transported to the laboratory where they were weighed, dipped in water, and weighed again. Wet clusters were hung on a metal rod and air-dried at room temperature. Drying rate was calculated as the difference in cluster weight, which was taken after 5, 10, 15, 45, 60, 90, 120,

150, and 180 min. For this purpose, complete dryness was defined as the moment when clusters had returned to their initial weight. To calculate cluster compactness, berries were separated from the rachis and the total numbers of both berries and branches were counted and the rachis length was measured.

2.2.8 Statistical Analysis

Data were analyzed using one-way ANOVA in PROC MIXED procedure, SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). Using the tagged shoots, measurements of shoot length were taken weekly and then analyzed using the REPEATED statement function in PROC MIXED.

Normality of the residuals was assessed by visual inspection of the normal probability plot and Kolmogorov test. Whenever the distribution of the residuals was found to significantly diverge from the normal distribution, data were subjected to either logarithmic or square root transformation. Homogeneity of variances was checked using the side-by-side box plot and Levene's test. Models with equal and unequal variances as well as models with different variance - covariance structures in repeated measurements were compared using the goodness-of-fit indicators. The model that showed the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) was used for further analysis. When the treatment effect was found to be statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the treatments were conducted using the t-test. When the treatment effect was not statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the treatments were conducted using Tukey's HSD.

Significance of linear regressions was checked using the Regression Wizard in the scientific data analysis and graphing software package Sigma Plot 11.0 (Systat Software, Inc., San Jose, CA, USA).

Table 2.1 Timing of developmental stage (bud burst, bloom, fruit set and harvest) in 2011, as a calendar date and day of the year.

Year 2011		
Developmental stage	Date	Day of the year
Budburst	May 10	130
Bloom	June 15	166
Pea size berry	July 7	188
Harvest	September 23	266

Table 2.2 Number of inflorescences and shoots per vine before treatment application in 2011.

Treatment[z]	Number of inflorescences per vine[y]	Number of shoots per vine
LR-0	77.3	79.3
LR-4	77.5	91.2
LR-6	78.7	97.2
LR-8	77.8	77.2
LR-10	74.8	88.0

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes.

[y] Means were based on 6 replicates.

Figure 2.1 Top: a vine with no leaves removed (LR-0); Middle: a vine with leaves removed from 4 basal nodes (LR-4); Bottom: a vine with leaves removed from 6 basal nodes (LR-6).

Figure 2.2 Top: a vine with leaves removed from 8 basal nodes (LR-8); Bottom: a vine with leaves removed from 10 basal nodes (LR-10). Photos were taken in fruit set in 2011.

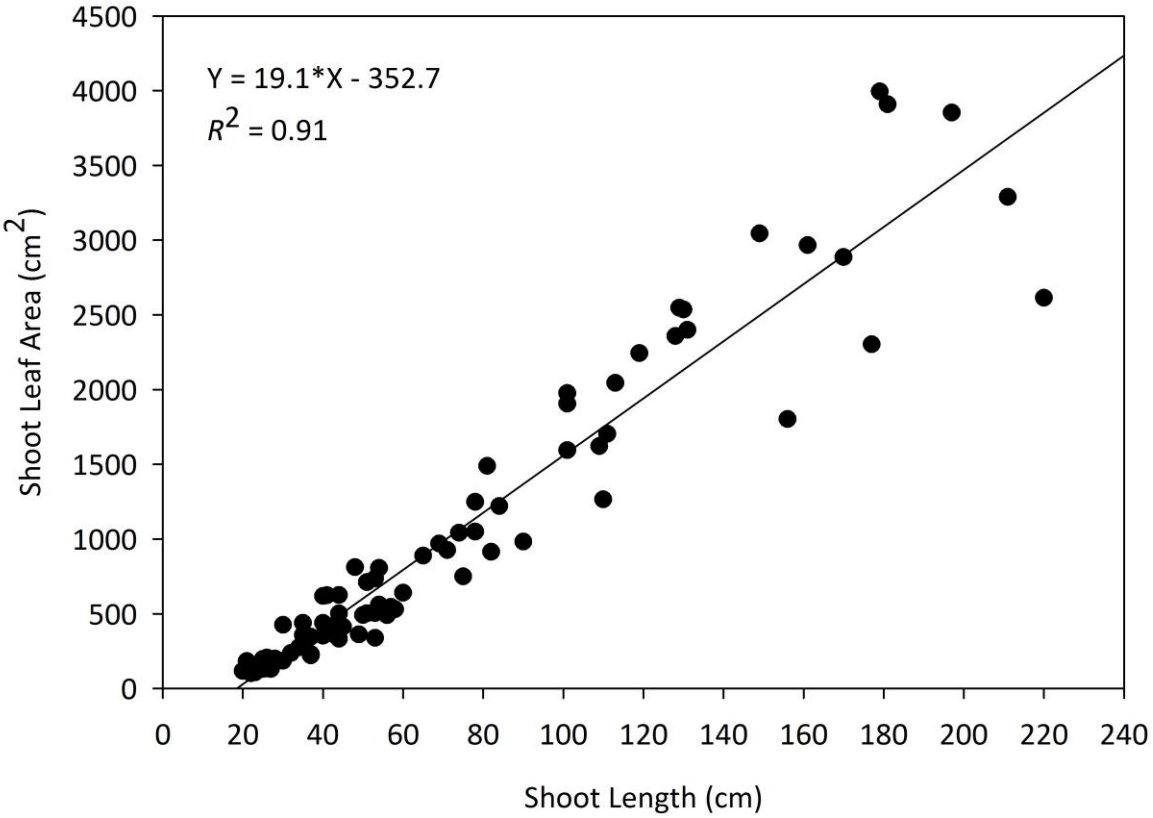

Figure 2.3 Linear correlation between shoot length (cm) and shoot leaf area (cm^2) established on 80 shoots, collected from May 31 to July 14, 2011.

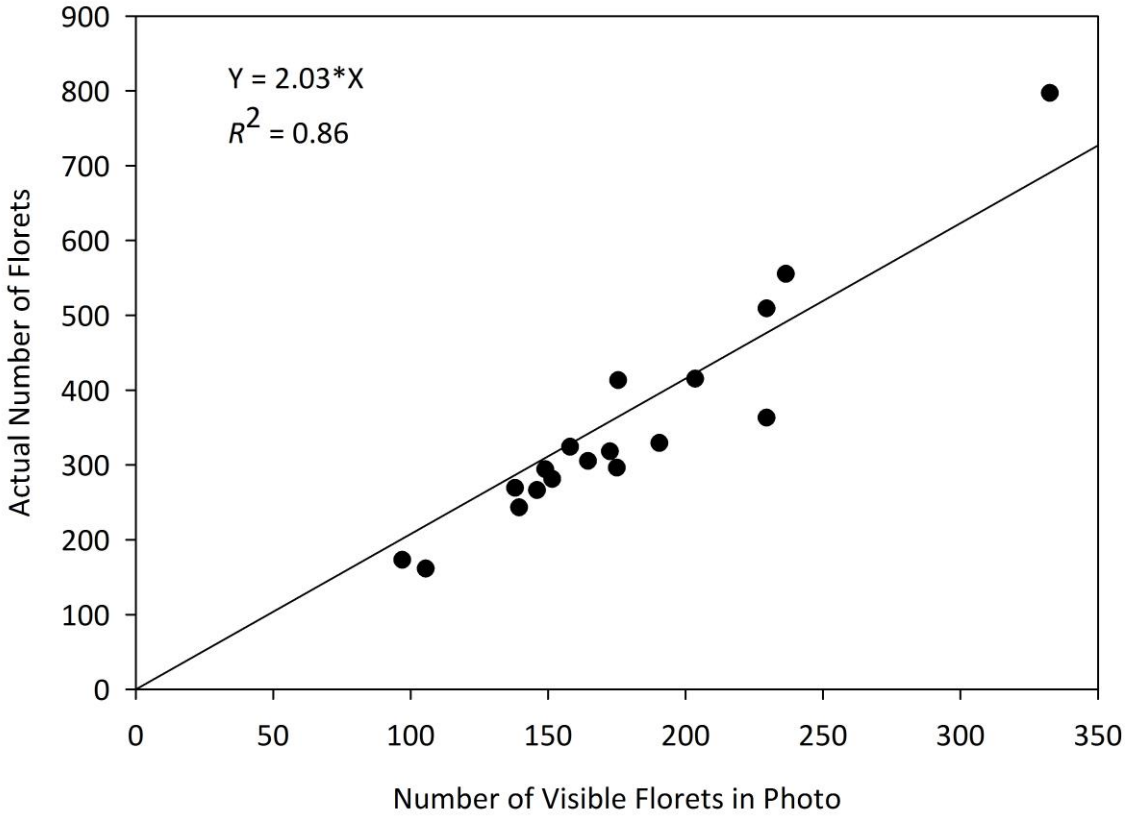

Figure 2.4 Linear correlation between number of visible florets in photos and number of actual florets per cluster based on a sample of 20 inflorescences collected at developmental stage 20, after Eichhorn and Lorenz (1977).

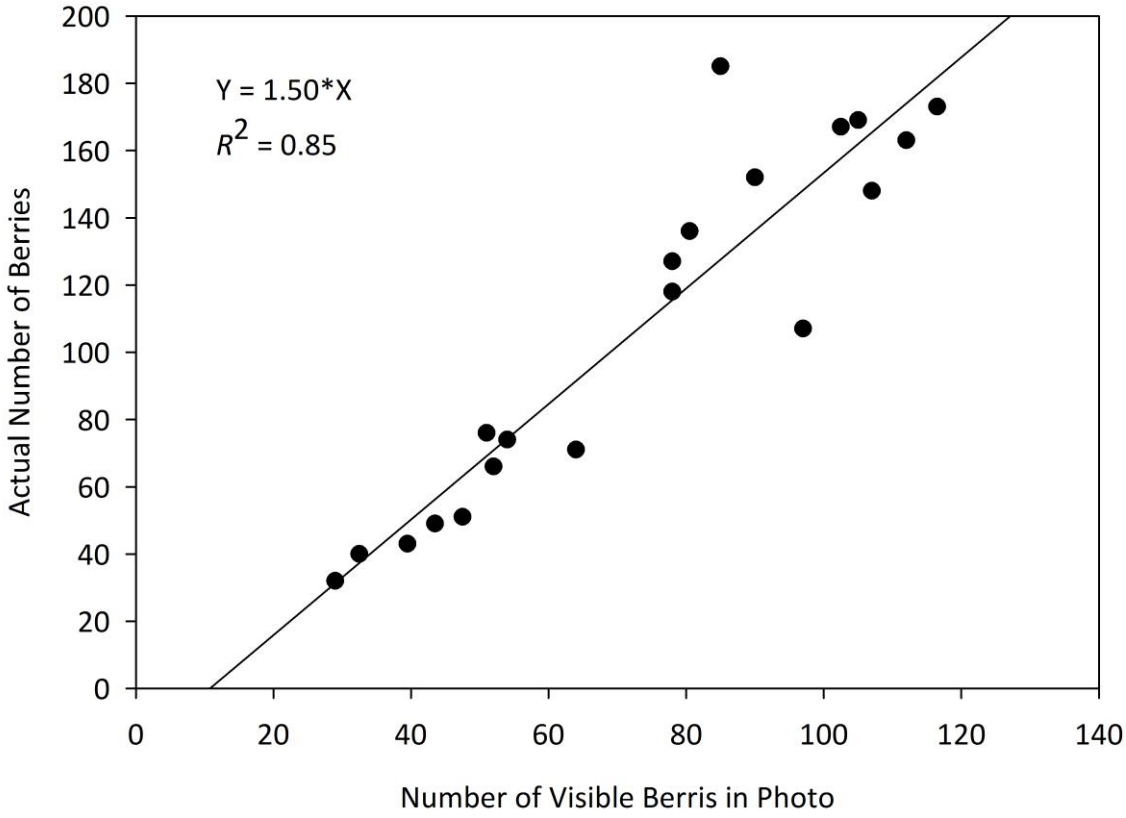

Figure 2.5 Linear correlation between number of visible berries in photos and number of actual berries per cluster based on sample of 20 clusters, collected at developmental stage 31, after Eichhorn and Lorenz (1977).

2.3 Results

2.3.1 Weather Conditions During the Growing Season of 2011

Provoked by warm days in April 2011, budburst occurred on May 10, early in relation to historical means (Enviro-Weather, 2013). However, the early growth was not followed by frost as usually happens in Michigan's climate. The lowest daily temperature of 2.8°C was recorded on May 16 and it was not harmful to young developing shoots. The maximum daily temperature of 35.5°C was recorded on July 20, which coincided with a lag phase in berry development (stage 2 after Coombe and McCarthy 2000). Additionally, this was the only day in the season with a temperature above 35°C. Therefore, 2011 was without temperature extremes that could impair vine growth and fruit development. However, in comparison to an average growing season at SWMREC, 2011 had a lower heat accumulation with only 1467 GDD. Total precipitation for the season was 592 mm (Figure 2.6), which corresponded to the historical precipitation mean in Michigan.

Over the period before bloom and to the conclusion of fruit set, maximum daily temperatures did not exceed 34.6°C. This maximum occurred on June 6, which was 8 days before full bloom. The daily minimum never dropped below 10°C. Over this period, rain occurred approximately twice per week for a total of 62.2 mm. The mean temperature fluctuated between 15 and 25°C, providing optimum conditions for flowering and fruit set (Figure 2.7).

Three weeks before harvest, mean temperature fluctuated from 10 to 29°C. The maximum of 33.9°C was recorded on September 1 and the minimum of 3.7°C occurred on September 16. Precipitation of 23.4 mm was distributed over seven rainy days during this period (Figure 2.8). The rain forecast for the last week of September prompted us to harvest on September 23. By doing so, we prevented bunch rot from spreading on the clusters.

2.3.2 Removed and Retained Leaf Area After Treatment Application

Immediately before defoliation, the total LA per shoot was the same among treatments (Table 2.3).

After defoliation, the most LA was removed in treatment LR-10, and then the treatments LR-8, LR-6, LR-4 followed it in descending order (Table 2.3). When retained LA is expressed as a percentage of total LA in the control (LR-0), all treatments significantly differed from each other with LR-4 having the highest percentage of retained LA (71%) and LR-10 having the lowest percentage of retained LA (14%).

2.3.3 Defoliation Impact on Shoot Growth

Shoot growth was measured from 2 weeks before bloom (May 31), until 4 weeks after bloom (July 13). As shown in Figure 2.9, over the 2 weeks before defoliation, at each measurement time, shoots had the same length. Even one week after defoliation (June 21), none of the treated vines differed from each other nor from the control. The first significant difference in shoot length was found on June 29, two weeks after defoliation, when treatment

LR-10 showed a lag in growth compared to LR-4, but not significantly different when compared to the control. During the following 2 weeks, LR-4, LR-6 and LR-8, were compensating for removed LA by increasing the shoot length and developing new leaves. Although not statistically different, these treatments had longer shoots than the control. On the other hand, defoliation of the 10 nodes was the threshold, above which vines could not compensate by investing in new foliage. Consequently, a month after defoliation (June 13), LR-10 showed reduced shoot growth resulting in significantly shorter shoots compared to all other defoliated vines. However, non-defoliated vines were not significantly different in shoot length from any of the defoliated vines (Figure 2.9).

2.3.4 Defoliation Impact on Retained Leaf Area

Immediately after the leaf removal treatments were applied, the untreated control, LR-4, LR-6, LR-8 and LR-10 treatments had 874.6, 627.3, 534.0, 332.6, and 128.8 cm^2 retained leaf area (LA), respectively (Table 2.4). Interestingly, LR-4 did not have significantly different removed LA compared to the control or LR-6. Over the next 2 weeks, LR-6 and LR-4 expanded LA by a considerable increment, which resulted in 1030.7 and 820.1 cm^2 retained LA, respectively. Leaf area development in defoliated vines provided LR-4 and LR-6 with statistically similar amounts of retained LA compared to the control after one month post-bloom. Also, the retained LA of LR-8 did not significantly change in comparison to LR-4 and LR-6. Only LR-10 showed a significant reduction in leaf area development compared to all other vines during the

entire period. Even at a month post-defoliation, the retained LA on LR-10 was comparable to the amount of leaves that were present on vines 1 week before bloom.

Immediately after defoliation and relative to the total LA, LR-4, LR-6, LR-8 and LR-10 had 72.0, 57.5, 36.6, and 17.9 percent of retained LA, respectively. One month later, LR-4, LR-6 and LR-10 had 82.8, 74.7 and 62.8 percent of recovered LA, respectively, while in contrast, LR-10 stayed below 50 percent of retained LA (Table 2.5).

2.3.5 Impact of Defoliation on Fruit Set

Using the linear correlation between the number of visible florets in the photos and the number of actual florets per cluster, we calculated the estimated number of florets per cluster. In 2011, the treatments and control showed variation from 389 to 442 estimated florets per cluster (Table 2.6).

Leaf removal at bloom and during fruit set caused significantly lower estimated numbers of berries per cluster in treatments LR-8 and LR-10 (Table 2.6). However, removing 4 and 6 leaves (LR-4 and LR-6) could not cause essential source reduction, so these treatments had statistically similar estimated numbers of berries per cluster compared to the control; specifically, the non-defoliated control, LR-4 and LR-6 had approximately 109 estimated berries per cluster. In contrast, the percentage of fruit set at developmental stage 31 (FS-31) of LR-8 and LR-10 resulted in an estimated average of 75 berries per cluster, a difference of 31%. The early defoliation caused significantly reduced FS-31 in LR-8 and LR-10 compared to the control, LR-4 and LR-6 (Table 2.6). The defoliation of 8 basal nodes seemed to be a limitation threshold

at which the vines were no longer able to compensate effectively, and this resulted in significantly lower FS-31 and, thus, fewer berries per cluster.

Comparing the estimated number of berries (Table 2.6) and the actual number of berries (Table 2.8), we noticed an additional drop in berry numbers, which occurred between developmental stage 31 and 38. That additional drop resulted in a 2, 13, 13, 25 and 40% decrease of berry numbers in LR-0, LR-4, LR-6, LR-8 and LR-10, respectively. With further decreased actual numbers of berries per cluster, all vines showed lower percentage of fruit set at developmental stage 38 (FS-38) compared to FS-31. Moreover, LR-0, LR-4 and LR-8 had significantly higher FS-38 than LR-8 and LR-10, but FS-38 did not significantly differ among LR-0, LR-4 and LR-8 (Table 2.6).

2.3.6 Change in Cluster Weight Caused by Defoliation

Early leaf removal caused significantly reduced cluster weights in all defoliated vines (Table 2.7). Treatments LR-4 and LR-6 had the same mean cluster weight, and significantly heavier clusters than LR-8 and LR-10. Practically, the vines with 8 and 10 defoliated nodes had clusters that were more than 55% lighter than the control.

The same reduction trend was observed in total berry weight and rachis weight (Table 2.7). In comparison to the control, all defoliated vines had lower berry and rachis weights. LR-4 and LR-6 had approximately 25% lighter total berries and rachises than control. The more severe defoliation in LR-8 and LR-10 caused an approximately 60% decrease in total berry weight and rachis weight compared to the control.

The tremendous reduction in cluster size was a result of fewer berries per cluster (Table 2.8) and not significantly reduced berry size (Table 2.7). However, there was a decreased trend in berry size, which corresponded to an increased amount of removed leaves.

The limitation of source availability during the early stages of cluster development did not significantly affect rachis length (Table 2.7). It was observed that LR-8 and LR-10 had slightly shorter rachises than other treatments regardless of the fact that the statistical analysis showed no significant differences among vines.

2.3.7 Early Defoliation Affected Cluster Morphology

Eight or more nodes defoliated at bloom resulted in reduced berry numbers at harvest (Table 2.8). Also, only removal of leaves from 8 and 10 nodes caused enough of a source limitation that branches on the rachis began to shrivel and drop off.

The early leaf removal resulted in decreased compactness index (CI) in the treatments LR-8 and LR-10 (4.4 and 3.6, respectively, Table 2.8). Additionally, the treatments LR-8 and LR-10 had in a lower number of branches per rachis length compared to the control and LR-4. However, even though the treatments LR-8 and LR-10 had a lower cluster compactness, they did not differ in rot severity compared to the control (Table 2.8). It is noteworthy that treatments LR-4 and LR-6 did show a significant decrease in rot severity even though there was no significant difference in cluster compactness when compared to the control.

2.3.8 Impact of Early Defoliation on Fruit Chemistry and Color

All defoliated vines had significantly higher soluble solids in the juice than the control, except for LR-6 (Table 2.9). The highest soluble solids, i.e., 24 ^{o}Brix, were found in the treatment LR-10. This treatment also showed significantly higher juice pH compared to other treatments and to the control. When compared to non-defoliated vines, a significantly lower TA was found in treatments LR-8 and LR-10, 5.4 and 5.9 g/L respectively (Table 2.9).

Although early leaf removal exposed Pinot noir clusters to the sun for the full season, a beneficial effect of defoliation on improved juice color was not found. No differences in anthocyanin content between the control and any of the defoliated treatments were found. On average, all vines had approximately 0.3 mg of anthocyanins per gram of berry fresh weight, which is relatively low for wine grapes but very common for Pinot noir (Table 2.9). However, the early defoliation of 10 basal nodes significantly increased phenolics, which amounted to 1.20 a.u./g compared to 0.95 a.u./g found in the control samples.

2.3.9 Yield Components

During harvest 2011, all clusters on a vine were counted and yield per vine was measured. Results showed that defoliated vines did not differ from the control in the total number of clusters per vine. A higher number of clusters per vine was found only in LR-6 compared to LR-10 (Table 2.10). However, early leaf removal successfully decreased yield per vine in treatments LR-8 and LR-10. Yield was 16.9, 14.7, 18.0, 11.2 and 7.5 t/ha for the control, LR-4, LR-6, LR-8 and LR-10, respectively. Since defoliation did not affect the number of clusters

per vine, we concluded that a significant yield reduction in treatments LR-8 and LR-10 was the direct consequence of the average of 55% decrease in cluster weight that was found.

While counting all clusters on the vines, we also counted the number of clusters with rot. Defoliation did not prevent rot incidence or rot severity in 2011 (Table 2.10). The percentage of rotted clusters varied from 11 to 14% overall per vine. This level of rot incidence was relatively low and likely a consequence of the reduced amount of precipitation from veraison to harvest.

Limitation of source availability at bloom did not have a significant effect on pruning weight (Table 2.10). The highest shoot mass was measured in LR-6, while LR-10 had the lowest pruning weight 0.3 kg. Also, statistical analysis indicated no differences in crop load, as shown by the Ravaz Index (RI) that ranged from 27.3 in LR-0 to 12.5 in LR-10 (Table 2.10).

2.3.10 Water Retention

Five minutes after complete wetting, clusters of LR-0 and LR-10 retained water that resulted in a 3.2 and 3.7% increase of their initial weight, respectively (Figure 2.10). During the next 10 min, clusters lost considerable amount of water and their weight was 1.5 (LR-0) and 1.2% (LR-10) heavier than their initial weight, respectively. Forty-five and 60 min after wetting, the drying rate was significantly higher in LR-10 than in LR-0. That led to a complete drying of LR-10 in which clusters reached their initial weight after 60 min. In contrast, clusters of LR-0 reached that state after 150 min. In other words, LR-0 clusters required a period 2.5 times longer than LR-10 to dry fully under laboratory conditions.

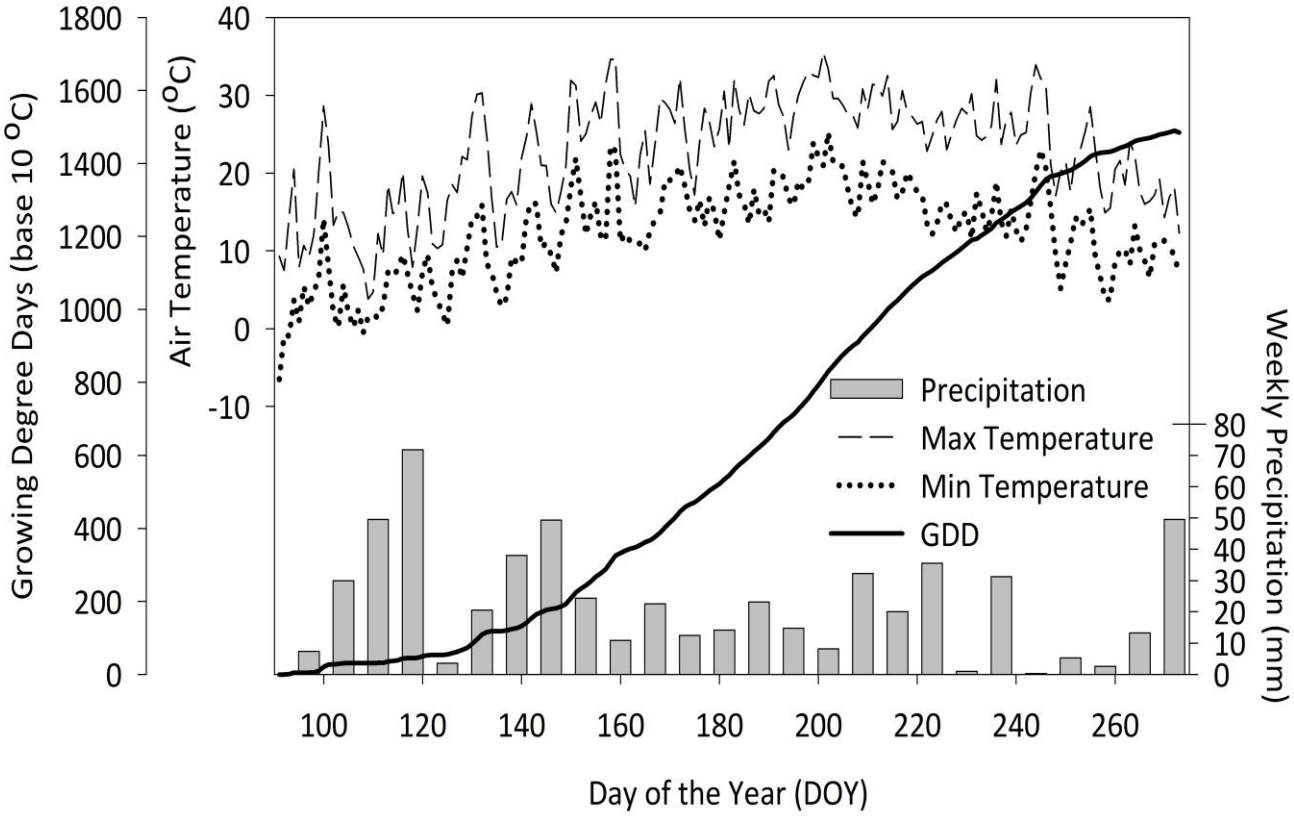

Figure 2.6 Weekly precipitation, minimum and maximum daily air temperature, and growing degree days from April 1st to October 1st in 2011, SWMREC.

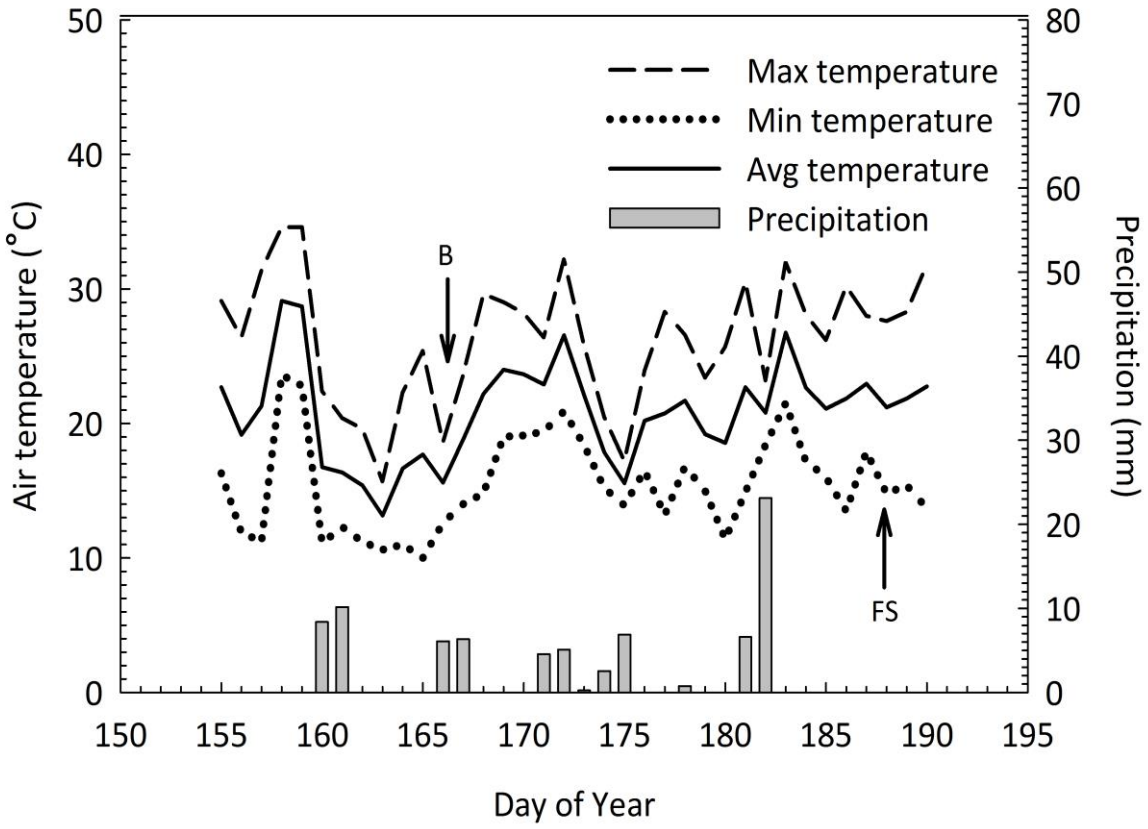

Figure 2.7 Daily precipitation and minimum, maximum and mean air temperature before bloom (B) and during fruit set (FS) in 2011, SWMREC.

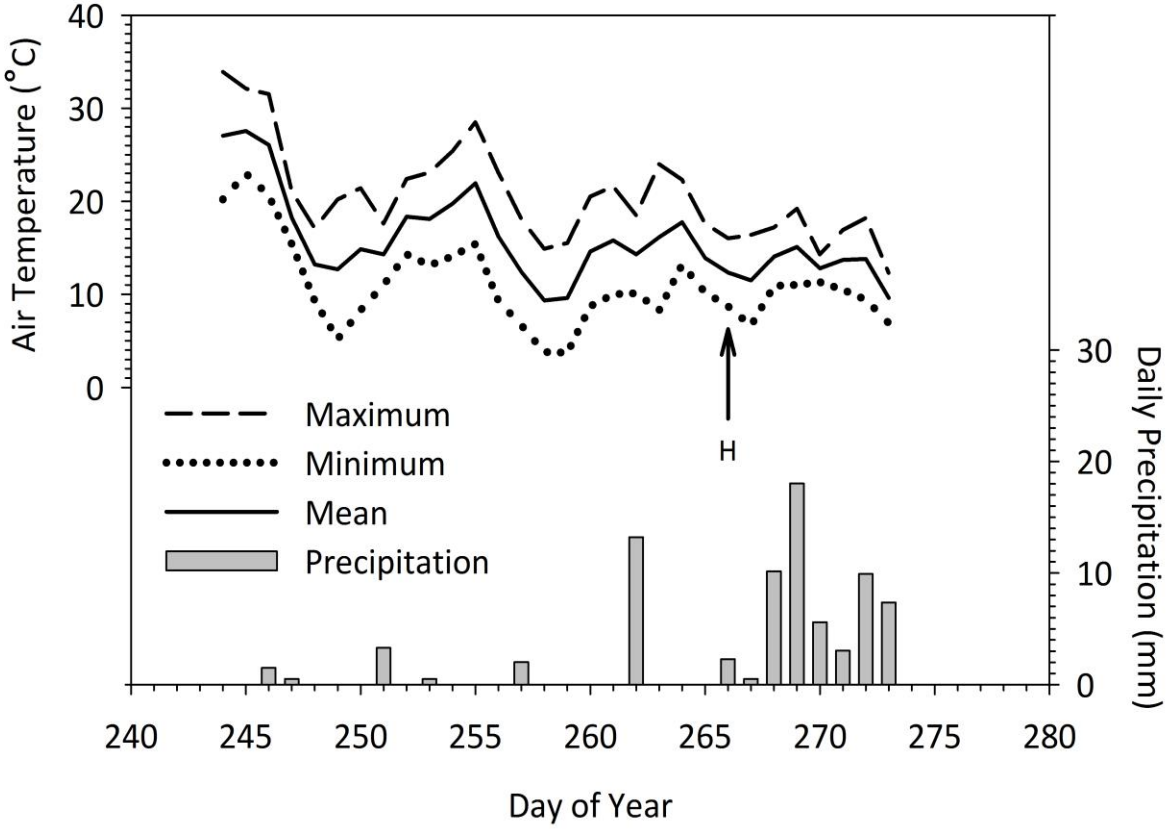

Figure 2.8 Daily precipitation and minimum, maximum and mean air temperature two weeks before harvest (H) in 2011, SWMREC.

Table 2.3 Total leaf area before treatment application; and, retained LA per shoot after defoliation in 2011.

Treatment[z]	Total LA[w] (cm^2)[y]		Retained LA (cm^2)		Retained LA as a percentage of the control (%)	
LR-0	811[x]	ns	811	a	-	
LR-4	809		576	b	71	a
LR-6	823		465	b	56	b
LR-8	814		275	c	35	c
LR-10	694		75	d	14	d

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within a column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] LA = Leaf area.

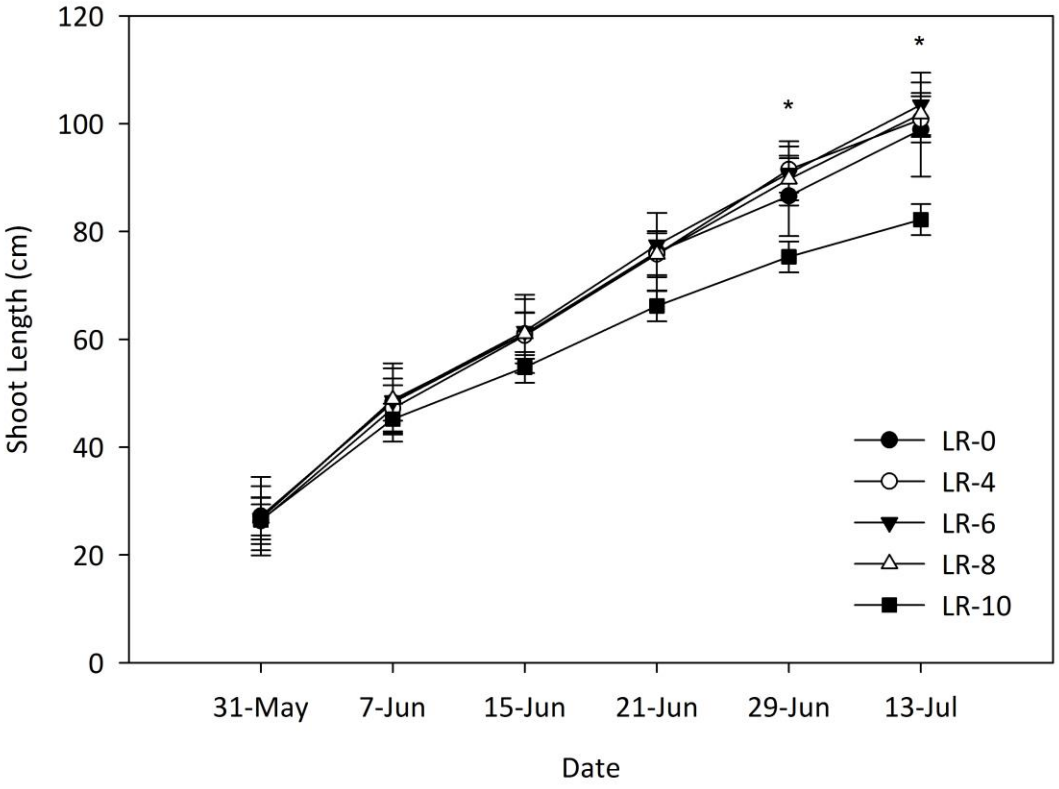

Figure 2.9 Effect of early defoliation on weekly shoot growth measured before and after treatment application in 2011. Means were based on 6 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SEM). LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes. Defoliation was performed on June 15 in 2011.

Table 2.4 Effect of early defoliation on retained leaf area before and after treatment application.

Treatment[z]	Retained leaf area (cm^2)[y]											
Day	May 31		June 7		June 15[w]		June 21		June 29		July 13	
LR-0	164[x]	ns	567	ns	875	a	1103	a	1381	a	1730	a
LR-4	144		546		627	ab	862	ab	1158	a	1252	ab
LR-6	162		571		534	b	774	bc	1031	ab	1272	ab
LR-8	160		582		333	c	552	c	820	b	1050	b
LR-10	157		512		129	d	292	d	465	c	593	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Defoliation was performed on June 15 in 2011.

Table 2.5 Effect of early defoliation on percent of retained leaf area compared to the non defoliated control before and after treatment application.

Treatment[z]	Retained leaf area (%)[y]							
Day	June 15[w]		June 21		June 29		July 13	
LR-4	72[x]	a	78	a	82	a	83	a
LR-6	57	b	66	b	72	b	75	b
LR-8	37	c	49	c	58	c	63	c
LR-10	18	d	31	d	41	d	46	d

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Defoliation was performed on June 15 in 2011.

Table 2.6 Early defoliation effect on number of berries per cluster and percentage of fruit set at developmental stages 31 and 38 in 2011.

Treatment[z]	Estimated number of florets per cluster[y]	Estimated number of berries per cluster		FS-31 (%)[w]		FS-38 (%)[v]	
LR-0	425.4	113.3[x]	a	27.1	a	26.5	a
LR-4	388.6	106.2	a	28.2	a	24.6	a
LR-6	441.8	106.1	a	24.6	a	21.6	a
LR-8	423.7	76.2	b	18.6	b	14.0	b
LR-10	442.5	73.2	b	17.5	b	10.6	b

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Percentage of fruit set, which is derived from berry number at developmental stage 31, after Eichhorn and Lorenz (1977).

[v] Percentage of fruit set, which is derived from berry number at developmental stage 38, after Eichhorn and Lorenz (1977).

Table 2.7 Impact of early defoliation on cluster size, berry weight, and rachis weight and length in 2011.

Treatment[z]	Cluster weight (g)[y]		Total berry weight (g)		Berry weight (g)		Rachis weight (g)		Rachis length (cm)	
LR-0	132.2[x]	a	124.7	a	1.08	ns	6.4	a	16.9	ns
LR-4	102.7	b	96.3	b	1.04		4.9	b	14.5	
LR-6	101.3	b	94.8	b	1.04		4.8	b	16.7	
LR-8	60.1	c	56.5	c	1.01		3.0	c	13.5	
LR-10	46.2	c	42.8	c	0.98		2.4	c	13.0	

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

Table 2.8 Impact of early defoliation on components of cluster morphology and bunch rot severity in 2011.

Treatment[z]	Actual berry number[y]		Branch number		CI[w]		Number of branches per rachis length		Rot severity[v] (%)	
LR-0	111.0[x]	a	16.6	a	7.0	a	1.0	a	7.8	a
LR-4	92.6	a	13.9	a	6.6	a	1.0	a	1.7	c
LR-6	91.8	a	14.0	a	6.1	a	0.9	ab	3.1	bc
LR-8	57.3	b	11.0	b	4.4	b	0.8	b	4.5	ab
LR-10	44.1	b	10.2	b	3.6	b	0.8	b	7.4	ab

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] CI = Compactness index expressed as number of berries per rachis length.

[v] Rot severity was calculated as a percentage of affected berries per tagged cluster.

Table 2.9 Impact of early defoliation on fruit chemistry and color content in 2011.

Treatment[z]	Soluble solids (°Brix)[y]		pH		TA[w] (g/L)		Anthocyanin (mg/g)		Phenolics (a.u./g)	
LR-0	20.9[x]	c	3.46	b	6.09	a	0.34	ns	0.95	bc
LR-4	22.2	b	3.44	b	5.87	ab	0.30		0.87	c
LR-6	21.9	bc	3.49	b	5.49	abc	0.29		0.86	c
LR-8	22.8	b	3.51	b	5.44	bc	0.35		1.12	ab
LR-10	24.0	a	3.69	a	4.95	c	0.37		1.20	a

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value ≥ 0.05 by t-test; ns = no significant difference.

[w] TA = Total acidity.

Table 2.10 Impact of early defoliation on yield components in 2011.

Treatment[z]	Number of clusters per vine[y]		Yield per vine (kg)		Yield per hectare (t/ha)		Rot incidence[w] (%)		Pruning weight (kg)		RI[v]	
LR-0	91.7[x]	ab	9.1	a	16.9	a	13.4	ns	0.4	ns	27.3	ns
LR-4	96.3	ab	7.9	a	14.7	a	12.3		0.6		17.8	
LR-6	107.0	a	9.7	a	18.0	a	11.5		0.7		14.1	
LR-8	92.5	ab	6.1	b	11.2	b	14.2		0.4		16.6	
LR-10	81.5	b	4.1	c	7.5	c	11.0		0.3		12.5	

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 6 replicates.

[x] Means within the column followed by the same letter are not significantly different at *p*-value ≥ 0.05 by t-test; ns = no significant difference.

[w] Calculated as the number of clusters with more than 2-3% of rot per total number of clusters.

[v] RI = Ravaz index, calculated as a ratio between yield per vine and the vine pruning weight.

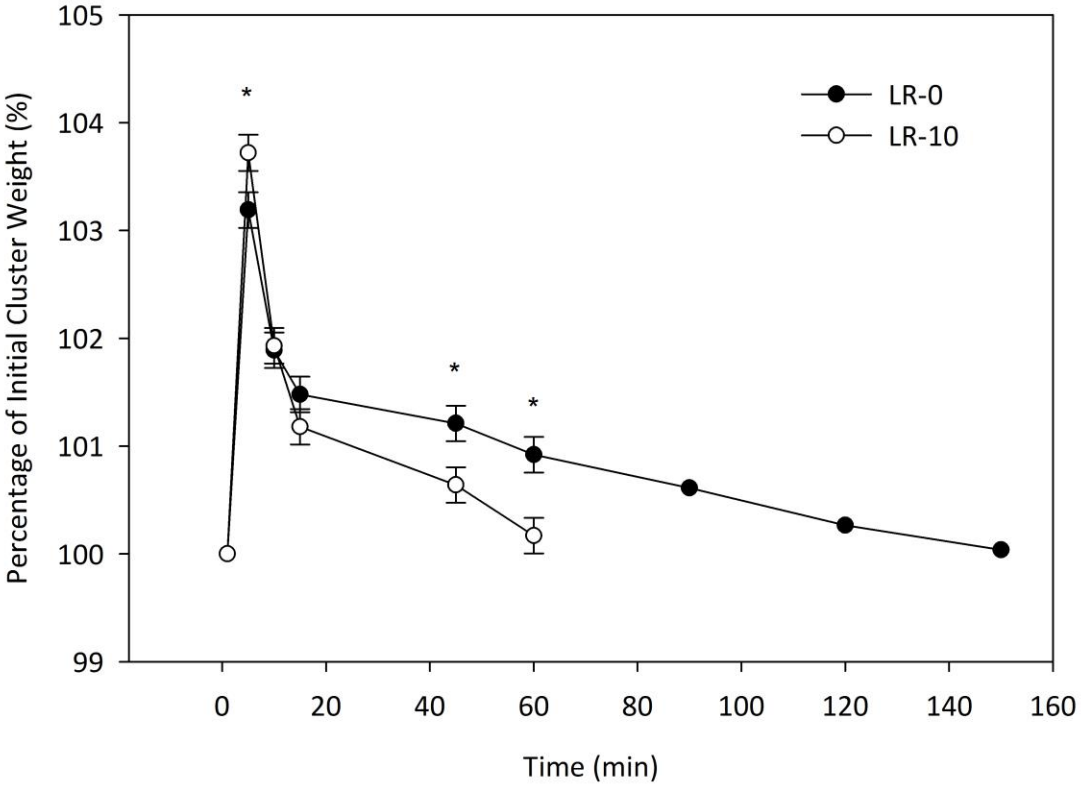

Figure 2.10 The rate of clusters drying after being dipped in water as the percentage of initial cluster weight plotted against the time interval required for clusters to return to their initial weight or to complete dryness. Means were based on 10 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE).

Figure 2.11 Pinot noir's clusters: LR-6 (above) and LR-10 (below) with dry florets and cluster branches at fruit set 2011, SWMREC.

2.4 Discussion

The development of a grape inflorescence depends on the presence of carbohydrates, which originate from reserves, leaves or the inflorescence itself (Morinaga et al., 2003; Vasconcelos et al., 2009; Vaillant-Gaveau et al., 2011). During bloom, the inflorescence not only generate new assimilated carbon for itself, but also a surplus that is distributed to the growing leaves (Vaillant-Gaveau et al., 2011). However, parallel to the substantial decrease in chlorophyll content, inflorescence photosynthesis declines and becomes negligible at fruit set (Lebon et al., 2005). From this stage forward, the inflorescence/cluster development relies mainly on what the leaf assimilates. The important role of supplying the cluster is assigned to the leaves on the adjacent nodes below or above the cluster's own (Hale and Weaver, 1962), with an emphasis that leaves on the cluster side of the shoot make a larger contribution (Motomura, 1990). If the supply of carbohydrates for the clusters is restricted at bloom by defoliation, poor fruit set and the abortion of fruitlets are inevitable (Coombe, 1959; Candolfi-Vasconcelos and Koblet, 1990). This was consistent with what we observed in our study where defoliated Pinot noir vines, on which leaves had been removed from 8 or 10 nodes, showed a considerably reduced percentage of FS-38. Also, early defoliation at 8 or more basal nodes resulted in the effective reduction of the number of berries per cluster at developmental stage 38.

Immediately after defoliation, our experiment showed that removed leaf area was negatively correlated ($r = -0.83$) with percentage of FS-38. Also, we found a strong negative correlation ($r = -0.81$) between the removed leaf area and the numbers of actual berries per cluster (Figure A.1). Additionally, there is 66 and 68% of total variation of actual berry number

and FS-38, respectively, which is explained by removed LA (Figure A.1). Similar coefficients of determination were found when the percentage of removed leaf area was correlated to both actual berry number and percentage of FS-38 (Figure A.3). Slightly lower coefficients of determination (0.60 and 0.63) were found when the actual numbers of berries per cluster and percentage of FS-38 were correlated with retained leaf area (Figure A.2). This suggested that removed and retained LA expressed either as an absolute or relative number were of similar importance to the actual number of berries per cluster and, thus, FS-38.

We also noticed that only defoliation at 8 or more nodes led to the significant reduction of estimated berry numbers and, consequently, to the reduced percentage of FS-31. Comparing FS-31 with FS-38, the control showed a slight decrease, whereas lower FS-38 was more prominent in defoliated vines. The existing difference between FS-13 and FS-38 in defoliated vines can be explained by the difference between the estimated and the actual number of berries (Table 2.4 and 2.6). Furthermore, comparing these two stages, control clusters showed little change in the number of berries from the pea size berry stage until harvest. On the other hand, every defoliated vine had clusters with fewer berries at stage 38 than at stage 31. Moreover, this difference in berry number was amplified as the number of defoliated nodes increased. Defoliation of 4 and 6 nodes caused an additional drop of approximately 14 berries per cluster, while clusters on shoots with 8 and 10 defoliated nodes lost 19 and 29 berries, respectively (Table A.1). Similar results were reported by Candolfi-Vasconcelos and Koblet, (1990). The intensive berry drop lasted over the second and third week after bloom. It continued further at lower intensity and finally stopped 6 weeks after bloom. Candolfi-Vasconcelos and Koblet (1990) pointed out the cessation of cell division in berries and the

beginning of cell differentiation as the likely reason that berry drop stopped after the sixth week.

Interestingly, we found that early defoliation did not impact mean berry weight (Table 2.7). Many authors reported a decrease in berry weight as the consequence of source limitation during the early stages of berry development (Poni et al. 2006; Intrieri et al. 2008; Poni et al. 2008; Lohitnavy et al. 2010; Tardaguila et al. 2010). Conversely, there are also reports that berry size may increase due to the compensation effect that promotes berries to reach full size (Tardaguila et al. 2010; Tardaguila et al. 2012). The restriction of source availability in our experiment was created at the point of full bloom and, thus, this restriction could hypothetically affect the first phase of fruit development, i.e. cell division. However, according to Keller (2010), the process of cell division is mostly under the control of genetic factors and that it is cultivar specific with environmental factors having a negligible effect. In contrast, the period of cell expansion is driven by environmental conditions. On July 13, the last time when shoot length was measured, the most extreme defoliation treatment resulted in approximately 66% less retained LA than the control. However, even that tremendous reduction in LA did not significantly reduce berry weight. Additionally, we found a weak correlation between removed leaf area and berry weight (Figure A.8). The final berry size is not only dependent on cell number and cell volume, but also on the soluble solids content (Candolfivasconcelos and Koblet 1990; Petrie et al. 2000; Palliotti et al. 2010). While the berry matures, soluble solids increase to a certain point after which any further increase is due to berry dehydration and shriveling, which in turn affects berry size (Keller 2010). In support, we found that berry weight in our research was strongly correlated (R^2 = 0.98) with soluble solids (Figure A.9).

The primary purpose of this research was the investigation of the effect of leaf removal on cluster compactness. While many researchers report cluster compactness, using the ratio between berry number or berry weight and rachis length, little is known about the effect of defoliation stress on rachis development. We found that early leaf removal did not significantly change rachis length. This could be due to the fact that rachis length was measured as the sum of central axis length, lateral wing, and secondary branch length if the secondary exceeded 5 mm. Therefore, any reduction of branch numbers would lead to a simultaneous reduction of rachis length. Additionally, we found a weak correlation between the percentage of removed leaf area and rachis length (Figure A.7). On the other hand, all levels of defoliation resulted in a significant rachis weight decrease (Table 2.7). The decrease of rachis weight was caused by a reduction in the number of branches per cluster (Table 2.7). In the grape inflorescence, flowers were grouped in the dichasium, which contains a central, king flower and two lateral flowers (May, 2004). A group of dichasiums attached together to the same base make a branch. In all likelihood, source limitation caused abortion of the flowers/berries that were weaker, i.e. the lateral when reducing the number of berries per branch, braches became lighter. In the extreme case, if a branch contained a couple of dichasia, the whole branch could dry and drop off, leading to fewer branches per cluster (Figure 2.11). The present experiment showed that early defoliation at 8 or more nodes could cause such a significant decline in branch numbers.

Although LR-8 and LR-10 reduced cluster compactness, none of them showed reduction in bunch rot neither rot incidence nor severity, likely due to the fact that these treatments were advanced in ripening and more soluble solids (Table 2.9) were present in the grape juice (Hill et al., 1981). Moreover, the weather conditions before harvest were not favorable for bunch rot

development. Therefore, even the control had a relatively moderate level of rot incidence and severity (Table 2.8 and 2.11).

Under the wet simulated conditions in the laboratory, clusters from non-defoliated vines showed lower drying rates (Figure 2.10). While LR-10 clusters required only 60 min to reach full dryness (initial weight), it took 150 min for LR-0 clusters to get to that condition. According to Vail and Marois (1991), cluster architecture plays an important roll in cultivar susceptibility to bunch rot, modifying the microclimate conditions at the berry's surface, i.e, tight clusters dry at a slower rate. These authors also point out that cluster weight makes the greatest contribution to cluster tightness; more than the compactness index (CI), which is often used to quantify cluster tightness. Per Vail and Marois, cluster tightness was estimated using a firmness tester equipped with an Ametek gage. In our research, we discovered that the time needed for the cluster to dry fully is correlated to both cluster compactness ($r = 0.66$) and total berry weight ($r = 0.61$), i.e., cluster weight (Figure A.11).

As shown in Table 2.5, all levels of defoliation significantly reduced cluster weight. This reduction in cluster weight was due to defoliation decreasing the number of berries per cluster, while mean berry weight showed little significant change (Table 2.7). We also found that the percentage of removed leaf area per shoot was strongly correlated to cluster weight ($r = 0.87$; Figure A.5). Furthermore, none of the defoliation treatments changed the number of clusters per vine compared to the control. We concluded that the yield per vine was considerably reduced in LR-8 and LR-10 only due to the reduction in cluster size. The control, LR-4 and LR-6 produced on average 16.5 t/ha, while LR-8 and LR-10 yielded 11.2 and 7.5 t/ha, respectively, which is still considered high (Table 2.10).

The limitation of source availability at bloom had an impact on shoot length (Figure 2.9). LR-10, from which 87% of LA had been removed, had shorter shoots than other defoliated vines but not the control (LR-0) on July 13. Hunter and Visser (1990) found no differences in shoot length, which was measured at veraison, when 0, 33 and 66% of LA was removed at berry set. Although, LR-10 had shorter shoots than other defoliated vines, early leaf removal did not have a significant effect on pruning weight (Table 2.10). However, the mass of the shoots that was removed by summer hedging was not taken into account. Therefore, we cannot speculate about the final contribution of shoot length to pruning weight. With no impact on pruning weight but significant yield reduction, more extensive leaf removal resulted in a lower RI. However, even with the most severe defoliation, yield was at such a level that the RI never dropped below 12.5 indicating that the vines were generally overcropped (Table 2.10).

High soluble solids in the grape juice are primarily caused by low yield (Figure A.10), meaning that the retained leaf area was sufficient to support cluster development and fruit ripening in the severely defoliated vines. As shown in Table 2.4, retained LA measured 1 month after defoliation in LR-10 was 66% less than in the control. At that time, basal leaves in the control were at least 30 days old and, thus, less photosynthetically active. Similarly, cluster weight of LR-10 was also 65% less than in the control (Table 2.7). Therefore, the ratio between retained LA per shoot and cluster weight was the same for LR-10 and the control. However, the leaves of LR-10 were younger and presumably more productive, which made a difference in the related fruit ripening stage. Statistical analysis showed that all defoliated vines had significantly higher soluble solids than the control, except LR-6 (Table 2.9). Interestingly, we observed that vines randomly chosen for treatment LR-6 were also the ones that had both the highest

number of clusters and yield per vine. In comparison to the control all other defoliated vines had lower yield due to source limitations, and, therefore, more assimilates were available per unit of fruit, which in turn resulted in higher ^{o}Brix.

Although early leaf removal exposed Pinot noir clusters to the sun for the whole season, a beneficial effect of defoliation on improved juice color was not found (Table 2.9). To find an explanation, it is worth noting that defoliation did not significantly decrease berry size, which would have lead to a higher skin to pulp ratio and thus higher anthocyanin content (Poni et al., 2006). Six leaves removed at berry set and veraison increased total anthocyanins in Pinot noir grown in Slovenia in two different locations (Lemut S. et al., 2011). The early defoliation at 10 basal nodes significantly increased phenolics (Table 2.9). Grape seeds are the major source of phenols in the fruit. Therefore, increased phenolic content in LR-10 might be a consequence of the seeds advanced ripening stage or/and a higher number of seeds per berry.

2.5 Conclusion

The purpose of this experiment was to investigate what level of leaf removal would result in a significant decrease of both fruit set and cluster compactness. The restricted supply of carbohydrates for the clusters at bloom caused with defoliation at 8 and 10 nodes, showed a considerably reduced percentage of FS-31 and FS-38. Also, early defoliation at 8 or more basal nodes resulted in the effective reduction of the number of berries per cluster at developmental stage 38. However, non of the imposed level of defoliation presented such a source reduction which would have an impact on the mean berry weight. We found that early leaf removal did not significantly change rachis length, but all levels of defoliation resulted in a significant rachis weight decrease.

Although LR-8 and LR-10 reduced cluster compactness, none of them showed reduction in bunch rot neither rot incidence nor severity, likely due to the fact that these treatments were advanced in ripening. Additionally, the weather conditions before harvest were not favorable for bunch rot development and even the control had a relatively moderate level of rot incidence and severity. Treatments LR-8 and LR-10 showed a reduction in the yield per vine, which was due to a reduction in the number of berries per cluster. Although early leaf removal exposed Pinot noir clusters to the sun for the whole season, a beneficial effect of defoliation on improved juice color was not found, but the early defoliation at 10 basal nodes significantly increased phenolics.

3 SUSTAINABILITY OF EARLY DEFOLIATION ON PINOT NOIR CLONE 777 IN 2012

3.1 Introduction

Leaf assimilates as a source are used to support growth and development of vine sinks, and to maintain basic metabolic activities of all organs. The excess of carbohydrates produced in leaves are converted into starch and stored in canes, trunks and roots. If the production of assimilates is restricted (e.g. reducing leaf photosynthesis or surface area), vine sinks will then compete for the limited amount of carbohydrates available.

The partitioning of assimilates depends primarily on sink strength, which is determined by the phenological stage of the given sink. For instance, flowers and young berries are weak sinks compared to shoot tips (Candolfi-Vasconcelos and Koblet 1990). However, as seeds develop clusters become a stronger sink that easily attracts available assimilates at the expense of growing shoot tips.

At the beginning of ripening, Pinot noir clusters have the capacity to attract stored reserves from perennial organs when the vines are under defoliation stress (Candolfi-Vasconcelos et al, 1994). The authors stated that defoliation at the onset of ripening led to the translocation of stored carbon from lower portions of the vines to the ripening fruits. Using a labeling technique for targeting the carbon reserves in the trunk and roots, Candolfi-Vasconcelos et al. (1994) found that 32% of the labeled carbon in the roots moved to the fruit

during the middle of the ripening period. In contrast, only 0.7% of the labeled carbon reserves were found in the fruit of control plants. Later defoliation approximately 3 weeks before harvest had no effect on labeled carbon partitioning.

Restricted availability of leaf assimilates affects the development of all vine organs. A study on potted vines showed that limiting leaf area from fruit set until harvest slightly reduced trunk and shoot dry weight. However, the reduction of leaf area had a significant effect on berry dry weight and decreased the dry weight of roots also (Buttrose, 1966). Reduced root growth may cause reduced water and nutrient uptake, which impairs development of the aboveground vine organs.

It is worth noting that at the beginning of the season permanent structures (roots, trunk, canes) act as a *source*, supporting bud burst and supplying growing shoots and undeveloped leaves with carbohydrate reserves stored over the winter. If the replenishment of the reserves in the previous growing season was affected by source limitation, insufficient reserves in the following spring would impact vine growth and fruit development. Pinot noir's vines that were exposed to different leaf removal treatments in the previous year showed a decline in berry size and an increase of shootless nodes on defoliated positions (Howell et al., 1994). Although these authors did not find any observable impact upon bud fertility, other research on leaf removal in Sultana vines found that, four weeks after bloom, the number of bunches per shoot were greatly reduced in the following season and the effect was more pronounced as defoliation was more severe (May et al., 1969).

The second year objective of our study was to investigate the influence of the long-term application of early leaf removal on the performance of Pinot noir in the cool growing climate in

Michigan. For this reason, we imposed the same level of defoliation on the same vines in two consecutive years. In that way we were able to monitor vine performance under continuous and consistent defoliation stress, measuring the growth parameters, assessing the fruit quality, and analyzing the yield components.

In the first year of our experiment, low humidity at harvest significantly reduced bunch rot infestation hiding the expected beneficial effect of reduced cluster compactness on disease incidence and severity. However, by expanding to two-year observations of treatment effect on fruit set and cluster compactness, we aim to see an interaction between cluster morphology, disease pressure, and environmental factors.

3.2 Material and Methods

3.2.1 Plant Material and Experimental Design

Pinot noir vines (clone 777 grafted on C3309 rootstock), which were used for early defoliation during 2011 in SWMREC, were pruned to 60 buds per vine during March 2012. The experiment in 2012 was arranged as a randomized complete block design with one categorical factor, leaf removal (LR) with five levels of defoliation: no leaves removed (LR-0); leaves removed from 4 basal nodes (LR-4); leaves removed from 6 basal nodes (LR-6); leaves removed from 8 basal nodes (LR-8); and, leaves removed from 10 basal nodes (LR-10). Approximately 3 weeks before bloom, a sub-sample of 4 shoots per vine was randomly chosen and tagged for detailed measurements of shoot length, degree of fruit set, cluster parameters, and fruit chemistry. Table 3.1 presents the timing of budburst, bloom, berry pea size and harvest. In 2012, described treatments were applied at full bloom, developmental stage 23 after Eichhorn and Lorenz (1977), on the exactly same plants as they were in 2011.

No additional shoot or cluster thinning was performed before treatment application. Recommended crop protection practices were followed and the pest management program was based on scouting, experience and weather conditions, except during bloom time to avoid potential mechanical damage to flowers by the sprayer. A combination of fungicides and insecticides used for control were rotated to avoid resistance. Pertinent temperature data were recorded during the experiment by an automated weather station from the Michigan Automated Weather Network (MAWN) located on the site at 120 m from the experimental vineyard. We tracked total monthly precipitation, daily precipitation, daily minimum, maximum, and average temperature, and Growing Degree Days (GDD) calculated with the Baskerville-Emin

method using a base temperature of 10°C (Baskerville and Emin 1969). On the few days when SWMREC's meteorology station was not operational, weather data were provided from the nearest meteorology station in Scottdale, Michigan. No irrigation was used and standard summer vineyard cultural practices were applied.

3.2.2 Estimation of Leaf Area

Shoot length was measured weekly, starting one week before bloom until June 19. A sample of ten shoots, collected weekly from guard vines, was used to estimate total leaf area (LA) per shoot. Leaves removed using each defoliation level were collected in ziplock bags and transported to the campus laboratory. In the laboratory, total LA was determined by measuring the single LA with a leaf area meter (LI-3050AHS, Lambda Instruments Corporation, Nebraska). A linear relationship between the LA (y) and shoot length (x): $y = 17.51x - 87.52$, $R^2 = 0.82$, was used for estimation of total LA (Figure 3.1). After defoliation, LA removed per shoot was measured and subtracted from total LA in a final calculation for the retained LA.

3.2.3 Estimation of Fruit Set

Each basal cluster per tagged shoot (n=120) was photographed in the field at developmental stage 20 (onset of bloom) and at developmental stage 31 (berries pea size), after Eichhorn and Lorenz (1977). From the guard vines, samples of twenty clusters at developmental stage 20 and twenty clusters at stage 31 were photographed in the field against a dark background and then separately collected in ziplock bags and transported to the

laboratory. Using the same methodology described by Poni et al. (2006), the actual number of florets and berries were destructively counted. The number of florets and berries visible in the photos were counted using Microsoft Office Paint (Windows XP). The linear relationships between the actual number of florets (y) and the counted florets (x): y = 1.48x, R^2 = 0.95 (Figure 3.2); and actual number of berries (y) and counted berries (x) on the photos: y = 1.38x, R^2 = 0.91 (Figure 3.3) were used to estimate the initial number of florets and set berries of each basal cluster per tagged shoot.

The percentage of fruit set was expressed in two ways: percentage of fruit set at developmental stage 31 (FS-31) and percentage of fruit set at developmental stage 38, harvest (FS-38). FS-31 was calculated as the ratio between the estimated number of set berries three weeks after bloom and the estimated number of florets. FS-38 was calculated as a ratio between the number of berries at harvest and the estimated number of florets.

3.2.4 Cluster Parameters and Morphology

After harvest, basal clusters from tagged shoots were collected and weighed. Berries were separated from the rachis and then total berry numbers, total berry weights, and rachis weights were recorded. Rachis length was calculated as the sum of the central axis length (inner arm), lateral wing or shoulder length (outer arm), and secondary branch length (if they were longer than 5 mm). The number of secondary branches on the inner and outer were also recorded. Cluster compactness was expressed in two different ways: as the ratio between the

number of berries and rachis length (compactness index, CI) and as the ratio between the number of branches and rachis length.

3.2.5 Basic Fruit Chemistry and Color Analysis

Basic fruit chemistry and color analysis were analyzed as described in Iland et al. (2004). Approximately 20 mL of juice was collected for soluble solids (^{O}Brix) analysis with an Atago PAL-1 refractometer (Kirkland, WA) and pH measurement with a Thermo Scientific Orion 370 pH meter (Beverly, MA). For total acidity (TA) determination, 10 mL of juice was titrated against a standardized 0.1N sodium hydroxide solution in an automatic titrator coupled to an auto sampler and control unit (Titroline 96, Schott, Germany) and expressed as g/L of tartaric acid equivalents. The remaining portions of the berries were briefly frozen and subsequently cold-ground with a tissue grinder (Model PT 10/35, Brinkmann Instruments Co, Switzerland). Approximately 1 g of each sample was transferred to a 15 mL centrifuge tube. Anthocyanins and total phenolics of the berries were extracted in a 50% ethanol solution, pH 2, for 1 hr and then centrifuged at 20,000 rpm. One mL of supernatant was diluted into 10 mL of 1M HCl and stabilized for 3 hr. The absorbances of extracts were read at 520 and 280 nm on a spectrophotometer (UV-1800, Shimadzu, Japan) and expressed as mg of anthocyanins per gram of berry weight and absorbance units of phenolics per gram of fresh berry weight.

3.2.6 Yield Components

At harvest, yield per vine and total number of clusters per vine were recorded. Harvest cluster rot was calculated and recorded as rot incidence (percentage of affected clusters per

vine), where every cluster was considered to be affected if it was visually judged to have more than 2-3% of visible rot. During winter pruning post-harvest, pruning weight per vine was recorded. Crop load, expressed as a Ravaz Index (RI; Ravaz 1911), was calculated as a ratio between yield per vine and the vine pruning weight.

3.2.7 Rot Rating

For detailed ratings of Botrytis (Botrytis cinerea, De Bary), black rot (Guignardia bidwellii, Ellis) and sour rot [Kloeckera apiculata (Rees emend. Kloker), Saccharoycopsis vini (Kreger-van Rij), Hanseniaspora uvarum (Niehaus), Kluyveromyces lactis (Dombrowski), Candida pseudotropicalis (Cast.), Candida valida (Leberle), candida steatolytica (Yarrow), Torulaspora delbrueckii (Leberle), Issatchenkia terricola (van der Walt), Hansenula jadinii (A. et R. Sartory, Weill et Meyer), Zygosaccharomyces bailii (Lindner), Acetobacter spp., Gluconobacter spp., and Bacilus spp.; Bisiach (1986)] 10 random clusters from each of 30 vines were separately collected in ziplock bags during harvest. Clusters were transported to the laboratory, stored at 4°C over night, and ratings of the amount of rot were performed the next day. Berries were detached from the rachis, then the total number of berries per cluster, and the number of berries affected by either Botrytis, black and sour rot were recorded. The severity of different types of rot was calculated as the ratio between the number of affected berries and the total number of berries per cluster.

3.2.8 Berry Analysis

Five random berries from the basal clusters of each tagged shoot were collected, with a small part of pedicels attached, immediately after harvest. Berries were kept in sample bags with puncture proof tabs and stored at 4°C until the time of analysis. Berries without pedicel attached were separately weighed. Each berry was then sliced in half with a razor blade and berry flesh was gently separated from the skin and seeds with a metal spatula. Both skin and seeds were rinsed in distilled water and blotted with Kimwipes tissues until dry. Seeds were counted and the number was recorded, while skin and seed were separately weighed.

3.2.9 Statistical Analysis

Data were analyzed using one-way ANOVA in PROC MIXED procedure, SAS 9.3. Using the tagged shoots, measurements of shoot length were taken weekly and then analyzed using the REPEATED statement function in PROC MIXED.

Normality of the residuals was assessed by visual inspection of the normal probability plot and Kolmogorov test. Whenever the distribution of the residuals was found to significantly diverge from the normal distribution, data were subjected to either logarithmic or square root transformation. Homogeneity of variances was checked using the side-by-side box plot and Levene's test. Models with equal and unequal variances as well as models with different variance - covariance structures in repeated measurements were compared using the goodness of fit indicators. The model that showed the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) values was used for further analysis. When the treatment effect was found to be statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the

treatments were conducted using the t-test. When the treatment effect was not statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the treatments were conducted using Tukey's HSD.

Significance of linear regressions was checked using the Regression Wizard in a scientific data analysis and graphing software package Sigma Plot 11.0.

Table 3.1 Timing of developmental stage (bud burst, bloom, fruit set and harvest) in 2012, as a calendar date and day of the year.

Year 2012

Developmental stage	Date	Day of Year
Bud burst	March 30	89
Bloom	June 6	157
Berry pea size	June 27	178
Harvest	September 6	249

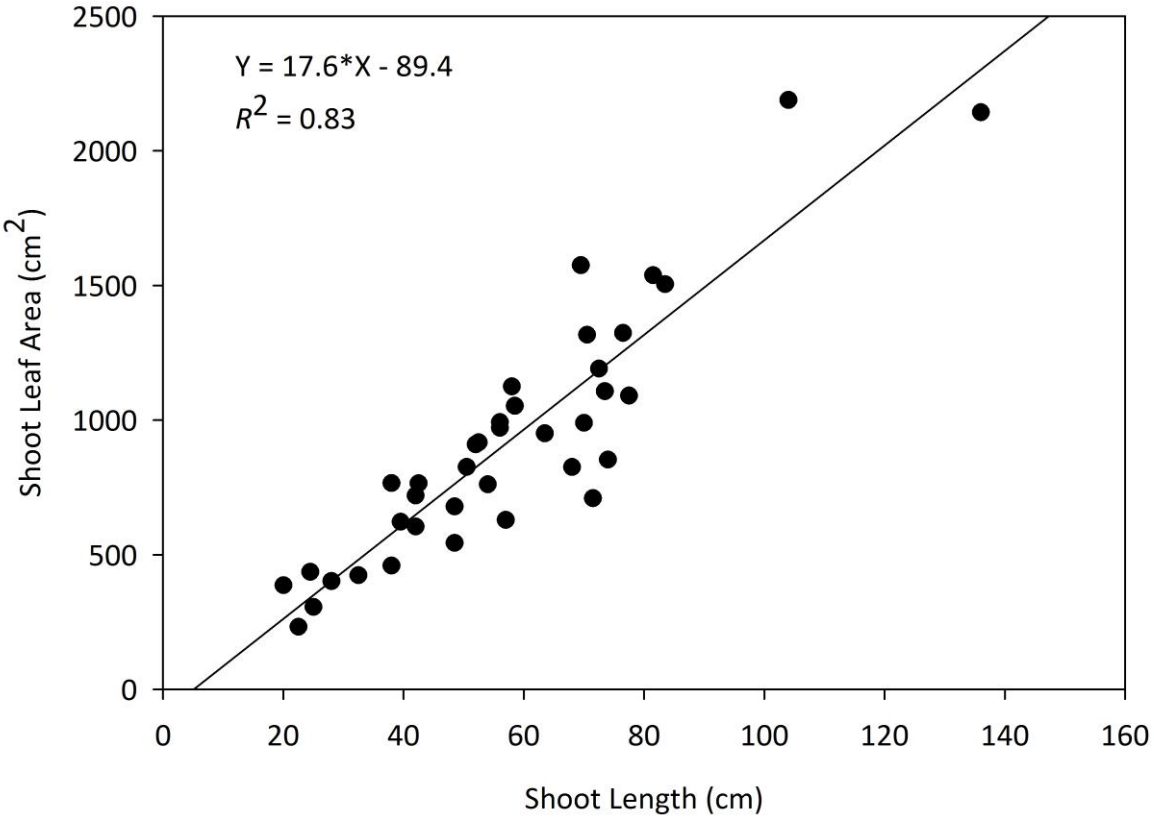

Figure 3.1 The linear correlation between shoot length (cm) and shoot leaf area (cm^2) established on 40 shoots, collected from June 3 to June 19.

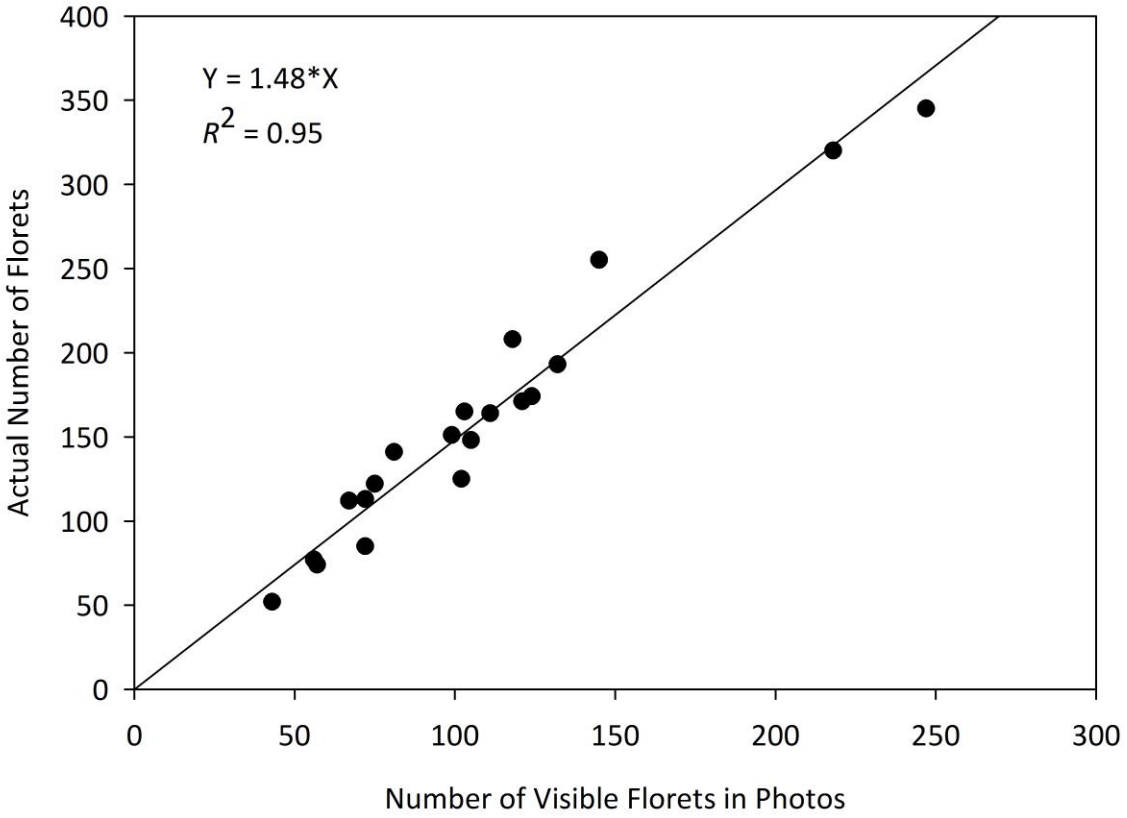

Figure 3.2 Linear correlation between number of visible florets in photos and number of actual florets per cluster based on the sample of 20 inflorescences. Inflorescences were collected at developmental stage 20, after Eichhorn and Lorenz (1977).

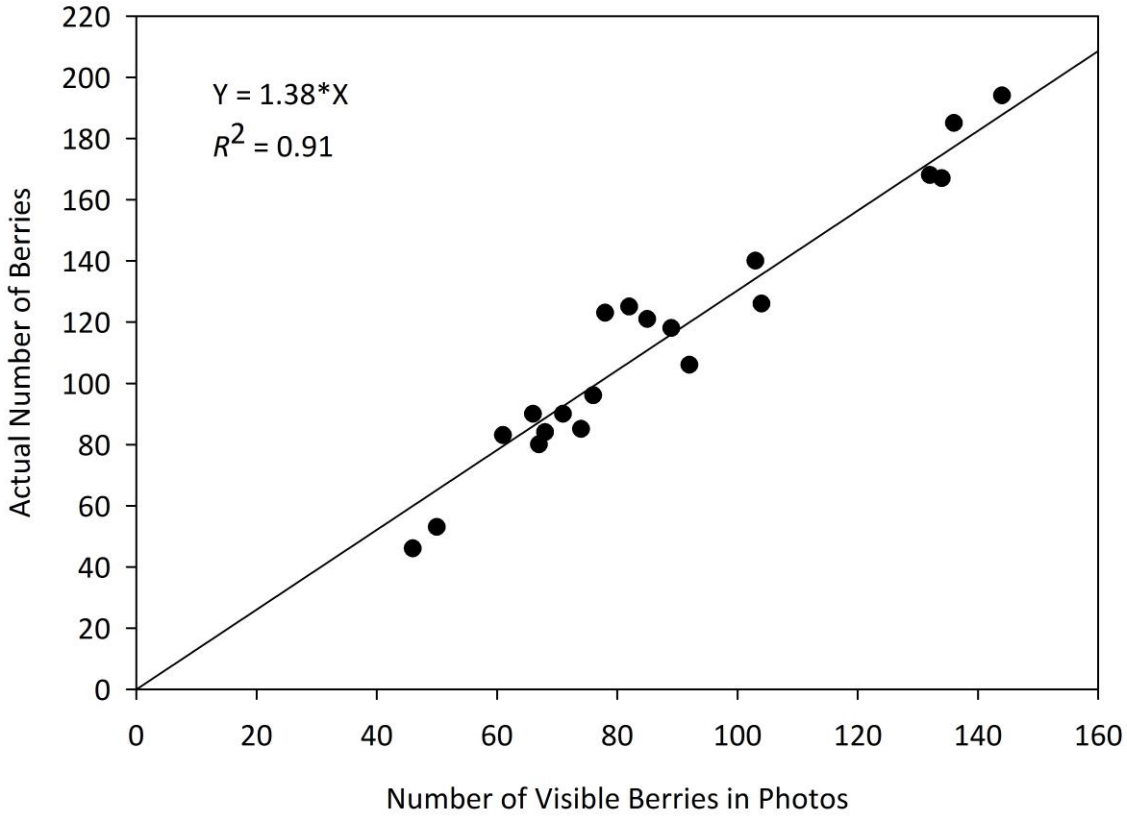

Figure 3.3 Linear correlation between number of visible berries in photos and number of actual florets per cluster based on the sample of 20 clusters. Clusters were collected at developmental stage 31, after Eichhorn and Lorenz (1977).

3.3 Results

3.3.1 Growing Season Weather Conditions

The 2012 growing season started with a very unusual and rapid heat accumulation in March (http://www.agweather.geo.msu.edu/mawn/). This late winter - early spring warm spell triggered an early bud burst (March 30; Table 3.1) on Pinot noir vines. Bud burst occurred approximately one month earlier than usual and 40 calendar days earlier than in 2011. The warm start of the season increased the chance of early spring frost and the potential for damage to emerging shoots. Therefore, at the beginning of April, we set and began operating overhead irrigation that succeeded in protecting the experimental plot. Low damaging temperatures occurred four times just in the first two weeks of April alone, while the last frost event with – 2.6 °C was recorded on April 27. By scouting in the vineyard on May 15, we estimated that the shoot loss was about 10% due to frost damage (data not shown). The last spring frost event occurred on April 27.

Daily temperature exceeded 35 °C on six days: May 27, July 4, 5, 6, 16, and 17. Heat accumulation by the October 1st recorded 1635 GDD, classing 2012 as a year well above the average and promising excellent conditions for grape ripening. However, total precipitation for this year was only 458 mm and, therefore, the plants were exposed to significant drought stress, especially in June and July. A very dry period recorded immediately after bloom lasted for 50 days during which vines were provided with a negligible twenty mm of rain (Figure 3.1).

For grapevines, the optimal temperature for bloom and fruit set is between 20 to 30 °C (Kliewer, 1977). Constant temperature below 15 or above 35 °C during bloom results in poor

fruit set (Kliewer, 1977). A few days before bloom until fruit set, vines received a little more than 10 mm of precipitation. During these three weeks, the maximum temperature never rose above 34 °C and the daily minimum never dropped below 7 °C, which occurred on DOY 165. The period's average temperature oscillated between 15 and 30 °C, and overall conditions ensured a favorable environment for flowering and fruit set (Figure 3.2).

A minimum temperature of 8 °C and a maximum of 33 °C were recorded during the course of three weeks before harvest, while the daily mean fluctuated between 15 and 25 °C (Figure 3.3). Almost 100 mm of rain was received before harvest, which presents 25% of the total precipitation from bud burst until fruit maturation.

3.3.2 Inflorescences and Shoot Number per Vine as a Result of Previous Year Defoliation

On May 15, shoots were approximately 10 cm long and inflorescences were visible allowing them to be counted. Defoliation in the previous year had no effect on the number of shoots per vine, which varied between 67 in LR-10 to 83 in LR-8 (Table 3.2). Nevertheless, the rigorous defoliation seemed to have had an effect on the number of inflorescences per vine. LR-10 had significantly fewer inflorescences compared to the control, LR-4 and LR-6. Both LR-0, LR-4 had 63 inflorescences per vine, whereas LR-6, LR-8 and LR-10 had 71, 52 and 43 inflorescences, respectively (Table 3.2).

3.3.3 Removed and Retained Leaf Area After Treatment Application

Before the treatments were applied, total LA per shoot varied between 854 and 986 cm^2, and was not affected by the previous year's treatment (Table 3.3). Leaf removal in LR-4, LR-6, LR-8 and LR-10, left 345, 547, 662 and 798 cm^2 of LA per shoot, respectively. Removed LA in LR-6 and LR-8 did not significantly differ, but was higher than in LR-4, and lower than in LR-10. Leaf removal treatments removed 38, 54 76 and 82 % of total shoot LA at bloom. Percentage of removed LA in LR-8 and LR-10 was significantly higher than in LR-6 and LR-4. Leaves per shoot after defoliation made 957, 584, 443, 192, and 159 cm^2 of retained LA in LR-4, LR-6, LR-8 and LR-10, respectively. Retained LA in LR-10 was similar to retained LA in LR-8 and these treatments had considerably less LA than the other treatments. Also, LR-6 had less retained LA than LR-4, and LR-4 had less retained LA compared to LR-0. After removing leaves from the 4, 6, 8 and 10 basal nodes, vines contained 62, 46, 24, and 18 percent of total LA, respectively (Table 3.3).

3.3.4 Defoliation Impact on Shoot Growth

In 2012, we started shoot length measurement one week before the defoliation was performed. On May 31 vines did not show any difference in shoot length, meaning that leaf removal in the previous year did not have an impact on the early shoot development rate (Table 3.4). Although the shoot growth was measured on a weekly base after the defoliation was applied, we did not observe significant differences in shoot length on any of dates of measurement. Even six weeks after defoliation, LR-0, LR-4, LR-6, LR-8, and LR-10 had 75, 74, 84,

67, and 79 cm, respectively, showing no significant difference among the treatments (Table 3.4). Over all, shoot length increase was very small for the period of rapid shoot growth, resulting in only an average of 17 cm of incremental shoot length for all treatments from June 6 to July 13.

3.3.5 Defoliation Impact on Retained Leaf Area

On May 31, LA per shoot was similar between treatments, ranging from 785 to 898 cm^2 (Table 3.5). Leaf removal on June 6 resulted in expected LA reduction in defoliated vines compared to the control. One week after defoliation LR-0, LR-4, LR-6, LR-8, and LR-10 had 1086, 718, 587, 319, and 325 cm^2, respectively. There were no significant differences between LR-4 and LR-6 or between LR-8 and LR-10. The same mean separation was preserved over the next five weeks. On July 13, LR-0, LR-4, LR-6, LR-8, and LR-10 had 1220, 860, 842, 444, and 510 cm^2, respectively. As shown in Table 5, even the control had an increment in LA that was only 258 cm^2, from the day of defoliation until July 13. A similar increase of the amount of LA was observed in defoliated vines. Thus the average increase for LR-4 and LR-6 from June 6 until July 13 was 335 cm^2, while the average LA of LR-8 and LR-10 rose up to 298 cm^2.

When retained LA is presented as the share of total LA, then LR-0, LR-4, LR-6, LR-8, and LR-10 had respectively 62, 44, 20, and 16 % on the defoliation day. The percentage of retained LA in LR-8 and LR-10 was statistically the same, but lower than in LR-6 and considerably less

than in LR-4. On July 13, LR-4 gained 68 % of retained LA, while that increase was 56, 35 and 37 % for LR-6, LR-8 and LR-10, respectively (Table 3.6).

3.3.6 Defoliation Impact on Fruit Set

Inflorescences contained fewer florets in 2012 than the ones in 2011 (389 to 442), and their number varied from 125 to 203. Early leaf removal in the preceding year affected the number of florets per cluster in 2012 (Table 3.7). The number of florets per cluster in LR-4, LR-6 and LR-8 was statistically similar to the control and it ranged from 157 to 203. However, the early defoliation of ten nodes resulted in fewer florets per inflorescence compared to the control and other defoliated vines. After leaf removal in 2012, clusters of LR-8 and LR-10 contained considerably fewer berries at stage 31 than LR-0, LR-4 and LR-6. Overall, the estimated number of berries for all treatments was distinctly lower than in 2011 and it varied from 41 to 83.

As a consequence of considerably smaller inflorescences in 2012, FS-31 was higher than in 2011, ranging from 34 % in LR-10 and LR-6 to 44 % in LR-4. None of the defoliation treatments resulted in lower FS-31 than the control. However, LR-6, LR-8 and LR-10 had 34, 36, and 35 % of FS-31 respectively, which was significantly lower than the 44 % in LR-4. Similar to 2011, we observed the difference between the estimated number of berries (Table 3.7) and the actual number of berries (Table 3.11), with one addition: even the control showed 12 % less berries per cluster at stage 38. Following the increase of defoliation intensity, the defoliated vines carried clusters with 8, 10, 19, and 26% less berries in stage 38. In contrast to FS-31, only

early leaf removal of ten nodes generated statistically lower FS-38 than the control, resulting in 26 % of set berries per cluster (Table 3.7).

3.3.7 Change in Cluster Weight Caused by Defoliation

Early leaf removal had an impact upon cluster weight. Treatments LR-8 and LR-10 significantly reduced cluster weights when compared to the control (LR-0). Additionally, LR-4 clusters were heavier than LR-6, LR-8 and LR-10 (Table 3.8). The same trend was reported for total berry weight. Also, early defoliation had an effect on single berry weight, skin and pulp weight, which were reduced in all defoliated vines (Table 3.10). Parallel to the increase in defoliation intensity, berry weight was decreased in the range of 11 to 21% and the same trend in weight decrease was observed for skin and pulp. On contrary, number of seeds and their weight stayed unchanged compared to control, while on the other hand we observed increase in seed to berry ratio. Since skin to berry ratio was unaffected, pulp to berry ratio consequently decreased with increased level of leaf removal. The early defoliation did not have an effect on skin to pulp ratio, which varied from 0.08 to 0.09 (Table 3.10).

Rachis weight and length were also reduced, particularly in LR-8 and LR-10 treatments. It also seemed that defoliation significantly shortened the rachis lengths of LR-10 compared to the control, but both LR-8 and LR-10 had shorter rachises than LR-4 and LR-6 (Table 3.8).

3.3.8 Early Defoliation Affected Cluster Morphology

The effect that early defoliation had on actual berry number and rachis length resulted in significantly lower compactness index (CI) only in LR-10, which was 4.7 compared to 6.7 in

the control. Early leaf removal in the second year significantly reduced the number of branches per cluster in LR-8 and LR-10, but that reduction did not lead to fewer branches per rachis length in any of the treatments. Although CI was successfully reduced with ten nodes of leaf removal that did not significantly change the percentage of berries that were affected by rot. Actually, none of the defoliated treatments performed significantly better than the non-defoliated control, and total rot severity varied between 9 and 14%. In particular, the defoliation treatments did not have any influence on Botrytis or Sour rot, but they did have an effect on Black rot (Table 3.14). However, total Black rot infestation was below 1% for all treatments and it did not make a significant contribution to the total rot severity.

3.3.9 Impact of Early Defoliation on Fruit chemistry and Color

Early leaf removal did not significantly impact soluble solids, which ranged from 20.7 to 22.2 ^{O}Brix (Table 3.12). However, early leaf removal had the influence on pH with every defoliation treatment showing significantly higher pH than the control. This was especially noticeable in LR-8 and LR-10, which had 4.09 and 3.99, respectively. Statistical analysis showed that defoliation did not change either TA or anthocyanin content. TA varied from 3.29 to 3.90 g/L, while the anthocyanin concentration was in the range from 0.38 to 0.50 mg/g, which was slightly higher than it was in 2011. In the second year of defoliation, the total phenolics of treated vines showed the consistent response , in which LR-8 and LR-10 had considerably more phenolics, 1.36 and 1.37 a.u./g, respectively, than the control, which had 1.07 a.u./g.

3.3.10 Yield Components

The cumulative effect of early defoliation of the 10 basal nodes in two consecutive years had the impact on the number of clusters per vine (Table 3.13). Only LR-10 had 60 clusters per vine and that was considerably less compared to LR-0, LR-4, and LR-6, which had 86, 90, and 98 clusters, respectively. Smaller and fewer clusters per vine in LR-8 and LR-10 resulted in the extremely reduced yield per vine and yield per hectare. LR-0, LR-4 and LR-6 had 11.1, 10.5 and 9.8 t/ha, whereas LR-8 and LR-10 had only 5.3 and 3.2 t/ha, respectively. None of the defoliation treatments managed to reduce rot incidence in 2012. However, all treated vines had between 22 and 29 % of their clusters infected compared to the control at 35 %. Interestingly, pruning weight was not significantly changed by two-years of defoliation and all the vines had approximately 0.3 kg of pruned shoots by the end of the second season. Although pruning weight stayed unchanged, the reduced yield in treatments LR-8 and LR-10 resulted in lower RI, which was respectively 12.4 and 6.3. All treated vines, except LR-10, showed very high RI, which in LR-0 and LR-4 reached 24, suggesting that vines were generally over-cropped two years in a row.

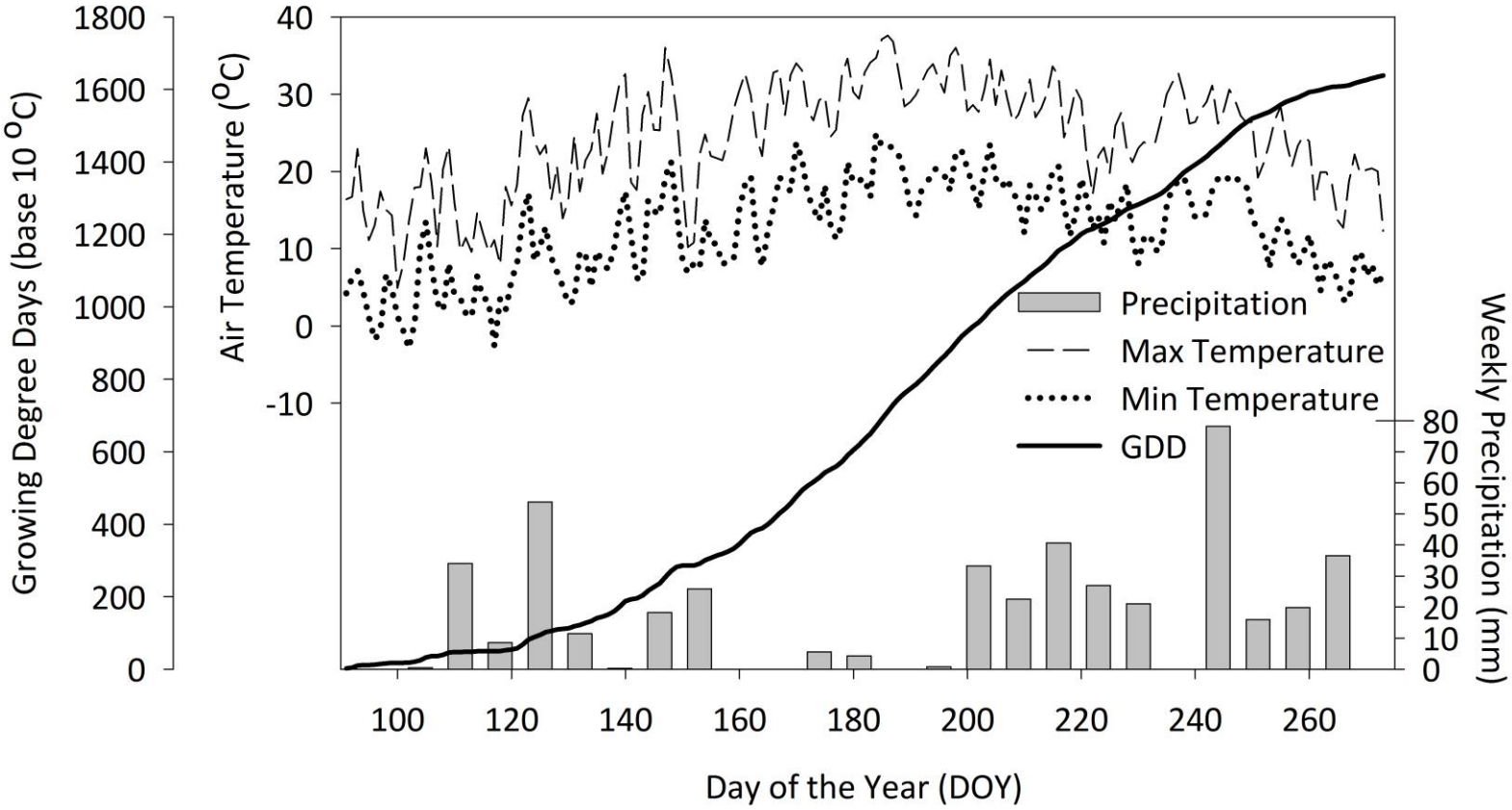

Figure 3.4 Weekly precipitation, minimum and maximum air daily temperature, and growing degree days from April 1st to October 1st in 2012, SWMREC.

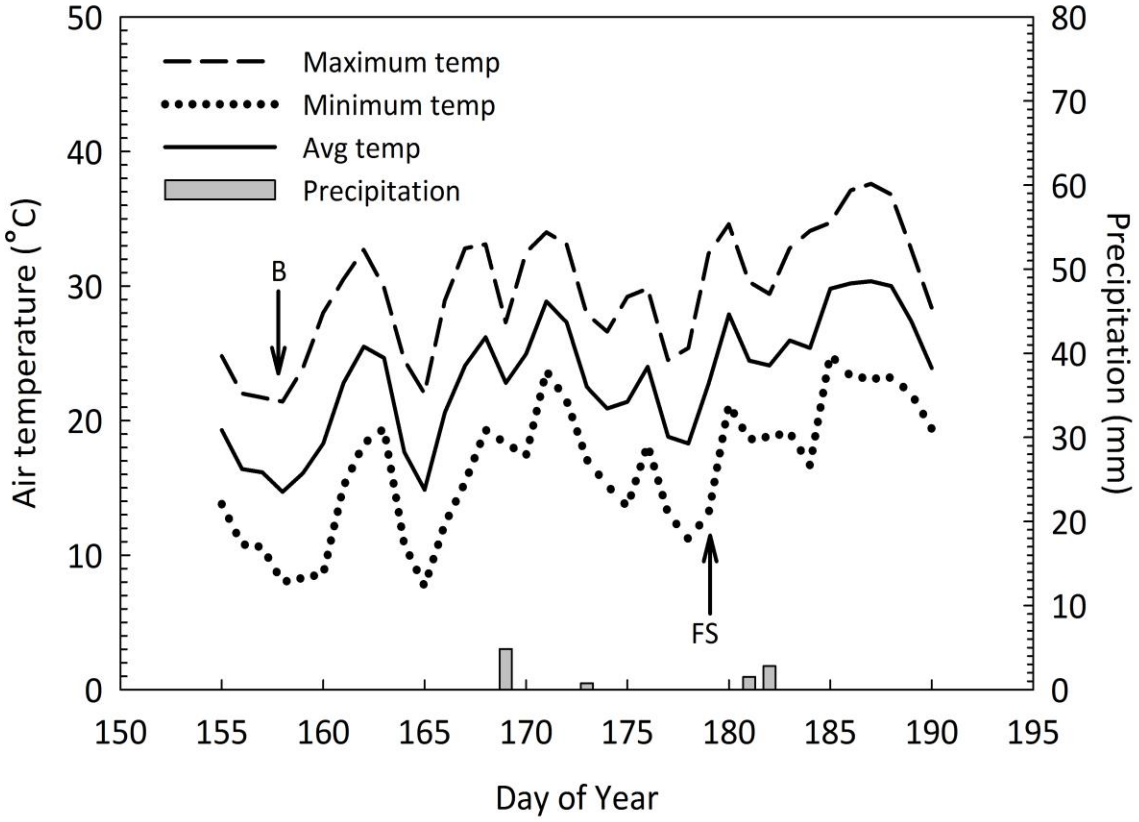

Figure 3.5 Daily minimum, maximum and mean air temperature, and precipitation before bloom (B) and during fruit set (FS) in 2012, SWMREC.

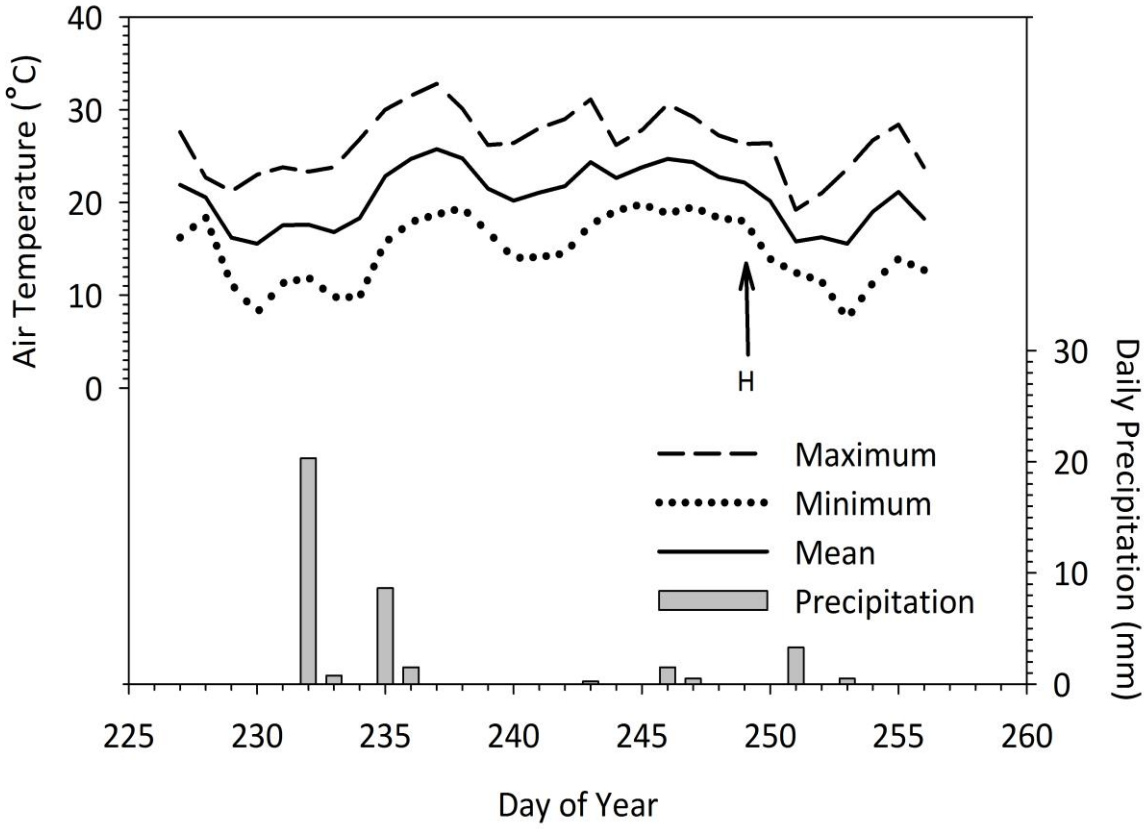

Figure 3.6 Daily minimum, maximum and mean air temperature and precipitation three weeks before harvest (H) in 2012, SWMREC.

Table 3.2 Number of inflorescences and shoots per vine before treatment application in 2012.

Treatment[z]	Number of shoots per vine[y]		Number of inflorescences per vine	
LR-0	73.2[x]	ns	63.3	ab
LR-4	82.5		63.5	ab
LR-6	76.8		70.7	a
LR-8	83.5		52.0	bc
LR-10	67.3		43.2	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 6.

[x] Means within the column followed by the same letter are not significantly different at *p*-value ≥ 0.05 by t-test; ns = no significant difference.

Table 3.3 Total leaf area before treatment application, removed LA with early defoliation, and retained LA per shoot after defoliation in 2012.

Treatment[z]	Total LA[w] (cm^2)[y]		Retained LA (cm^2)		Removed LA as a percentage of the control (%)	
LR-0	957[x]	ns	957	a	-	
LR-4	928		584	b	62	a
LR-6	986		443	c	46	b
LR-8	854		192	d	24	c
LR-10	955		159	d	18	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 24.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Leaf area.

Table 3.4 Effect of early defoliation on weekly shoot growth measured before and after treatment application.

Treatment[z]	Shoot length (cm)[y]					
Day	May 31	Jun 6[w]	Jun 13	Jun 19	Jun 29	Jul 13
LR-0	54[x] ns	60 ns	67 ns	71 ns	74 ns	75 ns
LR-4	53	58	66	69	73	74
LR-6	56	61	70	78	82	84
LR-8	50	54	61	65	68	67
LR-10	55	60	69	75	79	79

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 6.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by Tukey's HSD; ns = no significant difference.

[w] Defoliation was performed on June 6, 2012.

Table 3.5 Effect of early defoliation on retained leaf area before and after treatment application.

Treatment[z]	Retained leaf area (cm^2)[y]					
Day	May 31	Jun 6[w]	Jun 13	Jun 19	Jun 29	Jul 13
LR-0	861[x] ns	961 a	1086 a	1168 a	1209 a	1220 a
LR-4	839	585 b	718 b	782 b	842 b	860 b
LR-6	898	446 bc	587 b	734 b	818 b	842 b
LR-8	785	193 cd	319 c	397 c	449 c	444 c
LR-10	885	165 d	325 c	439 c	500 c	510 c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 6.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Defoliation was performed on June 6, 2012.

Table 3.6 Effect of early defoliation on percent of retained leaf area before and after treatment application.

Treatment[z]	Retained leaf area (%)[y]									
Day	Jun 6[w]		Jun 13		Jun 19		Jun 29		Jul 13	
LR-4	62[x]	a	66	a	67	a	68	a	68	a
LR-6	44	b	49	b	54	b	55	b	56	b
LR-8	20	c	30	c	33	c	35	c	35	c
LR-10	16	c	28	c	34	c	37	c	37	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 6.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

Table 3.7 Early defoliation effect on number of berries per cluster and percentage of fruit set at developmental stages 31 and 38 in 2012.

Treatment[z]	Estimated number of florets per cluster[y]		Estimated number of berries per cluster		FS-31 (%)[w]		FS-38 (%)[v]	
LR-0	185.2[x]	ab	76.4	ab	41.4	ab	36.3	ab
LR-4	203.5	a	83.7	a	44.0	a	40.0	a
LR-6	201.0	a	67.3	b	34.4	b	30.1	bc
LR-8	157.4	b	55.9	c	36.2	b	29.8	bc
LR-10	124.7	c	41.2	d	34.9	b	25.8	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 24.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Percentage of fruit set, which is derived from berry number at developmental stage 31, after Eichhorn and Lorenz (1977).

[v] Percentage of fruit set, which is derived from berry number at developmental stage 38, after Eichhorn and Lorenz (1977).

Table 3.8 Impact of early defoliation on cluster size, total berry weight, and rachis weight and length in 2012.

Treatment[z]	Cluster weight (g)[y]		Total berry weight (g)		Rachis weight (g)		Rachis length (cm)	
LR-0	82.6[x]	ab	79.7	ab	2.5	a	10.5	ab
LR-4	88.1	a	85.2	a	2.7	a	12.1	a
LR-6	66.6	b	64.1	b	2.1	a	10.8	a
LR-8	45.7	c	43.7	c	1.6	b	8.5	bc
LR-10	29.8	d	28.2	d	1.3	b	6.7	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 24.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

Table 3.9 Impact of early defoliation on berry weight and berry components in 2012.

Treatment[z]	Berry weight (g)[y]		Skin weight (g)		Seed weight (g)		Seed number		Pulp weight (g)	
LR-0	1.40[x]	a	0.102	a	0.067	ns	1.60	ns	1.25	a
LR-4	1.25	b	0.092	b	0.071		1.78		1.09	b
LR-6	1.24	b	0.092	b	0.072		1.76		1.07	b
LR-8	1.13	b	0.085	b	0.071		1.79		0.98	b
LR-10	1.11	b	0.086	b	0.068		1.66		0.95	b

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 96.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

Table 3.10 Impact of early defoliation on relative berry components in 2012.

Treatment[z]	Skin to pulp ratio[y]		Skin to berry ratio (%)		Seed to berry ratio (%)		Pulp to berry ratio (%)	
LR-0	0.08	ns[x]	7.46	ns	4.68	b	87.89	a
LR-4	0.08		7.38		5.59	a	87.03	ab
LR-6	0.09		7.45		5.68	a	86.76	bc
LR-8	0.09		7.60		6.16	a	86.24	bc
LR-10	0.09		7.86		6.10	a	86.04	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 96.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

Table 3.11 Impact of early defoliation on components of cluster morphology and bunch rot severity in 2012.

Treatment[z]	Actual berry number[y]		Branch number		CI[w]		Number of branches per rachis length	
LR-0	67.0[x]	ab	12.4	a	6.7	a	1.3	ns
LR-4	76.8	a	11.6	a	6.8	a	1.1	
LR-6	59.9	b	10.4	ab	5.6	ab	1.0	
LR-8	45.1	c	8.5	b	5.8	ab	1.0	
LR-10	30.2	d	8.2	b	4.7	b	1.3	

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 24.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Compactness index expressed as number of berries per 1 cm of rachis length.

Table 3.12 Impact of early defoliation on fruit chemistry and color content in 2012.

Treatment[z]	Soluble solids (°Brix)[y]		pH		TA[w] (g/L)		Anthocyanin (mg/g)		Phenolics (a.u./g)	
LR-0	21.6[x]	ns	3.66	d	3.77	ns	0.45	ns	1.07	b
LR-4	20.7		3.84	c	3.56		0.38		1.15	b
LR-6	21.9		3.93	bc	3.61		0.39		1.10	b
LR-8	22.1		4.09	a	3.29		0.45		1.36	a
LR-10	22.2		3.99	ab	3.90		0.50		1.37	a

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 24.

[x] Means within the column followed by the same letter are not significantly different at *p*-value ≥ 0.05 by t-test; ns = no significant difference.

[w] TA = Total acidity.

Table 3.13 Impact of early defoliation on yield components in 2012.

Treatment[z]	Number of clusters per vine[y]		Yield per vine (kg)		Yield per hectare (t/ha)		Rot incidence (%)[w]		Pruning weight (kg)		RI[v]	
LR-0	86.3[x]	a	6.0	a	11.1	a	35.0	ns	0.3	ns	23.6	a
LR-4	90.2	a	5.7	a	10.5	a	28.6		0.3		23.7	a
LR-6	98.2	a	5.3	a	9.8	a	28.0		0.4		14.8	ab
LR-8	77.3	ab	2.9	b	5.3	b	21.7		0.2		12.4	b
LR-10	60.5	b	1.7	c	3.2	b	29.3		0.3		6.3	c

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 6.

[x] Means within the column followed by the same letter are not significantly different at *p*-value ≥ 0.05 by t-test; ns = no significant difference.

[w] Calculated as number of clusters with ≥ 2-3% rot per total number of clusters.

[v] RI = Ravaz Index, calculated as ratio between yield per vine and vine pruning weight.

Table 3.14 Impact of early defoliation on Botrytis, Sour rot, and Black rot severity in 2012.

Treatment[z]	Botrytis rot (%)[y]		Sour rot (%)		Black rot (%)		Total rot severity[w] (%)	
LR-0	9.61[x]	ns	1.54	ns	0.20	bc	11.35	ns
LR-4	8.61		3.39		0.59	b	12.59	
LR-6	12.70		1.36		0.33	bc	14.38	
LR-8	7.12		1.29		1.35	a	9.76	
LR-10	12.22		0.12		0.03	c	12.37	

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means calculated on sample size of n = 60.

[x] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by t-test; ns = no significant difference.

[w] Rot severity was calculated as a percentage of affected berries per tagged cluster.

3.4 Discussion

Differentiation of the uncommitted primordia into inflorescence primordia occurs between bloom until veraison, determining the final inflorescence number on a vine for the following year (Keller, 2010). During this time, cumulative radiation and heat experienced by the buds, as well as the availability of assimilates, play important roles promoting inflorescence formation and consequently increasing bud fruitfulness for the following season (May et al., 1969; Sommer et al., 2000). Thus, restricted availability of leaf assimilates decreases bud fruitfulness, which in our experiment was observed in the most severe defoliation treatment. Only leaf removal on the 10 nodes in 2011 drastically reduced the number of inflorescences per vine. Although not significantly different than the control, a reduction in inflorescence number was recorded in LR-8. In previous studies, bud fertility, calculated as the number of bunches per number of buds retained at pruning, was stimulated by 33% leaf reduction after bud break, but reduced by the 66 % defoliation after berry set (Hunter and Visser, 1990). The authors referred to the severe defoliation as the cause of reduced nutrient availability for the initiation and differentiation of inflorescence primordia. However, we found a weak linear correlation between the removed leaf area per shoot in 2011 and the number of inflorescence per vine in 2012 (Table B.1). According to Bennett (2005), defoliation in the previous season reduced the number of inflorescence as the result of a whole vine's response, rather than node position effect. Reduction of the inflorescence number per shoot was reported when leaves above the fifth node or all main leaves on the shoots were removed in the previous season (Candolfi-Vasconcelos and Koblet, 1990; Bennett et al., 2005). However, removal of the main leaves up to the second node above the distal cluster at pre-bloom did not change the number of clusters

per shoot nor the number of clusters per vine in Ciliegiolo cultivar (Palliotti et al., 2012).

Although defoliation impacted the number of inflorescence, we did not record any reduction in the number of shoots per vine, which was consistent with results reported in other studies (Candolfi-Vasconcelos and Koblet, 1990; Hunter and Visser, 1990; Bennett et al., 2005). Candolfi-Vasconcelos and Koblet (1990) noticed that the removal of all main leaves on shoots or all leaves above the fifth node did not have an effect on shoot number in the season following the defoliation treatment, but it did reduce the shoot number after defoliation had been applied in the two consecutive years on the same vines. Contrary to these results, the other authors reported the increased number of shootless buds after one defoliation season, which was attributed to either inadequate carbohydrate supply for bud differentiation and shoot growth or to mechanical damage to the buds while leaves were stripped off the nodes (Howell et al., 1994; Sabbatini and Howell, 2010).

Regardless of the fact that defoliation in 2011 resulted in decreased retained leaf area per shoot, vine reserves were at a sufficient level to provide consistent shoot growth at the beginning of the following season and no differences in shoot length were detected in 2012 before the defoliation treatments were applied for the second time (Table 3.5). An expected decrease in shoot length was not found, however, after the same intensity of leaf removal had been performed on the same vines for two consecutive seasons. All vines had grown at the same gradual rate by July 13th when the last measurement was taken. This slow increase in shoot length was associated with the very dry and warm period that occurred in June and July, during which vines received only 20 mm of rain. Therefore, even non-defoliated shoots reached only 75 cm, while for the same period in 2011 the shoot length of the control was 99 cm.

During bud swelling and budburst, the branching of the inflorescence primordia resumes and flower initials are formed. This is the period when the final number of florets per inflorescence is determined by the environmental conditions and the reserve status of the vine (Vasconcelos et al., 2009). Ravaz Indices in 2011 indicated that all vines were over-cropped, which could induce low reserves in the vines and present the first reason for a generally lower number of flowers per inflorescence in 2012. It was also shown that higher temperatures during the two weeks before bud burst reduce the number of florets per inflorescence and the temperature influence on florets differentiation weakened as budburst advanced (Petrie and Clingeleffer, 2005). From the middle of March 2012, the maximum air temperature fluctuated from 24 to 29 °C for a week, which coincided to two weeks before bud burst. This unusually high temperature could be a reason for the generally lower number of florets per inflorescence in 2012 compared to 2011. Moreover, defoliation in 2011 showed a negative impact on the florets number, likely due to a reduction in the amount of stored reserves, which were of crucial importance for flower initiation and differentiation in early spring. Although removing more than 6 leaves resulted in lower number of florets compared to the control, only ten leaves removed showed the significant decrease, reducing the floret number by 32 %. Similar results were reported when 75 % defoliation of Chardonnay was applied earlier in the season in contrast to 12 weeks after bloom, which did not have any impact on the number of florets (Bennett et al., 2005). We found a significant linear correlation between floret numbers in 2012 and removed or retained leaf area per shoot ($r = 0.55$, Table B.1).

Defoliation in 2012 had an effect on both the estimated numbers of berries and the actual number of berries, FS-31 and FS-38. The threshold for producing the significant decrease

in the estimated and actual number of berries was a level of defoliation of eight nodes. Based on correlation coefficients, the amount of leaf area per shoot that was either removed or retained was of similar importance for the estimated and the actual number of berries (Table B.2). However, correlation coefficients implied that only 65% of the changes in berry number could be associated with changing the leaf area per shoot. The remaining 35% was attributed to other factors, which appeared as a result of the depletion of stored reserves and the vines' inability to restore them satisfactorily.

Comparing the estimated and actual number of berries at the same level of defoliation intensity we found that additional berry drop occurred in 2012. Interestingly, a difference of 12% between berries at fruit set and at harvest was noticeable even in the control. The additional berry drop in the control was most likely a response to environmental conditions and the lack of rain that occurred during the first stages of berry development (Hardie and Considine, 1976). Defoliated vines had lost 7 to 11 berries per cluster by harvest compared to the control, which had dropped an average of 9 berries; not enough to draw conclusions. The difference between the estimated and actual berry numbers is more obvious when presented as the percentage decrease between the two, and then it can be seen that the percent of berries that dropped proportionally increased with defoliation intensity (Table B.3). By 2012 the vines had been subjected to defoliation stress for two seasons and, percentage-wise, berry drop was more pronounced in 2011 than in 2012. This particularly refers to LR-8 and LR-10 and an explanation for it could be found in the different inflorescence sizes produced in each year. In 2011 inflorescences contained more than double the amount of florets found in 2012. Additionally, defoliation in 2011 decreased the number of florets in LR-8 and LR-10 in 2012

considerably. To obtain balance between source availability and sink requirement, the vines proportionally dropped more berries if clusters contained more florets before fruit set. Consequently, fruit set was higher in 2012 than it was in 2011 for the same reason: the effect of inflorescence size (May 2004).

In 2012, FS-38 was only significantly reduced in LR-10 compared to the control. Moreover, removing the leaves from only 4 nodes increased FS-31 and FS-38 compared to other defoliated vines (Table 3.7). We found significant linear regression between removed leaf area and FS-31; between removed leaf area and FS-38; between retained leaf area and FS-31; and, between retained leaf area and FS-38 (Table B.2). However, the low correlation coefficients suggested that 50% or more of the variability in FS-31 and FS-38 might be associated with other factors rather than the removed or retained leaf area. This primarily refers to the carry-over effect of defoliation in 2011 that negatively impacted inflorescence size.

Berry size in all vines was markedly affected by early leaf removal, which, depending on defoliation intensity, reduced berry weight by 11 to 21% in comparison to the control (Table 3.9). Moreover, we found significant linear regression between retained leaf area per shoot in 2012 and mean berry weight (Table B.4). As with other yield components, mean berry weight was under the influence of two-seasonal defoliation, which induced a level of source limitation to considerably reduce berry size. However, Bennett (2005) reported that defoliation performed 4, 8 or 12 weeks after bloom did not have an effect on berry weight in the year succeeding the treatment application. Also, Lee and Skinkins (2013) reported no changes in berry weight in Pinot noir vines at two locations when 5-6 leaves were removed at bloom in two consecutive years. In constrast, after the late-seasonal defoliation in the first and early

season defoliation in the second year, Candolfi-Vasconcelos and Koblet (1990) reported the decrease in berry weight when all main leaves were removed in Pinot noir vines.

Reducing berry size, early defoliation consequently affected skin and pulp weight decreasing them, while seed weight as well as seed number per berry remained unchanged. In previous studies, berry size was reduced in Sangiovese, increased in Barbera, and unchanged in Lambrusco with pre-bloom leaf removal (Poni et al., 2006; Poni et al., 2009). Inconsistent reports suggest that vine responses to early defoliation and impact upon berry size are cultivar specific. Significant linear regression between berry weight on the one hand and pulp, seed, and skin to berry ratio on the other was reported in Appendix B. Sixty-six, 78 and 63% of the changes in skin to berry ratio, pulp to berry ratio, and seed to berry ratio, respectively, can be associated with changing berry weight. Also, 71% of the change in skin to pulp ratio can be attributed to changing berry weight (Table B.5). There was a positive linear correlation between berry weight and pulp to berry ratio (Figure B.1). However, the correlation between skin and seed to berry ratio and berry weight was negative. Moreover, a correlation between berry weight and skin to pulp ratio was found to be negative (Figure B.2), which means that source limitation via early leaf removal led to the improved skin to pulp ratio. With the decreased pulp to berry ratio and the increased seed to berry ratio, we concluded that the main decrease in berry size came from the decrease in mesocarp cell number and/or size. Our finding corresponds with results obtained by Ollat and Gaudillère (1995). These authors reported that during the first stage of berry growth, carbon was accumulated in the same quantity in the seeds and the pericarp: flesh with skin (Ollat and Gaudillère, 1995). The limiting assimilate supply soon after fruit set, strongly affected cell enlargement in the pericarp and even though

the higher source to sink ratio was provided later, during lag phase, and the berry growth rate was found to be similar to the control, the compensation in the final berry size did not occur (Ollat and Gaudillere, 1998). In our study, defoliated vines never recovered the leaf area removed even with only 4 leaves removed (Table 3.6), and this could be the reason of a smaller berry size at harvest. This source limitation did not change the seed weight and according to Ollat and Gaudiller (1998) probably reduced pericarp cell enlargement that resulted in increased seed to berry ratio and decreased pulp to berry ratio.

Figure B.3 shows that in a frequency distribution of mean berry mass among five categories, ranging from 0.40 to 2.40 g, non-defoliated vines had more berries in the higher mass categories of 1.21 to 2.40 g than defoliated vines. Conversely, defoliated vines had more berries in the categories from 0.40 to 1.20 g, where approximately 55% of the berries fell in the small and small-middle mass groups. The effect of early defoliation on berry size in Pinot noir in our study contradicts the effect, which early defoliation had on berry size in Barbera vines (Poni et al., 2009). Unlike Barbera's and Lambrusco's berries, whose relative and absolute skin masses were increased with early defoliation, we found that Pinot noir's berries in defoliated vines had similar absolute and relative skin mass to the berries in non-defoliated vines across the five categories of berry size (Figures B.4 and B.5). Neither beneficial effect of improved cluster microclimate (temperature and light regime) with leaf removal in the cluster zone on increased skin mass, which was reported for Sangiovese berries (Poni et al., 2006), was found in Pinot noir. Palliotti et al. (2012) found reduced berry size caused by pre-bloom defoliation in Ciliegiolo in two years of experiments, but relative skin mass was increased only in the first year. Six leaves removed at fruit set in Merlot did not affect berry size, skin weight or the skin to

berry ratio, but reduced both berry size and skin weight in Cabernet Sauvignon (Kotseridis et al., 2012).

Pinot noir in our study responded to the early defoliation with increased total and relative seed mass across berry size categories (Figure B.6 and B.7) and that was in agreement with data obtained by Poni et al. (2009). However, the other authors reported no change in the seed number and seed weight caused by early defoliation (Kotseridis et al., 2012; Palliotti et al., 2012; Lee and Skinkis, 2013).

Additionally, we found no differences in absolute pulp mass between defoliated and non-defoliated vines, but relative pulp mass was reduced in defoliated vines in all berry size categories except for the largest berries (Figure B.8 and B.9). Furthermore, early leaf removal had no effect on the skin to pulp ratio in any of the berry size categories (Figure B.10). With this, we concluded that source limitation in the early stage of Pinot noir cluster development does not have a beneficial effect on increased skin or skin to pulp ratio, which would otherwise lead to higher resistance to bunch rot and improved wine color.

In 2012, cluster weight of LR-8 and LR-10 was reduced by 45 and 65 % compared to the control (Table 3.8). Clearly, fewer berries of a smaller size in general contributed to the lighter clusters in LR-8 and LR-10. The significant linear relationship illustrated that 79 % of the variation in cluster weight was attributed to the reduction of leaf area per shoot (Table B.4). Additionally, early leaf removal in the previous season affected the number of inflorescences per vine, which consequently led to fewer clusters per vine in LR-10. Therefore, the yield decrease in 2012 was the result of reduced berry and cluster weights caused by defoliation in 2012 and decreased cluster count per vine triggered by defoliation in 2011. Yield per vine was

strongly reduced with the defoliation of 8 and 10 nodes and was measured at 2.9 and 1.7 kg, respectively. Two season worth of defoliations of 8 and 10 nodes led to vineyard productions of 5.3 and 3.2 t/ha, below acceptable levels for sustainable Pinot noir production. In contrast, two-seasonal defoliation at the 6 nodes level resulted in slightly lighter clusters due to the decrease in berry weight, but was not the limiting factor causing significant change in yield.

Pruning weight, an index of vine size, was unchanged under the cumulative effect of defoliation over two years at approximately 0.3 kg per vine (Table 3.13). Generally, the low pruning weight in 2012 can be attributed to reduced shoot growth caused by lack of soil moisture. Although the yield per vine was overall lower in 2012 (Table 3.13) compared to 2011, except for LR-10, yield to pruning weight ratio was still considerably higher than it should theoretically be in balanced vines.

The two most severe levels of early leaf removal caused significant decreases in rachis weight, while the effect of early defoliation on rachis length was also significant, but only at the higher levels of defoliation (Table 3.8). We found significant linear correlation between the percentage of leaf area removed per shoot and the rachis weight (i.e. length). About 65 % of the variability in rachis weight can be associated with the degree of leaf area removed per shoot (Table B.4). However, only 50 % of the changes in rachis length can be associated with changing the percentage of leaf area removed per shoot in 2012.

The limitation of carbohydrates availability caused by 8 and 10 defoliated node treatments in the early stage of cluster development first affected berries causing their drop and, consequently, reducing the number of branches by which the berries were attached to the rachis. Consequently, by decreasing the number of branches, defoliation successfully reduces

both rachis weight and rachis length, including the length of the branches. A look at significant linear regression tells us that 60% of variability in branch number was attributed to the percentage of leaf area removed per shoot in 2012 (Table B.4).

Simultaneously decreasing berry number and rachis length, early defoliation affected cluster size and architecture, which in turn resulted in significantly lower CI only for LR-10 (Table 3.11). However, the number of brunches per rachis length was unchanged in 2012. Pallotti et al. (2012) reported that removal of 75-80% of the leaves in a canopy at pre-bloom reduced the number of berries, cluster compactness (expressed as yield per rachis length or OIV rating), and finally *Botrytis* rot in Ciliegiolo in two consecutive years. Two years of six leaves removal reduced cluster compactness (cluster density) in Merlot, while cluster compactness in Cabernet sauvignon was unaffected (Kotseridis et al., 2012).

Generally, unchanged cluster compactness and skin thickness with 2 years of early defoliation failed to improve cluster resistance to bunch rot (Table 3.11 and 3.14). Although not significant, all levels of defoliation did have a positive effect on the reduction of rot incidence as shown in Table 3.11. Weather conditions in 2012 were favorable for bunch rot development, which consequently led to 35% of rot incidence in the control vines. On the other hand, rot incidence was kept low in defoliated vines probably due to cluster microclimate improvement. Among three common types of grape rot, *Botrytis* had the prevailing contribution to total rot severity in our subject vines in 2012.

Early leaf removal did not affect juice soluble solids in 2012, although LR-8 and LR-10 generated slightly higher ^{o}Brix accumulation (Table 3.12). Moreover, ^{o}Brix accumulation was not correlated with vine yield (Figure B.11). A significant effect of early defoliation was found

on juice pH, where all defoliated vines had higher pH compared to non-defoliated ones. However, the same trend was not detected in juice TA, where treatments had no effect. Howell et al (1994) reported non-significant linear regression between the number of leaves that were removed six weeks after bloom in the first season of the experiment and ^{O}Brix, total acidity and pH in the following season when no defoliation was applied. Candolfi-Vasconcelos and Koblet (1990) measured significant increases in soluble solids, fruit coloration, and a decrease in total acidity after two-seasons of defoliation stress on Pinot noir.

The finding of no significant change in the amount of anthocyanins per gram of berry weight could be attributed to the fact that neither skin to berry ratio nor skin to pulp ratio were changed. Although, not changing berry mass, early defoliation in Merlot (Greece) and Pinot noir (Washington) increased the amount of anthocyanins (Kotseridis et al., 2012; Lee and Skinkis, 2013). Additionally, anthocyanins were increased in Cabernet Sauvignon and Ciliegiolo when berry weight was found to be lower in defoliated compared to non-defoliated vines (Kotseridis et al., 2012; Palliotti et al., 2012). In two years of our experiment we found consistent effects of early defoliation in on the amount of phenolics per gram of berry weight, which could be attributed to the increased seed to berry ratio, rather than to an increase in seed weight or seed number (Table 3.12).

3.5 Conclusion

The aim of this experiment was to evaluate the influence of the long-term application of early leaf removal on the performance of Pinot noir in the cool growing climate in Michigan such as the growth parameters, fruit quality, and yield components. The restricted availability of leaf assimilates in the first year of defoliation decreased bud fruitfulness (i.e. number of inflorescences per vine) in vines, which were defoliated at 10 nodes and also decreased the number of florets per inflorescence by 32% compared to the non defoliated control. However, the previous year defoliation did not have an impact on the number of shoots per vine nor the shoot growth at the beginning of the following season. In 2012, FS-38 was only significantly reduced in LR-10 compared to the control. Berry size in all vines was markedly affected by early leaf removal, and this reduction consequently affected skin and pulp weight, while seed weight and seed number per berry remained unchanged. In 2012, cluster weight of LR-8 and LR-10 was reduced by 45 and 65% compared to the control. The two most severe levels of early leaf removal caused significant decreases in rachis weight, while rachis length and CI were decreased only in LR-10. The significant yield decrease in 2012 was observed in LR-8 and LR-10 and it was due to the reduced number of cluster per vine, number of berry per cluster and reduced mean berry weight. The early defoliation did not help in bunch rot control in 2012. With no effect on skin thickness and skin to pulp ratio early defoliation did not change the amount of anthocyanins per gram of berry weight. However, the amount of phenolics per gram of berry weight were increased in LR-8 and LR-10.

4 IMPACT OF EARLY DEFOLIATION ON CARBON PARTITIONING AND LEAF PHOTOSYNTHESIS IN PINOT NOIR

4.1 Introduction

Triggered by defoliation, vines change their source to sink balance with a series of physiological responses. A reduction in main shoot leaf area provokes the production of more lateral shoots and retarding senescence of remained leaves (Candolfi-Vasconcelos and Koblet, 1990). The retained leaves are more efficient and increase mesophyll and stomatal conductance, water use efficiency, and chlorophyll content. This, in turn, results in temporary photosynthetic compensation initiated by defoliation or reduced sink availability (Candolfi-Vasconcelos and Koblet, 1991; Poni et al., 2006; Poni et al., 2008).

Translocation of nutrients from the permanent structures to the developing clusters is another way in which vines are able to respond to early defoliation stress. In a pioneer work of Quinlan and Weaver (1970) it was documented using isotopic enrichment that, during bloom, movement of ^{14}C labeled photoassimilates to proximal unfed shoots occurred when they were defoliated. The same authors found that the established direction of ^{14}C translocation to the uppermost fully developed leaf was reversed towards the basipetal when the leaves around the clusters were removed. Post- veraison clusters are strong sinks and, under defoliation stress,

ripening fruit attracted 12% of the ^{14}C stored in reserves compared to only 1.6% found in the clusters of non-defoliated vines (Candolfi-Vasconcelos et al., 1994).

The tendency of vines to achieve this new source-sink balance after removing leaves in the fruiting zone promotes a reduction in fruit set when defoliation is performed immediately before or at bloom (Poni et al., 2009; Tardaguila et al., 2012). For this reason, targeted early defoliation has become a common management strategy for the control of excessive cropping in high-yielding cultivars (Poni et al., 2006; Intrieri et al., 2008). It has also been used in efforts to improve fruit quality through the reduction of cluster compactness and subsequent bunch rot in cultivars where that is a known characteristic. Defoliation has the added benefit of increasing soluble solids, total phenolics, and anthocyanin content, which leads to higher quality grape must composition (Poni et al., 2004; Intrieri et al., 2008; Poni et al., 2009; Sabbatini and Howell, 2010; Lemut S. et al., 2011; Kotseridis et al., 2012; Palliotti et al., 2012; Lee and Skinkis, 2013).

However, little is known about the impact of retained leaves on <u>partially</u> defoliated shoots on the partitioning and distribution of assimilates and how changes in the distribution pattern affect fruit set and cluster morphology. Therefore, the objective of this study was to investigate the influence of early defoliation performed at bloom on leaf assimilate translocation along the shoots, on distribution dynamics, and on partitioning patterns among the sinks: clusters and shoot tips.

4.2 Material and Methods

4.2.1 Plant Material and Experimental Design

This research was carried out on 6 year old vines of *V. vinifera*, cv. Pinot noir (clone 777 grafted on C3309 rootstock) during 2011. The vineyard was located at the Southwest Michigan Research and Extension Center (SWMREC; lat. 40°09′ N; long. 86°36′ W; elevation 220 m) near Benton Harbor, Michigan. Vines were planted in a Spinks loamy fine soil (U.S. Department of Agriculture, Soil Conservation Service, 1957), with a spacing of 1.8 m between vines and 3.0 m between rows, and trained to a vertical shoot positioning system (VSP). Vines were spur-pruned during the winter leaving approximately sixty buds per vine. No additional shoot or cluster thinning was performed before treatment application.

The experiment was arranged as a randomized complete block design with one categorical factor, leaf removal (LR), with three levels of defoliation: no leaves removed (LR-0); leaves removed from six basal nodes (LR-6); and leaves removed from ten basal nodes (LR-10). Treatments were applied at full bloom, developmental stage 23, after Eichhorn and Lorenz (1977) on 15 June.

4.2.2 Application of ^{13}C

Four vine replicates of treatments LR-0, LR-6 and LR-10, previously described, were used for quantification of carbon distribution along the shoots one week after defoliation on 22 June 2011. Three shoots per vine were randomly chosen and separately enclosed in mylar bags. Each shoot was fed for 30 min with $^{13}CO_2$, which was generated by the reaction of 800 mg of

$Ba^{13}CO_2$ (98 atom %) with 5 mL of 85% lactic acid (Figure 4.1). Feeding with $^{13}CO_2$ was done between 1000 and 1400 hr on a cloudless day. After 30 min of feeding, the mylar bags were removed and shoots were exposed to the atmospheric air. Samples of shoot tips with immature leaves, main leaves along the pulsed shoots (approximately 3 cm^2), and clusters (approximately 1 g) were collected 1 hour after $^{13}CO_2$ labeling from the first shoot. Twenty-four hours later, the first shoot was completely harvested, while the second and third shoots were harvested 3 and 7 days after $^{13}CO_2$ labeling, respectively. Additionally, three shoots from non-labeled vines were collected for ^{13}C natural abundance determination. Harvested shoots were divided into shoot tips with immature leaves, main leaves and clusters. Shoot parts were oven-dried at 70°C for 2 days and their dry weights were recorded. Dry tissues were ground to a fine powder with mortar and pestle and sieved with mesh size 40. Approximately 1.5 mg of each sample was folded into small tin capsules, placed in trays, and sent to the Stable Isotope Facility, UC Davis, California for ^{13}C-analysis. The ^{13}C atom excess % and the percentage of ^{13}C distribution per organ were calculated as described by Morinaga et al, (2003).

4.2.3 Gas Exchange Measurements

Leaf assimilation (P_n), expressed as amount of CO_2 assimilated per unit leaf area and time (μmol m^{-2} s^{-1}), stomatal conductance (g_s), and intracellular partial pressure (C_i) were measured with a CIRAS 2 portable open system gas analyzer (PP System, Hitchin Herts, U.K.).

Four vines of treatments LR-0, LR-6 and LR-10, and two shoots per vine, were chosen from the eastern side of the cordon. Measurements were taken on leaves located on the 7^{th} and 11^{th} nodes from the base, between 1000 and 1300 hr, 1 day before defoliation (14 June 2011) and 7 and 10 days after defoliation (21 and 25 June 2011) with the exception of treatment LR-10, where only the 11^{th} leaf was observed. Main leaf length (LN) was taken on each measurement day and leaf area (LA) was calculated based on the equation LA = 21.06*LN − 69.26, R^2 = 0.91, after Sánchez-de-Miguel et al.

Respiration was measured on 15 June 2011 from 1100 to 1300 hr. For this purpose, five replicates of treatments LR-0, LR-6 and LR-10 were used. One shoot per vine was randomly chosen and measurements were taken on leaves located on the 7^{th} and 11^{th} nodes.

CO_2 response curves were generated with a CIRAS 2 gas analyzer at intercellular CO_2 concentrations of 0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 700, and 1000 µmol mol^{-1} of air on 26 June 2011. Using the software Photosyn Assistant (Dundee Scientific Ltd, Scotland UK), we assessed maximum rates of photosynthesis (A_{max}), respiration rate, CO_2 compensation point, and carboxylation efficiency. Measurements were performed between 1000 and 1200 hr, on leaves located on the 7^{th} and 11^{th} nodes of only one replicate of treatments LR-0, LR-6 and LR-10. Photosynthetic flux densities were above 1000 µmol m^{-2} s^{-1}.

Diurnal changes in photosynthetic rate were recorded on three replicates of treatments LR-0, LR-6 and LR-10. Two shoots per vine were chosen from the eastern side of the cordon and

measurements of P_n, g_s, and C_i were taken on leaves located on the 7^{th} and 11^{th} nodes from the base at 1000, 1200, 1430, 1630, and 1800 hr on 6 July 2011.

4.2.4 Statistical Analysis

Data were analyzed using one-way ANOVA in PROC MIXED procedure, SAS 9.3. Photoassimilate partitioning and diurnal measurements of P_n, g_s, and C_i were analyzed using the REPEATED statement function in PROC MIXED.

Normality of the residuals was assessed by visual inspection of the normal probability plot and Kolmogorov test. Whenever the distribution of the residuals was found to significantly diverge from the normal distribution, data were subjected to either logarithmic or square root transformation. Homogeneity of variances was checked using the side-by-side box plot and Levene's test. Models with equal and unequal variances, as well as models with different variance - covariance structures in repeated measurements, were compared using the goodness of fit indicators. The model that showed the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) was used for further analysis. When the treatment effect was found to be statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the treatments were conducted using the t-test. When the treatment effect or the interaction day times treatment were not statistically significant at $\alpha = 0.05$, the interaction was sliced by day and all-pairwise comparisons among the treatments were conducted using Tukey's HSD.

Figure 4.1 Shoots of LR-6 enclosed in mylar bags and fed for 30 min with $^{13}CO_2$ generated by the reaction of $Ba^{13}CO_2$ with lactic acid on 22 June 2011.

4.3 Results

4.3.1 ^{13}C Atom Excess% and Percentage of ^{13}C Distribution in Different Organs of the Shoot

Figure 4.2 shows ^{13}C atom excess% in fully developed leaves 1 week after defoliation. ^{13}C atom excess% was the highest 1 hour after the feeding when its concentration reached approximately 1.3% in LR-6 and LR-10 and 0.8% in LR-0. Twenty-four and 72 hours later, leaves of all vines showed the same concentration of ^{13}C, which varied between 0.2 and 0.4%. Only after 7 days did leaves of LR-10 show significantly higher ^{13}C atom excess% when compared to LR-6 and LR-0.

One hour after shoots had been fed with labeled $^{13}CO_{2,}$ ^{13}C atom excess% in the apical leaf and shoot tips was between 0.6 and 1.0% (Figure 4.3). A similar concentration was found for all treatments after 24 hours, while after 72 hours ^{13}C atom excess% in LR-0 and LR-6 significantly decreased compared to LR-10. Seven days after $^{13}CO_2$ application, ^{13}C atom excess% in the apical leaves and shoot tips dropped in all treatments but the decrease in LR-0 and LR-6 (0.2%) was significantly greater than in LR-10 (0.4%).

One hour after $^{13}CO_2$ application, clusters of the fed shoot in all treatments did not show any increase of ^{13}C atom excess%, where the concentration was approximately 0.0% (Figure 4.4). However, 24 hours later, ^{13}C atom excess% significantly increased in LR-0 and LR-6

(more than 0.4%), while LR-10 had only 0.1%. Three days later, ^{13}C atom excess% of LR-6 dropped to 0.2%, while clusters of LR-0 and LR-10 kept levels of ^{13}C atom excess% similar to what it was on the first day after $^{13}CO_2$ application. On the third day, ^{13}C atom excess% in LR-10 was significantly higher than in LR-0. Seven days after the shoots had been fed, clusters of all treatments had the same ^{13}C atom excess%, which ranged from 0.1 to 0.2%.

Figure 4.5 shows percentage of ^{13}C distribution for the fully developed leaves, apical leaves, and shoot tips and clusters 1 hr and 24 hr after pulsing. Immediately after pulsing, the leaves of LR-0, LR-6 and LR-10 had the same percent of ^{13}C, which varied between 83 to 89% of the total amount of ^{13}C in the shoot. Twenty-four hours later, the percentage of ^{13}C distribution in the leaves has been reduced to a range of 51 to 58% for all treatments. The percentage of ^{13}C distribution in the apical leaves and shoot tips was between 10 to 16% for all vines. After a day, the percentage of ^{13}C distribution increased to within the range of 22 to 30%. Although not significant, LR-0 had the lowest percentage of ^{13}C distribution in the apical leaves and shoot tips for both time points. Since the ^{13}C atom excess% for clusters, immediately after feeding, was similar to the natural abundance, the percentage of ^{13}C distribution for clusters in all treatments was around 0% (data not shown). However, the percentage of ^{13}C distribution increased to 18 and 19% in LR-6 and LR-0, respectively, after 24 hours. ^{13}C distribution in LR-10 clusters was only 9%.

4.3.2 Leaf Assimilation, Stomatal Conductance, Intracellular Partial Pressures of CO_2, and Respiration Measured Before and After Treatment Application

Table 4.1 and 4.2 show the leaf assimilation (P_n), stomatal conductance (g_s) and intracellular partial pressures of CO_2 (C_i) in the leaves located on the 7^{th} and 11^{th} nodes one day before leaf removal. Leaf assimilation for the 7^{th} and 11^{th} leaf were the same across the treatments at approximately 8.7 and 2.5 µmol m^{-2} s^{-1}, respectively. Neither stomatal conductance nor intracellular partial pressure of CO_2 differed between treatments for the same leaf position before treatment application.

One day after defoliation, respiration was measured on the 7^{th} and 11^{th} leaf position during the night (Table 4.3). No significant differences between treatments were noticed and respiration of the 7^{th} and 11^{th} leaves was in the range -0.40 to -0.46 and -0.62 to -0.82 µmol m^{-2} s^{-1}, respectively.

Significant differences in P_n, P_n/leaf, g_s and C_i were not detected on the 7^{th} leaf between LR-0 and LR-6 seven days after leaf removal (Table 4.4). However, we noticed increased P_n and P_n/leaf in LR-10 on the 11^{th} leaf 7 days after leaf removal (Table 4.5). On the other hand, g_s was decreased in LR-0 compared to LR-6 and LR-10.

Similar P_n and P_n/leaf in LR-0 and LR-6 were found on the 7^{th} leaf 10 days after defoliation, while g_s was decreased in LR-0 (Table 4.6). Ten days after defoliation, P_n and

P_n/leaf were still increased in LR-10 compared to LR-6 and LR-0 on the 11th leaf, while g_s was decreased in LR-0 and LR-6 (Table 4.7).

CO_2 response curves were taken on leaves positioned on the 7th and 11th nodes from the shoot base on 26 June 2011. From the data we obtained we assessed respiration rate, maximum assimilation, CO_2 compensation point and carboxylation efficiency and they are shown in Tables 4.8 and 4.9. The leaf on the 7th node in LR-0 had -5.32 µmol m^{-2} s^{-1} respiration rate, 21.6 µmol m^{-2} s^{-1} maximum assimilation, 57.6 µmol mol^{-1} CO_2 compensation point and 0.070 mol m^{-2} s^{-1} carboxylation efficiency (Table 4.8). The leaf position on the same node in LR-6 had -5.54 µmol m^{-2} s^{-1} respiration rate, 23.3 µmol m^{-2} s^{-1} maximum assimilation, 63.7 µmol mol^{-1} CO_2 compensation point and 0.069 mol m^{-2} s^{-1} carboxylation efficiency. Leaves located on the 11th node of LR-0, LR-6 and LR-10 had respectively -5.30, -5.34 and -6.35 µmol m^{-2} s^{-1} respiration rate; 19.4, 17.8 and 22.9 µmol m^{-2} s^{-1} maximum assimilation; 73.4, 78.6 and 62.5 µmol mol^{-1} CO_2 compensation point; and 0.067, 0.061 and 0.075mol m^{-2} s^{-1} carboxylation efficiency (Table 4.9).

On 6 July, diurnal measurements of P_n, taken on the 7th leaf from the shoot base, did not differ between LR-0 and LR-6 at any point of time (Figure 4.6). The highest photosynthetic rate was recorded at 1000 hr (approximately 165 µmol m^{-2} s^{-1}), after which it gradually decreased and reached 11.5 µmol m^{-2} s^{-1}at 1800 hr. Intracellular partial pressure was

fluctuating between 220 and 240 μmol^{-1} and it did not differ between treatments (Figure 4.7). Stomatal conductance was in the range of 240 to 210 and it was decreased in LR-0 at 1430 hr (Figure 4.8).

Diurnal measurements of P_n, taken on the 11th leaf from the shoot base on 6 July, showed that LR-0, LR-6 and LR-10 had respectively 14.2, 15.85 and 14.4 μmol m^{-2} s^{-1} at 1000 hr (Figure 4.9). Two hours later, P_n was in the range of 13.0 to 14.5 μmol m^{-2} s^{-1}. While P_n in LR-0 was gradually decreasing over the day, LR-6 and LR-10 kept the same level of about 14.0 μmol m^{-2} s^{-1} between 1200 and 1630 hrs. At 1430 hr, P_n in LR-10 was significantly higher than in LR-0. Although not always significantly higher from LR-0, LR-10 had increased P_n during the day. For the period 1000 to 1800 hrs, treatments had similar C_i, which fluctuated between 216 and 243 μmol mol^{-1} (Figure 4.10). Stomatal conductance was 240, 259 and 2664 μmol m^{-2} s^{-1} respectively for LR-0, LR-6 and LR-10 at 1000 hr, and slightly increased to 244 and 270 μmol m^{-2} s^{-1} in LR-0 and LR-6 and to 298 μmol m^{-2} s^{-1} in LR-10 at 1200 hr (Figure 4.11). LR-6 and LR-10 kept the same level of g_s until 1430 hr when it started to decrease, while stomata in LR-0 started to close after 1200 hr and g_s reached its minimum of 187 μmol m^{-2} s^{-1} at 1800 hr. Treatment LR-0 had lower g_s than LR-10 at 1430 hr and lower g_s than LR-6 at 1800 hr.

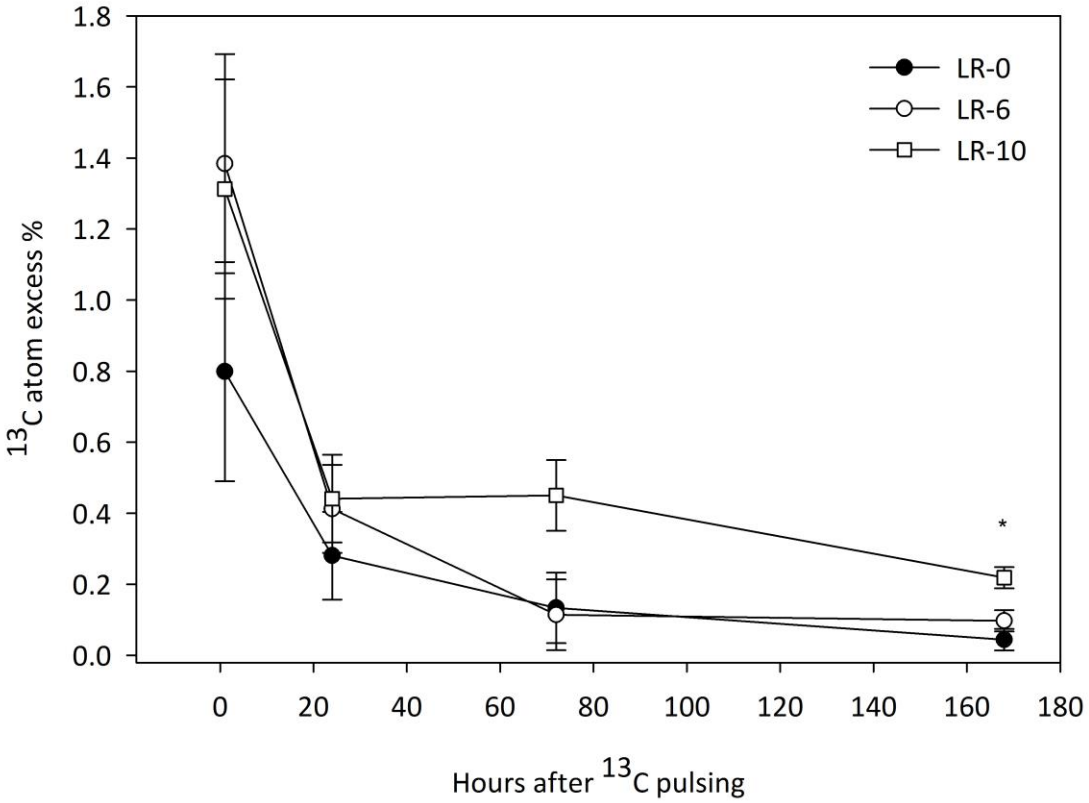

Figure 4.2 ^{13}C atom excess % analyzed in fully developed leaves during the week after pulsing. Means were based on 4 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

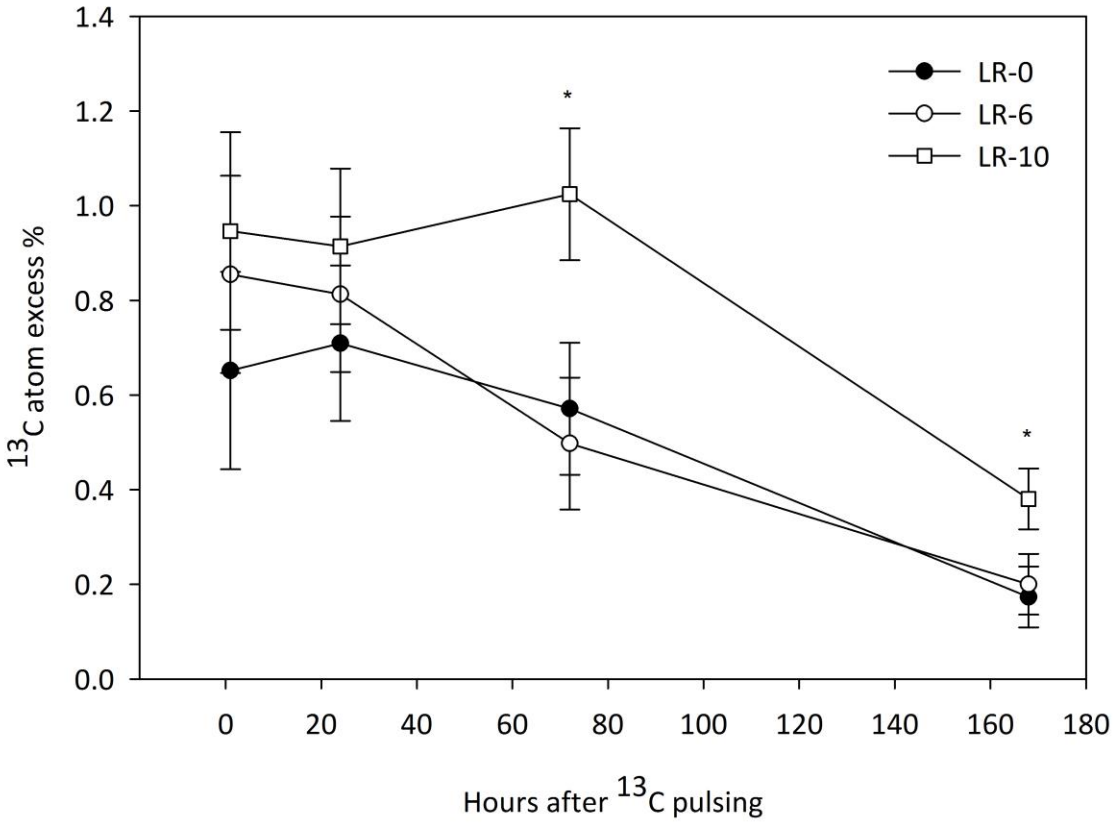

Figure 4.3 ^{13}C atom excess % in apical leaves and shoots tips during the week after pulsing. Means were based on 4 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

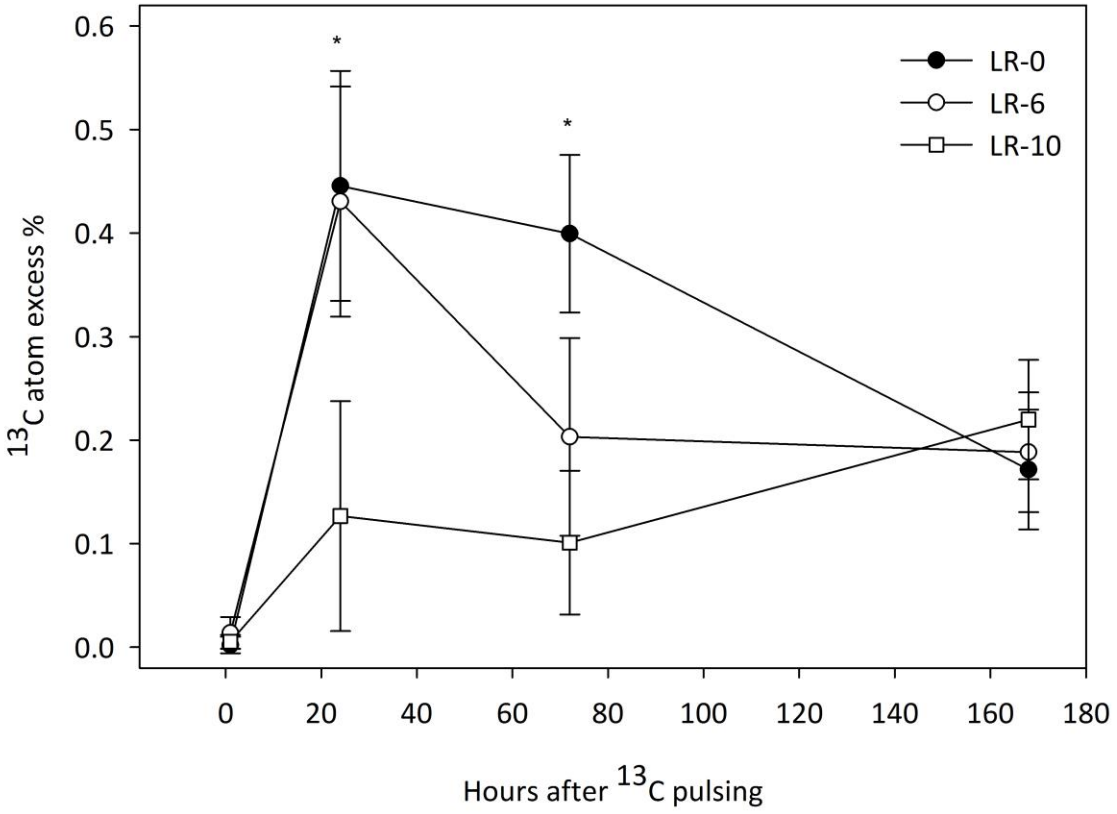

Figure 4.4 ^{13}C atom excess % analyzed in clusters during the week after pulsing. Means were based on 4 replicates. Significant effects of the treatment at time points were notified by an asterisk, p = 0.05. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

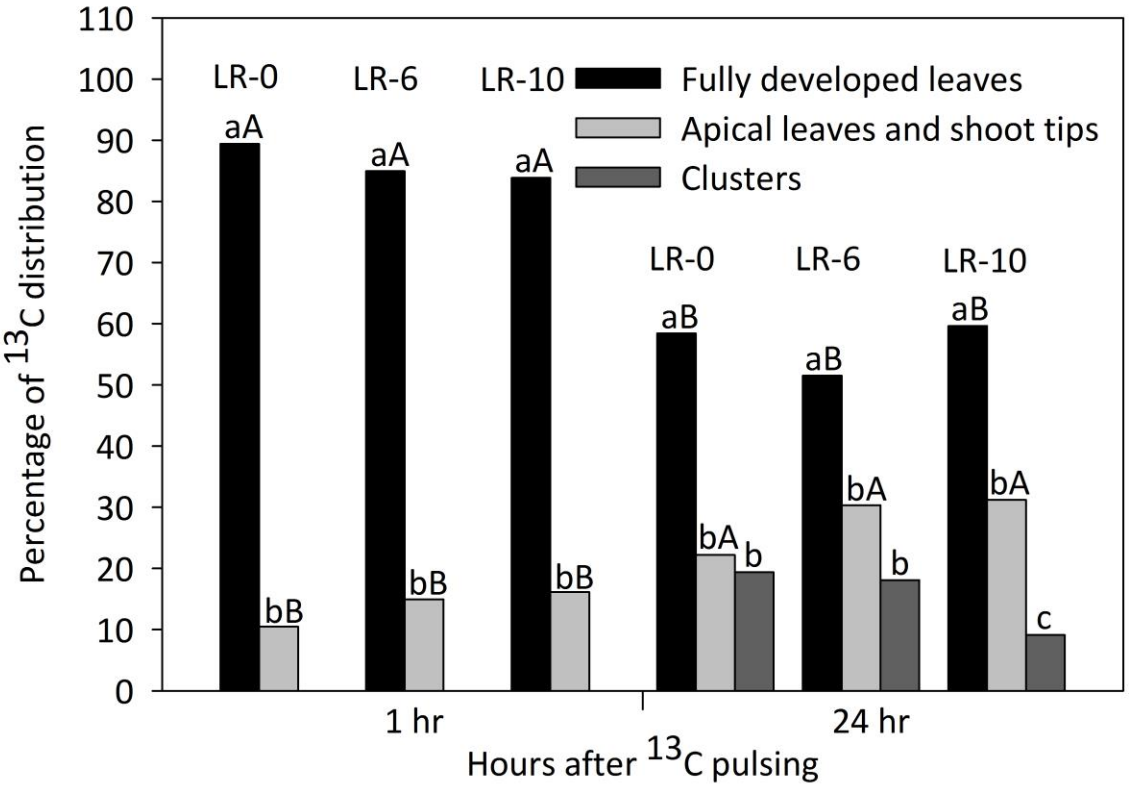

Figure 4.5 Percentage of ^{13}C distribution in fully developed leaves, apical shoots and clusters 1 hour and 24 hours after pulsing. Means were based on 4 replicates. The same upper case letters indicate no significant difference within the plant part, $p = 0.05$. The same lower case letter indicate no significant difference within the treatment, $p = 0.05$. LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

Table 4.1 Leaf assimilation (P_n), stomatal conductance (g_s), and intracellular partial pressure of CO_2 (C_i) on 14 June 2011.

Treatment[z]	P_n ($\mu molCO_2 m^{-2} s^{-1}$)[y]	g_s ($\mu molCO_2 m^{-2} s^{-1}$)	C_i ($\mu mol\ CO_2 mol^{-1}$)	Pn/leaf
LR-0	8.7[x] ns	150.4 ns	248.4 ns	0.0933 ns
LR-6	8.6	137.0	231.4	0.1082

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 4 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.2 Leaf assimilation (P_n), stomatal conductance (g_s), and intracellular partial pressure of CO_2 (C_i) on 14 June 2011.

Treatment[z]	P_n ($\mu molCO_2 m^{-2} s^{-1}$)[y]	g_s ($\mu molCO_2 m^{-2} s^{-1}$)	C_i ($\mu molCO_2 mol^{-1}$)	Pn/leaf
LR-0	2.5[x] ns	82.8 ns	291.5 ns	0.0033 ns
LR-6	2.8	91.0	294.6	0.0165
LR-10	2.5	84.4	294.8	0.0151

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 4 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.3 Influence of early defoliation on leaf respiration on 15 June 2011.

Treatment[z]	Respiration rate of the leaf on 7th node ($\mu molCO_2 m^{-2} s^{-1}$)[y]	Respiration rate of the leaf on 11th node ($\mu molCO_2 m^{-2} s^{-1}$)
LR-0	-0.46[x] ns	-0.84 ns
LR-6	-0.40	-0.62
LR-10	-	-0.78

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 5 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.4 Influence of early defoliation on leaf assimilation (P_n), stomatal conductance (g_s), and intracellular partial pressure of CO_2 (C_i) on 21 June 2011.

Treatment[z]	P_n ($\mu mol\ CO_2 m^{-2} s^{-1}$)[y]	g_s ($\mu mol\ CO_2 m^{-2} s^{-1}$)	C_i ($\mu mol\ CO_2 mol^{-1}$)	Pn/leaf
LR-0	13.7[x] ns	267.5 ns	234.7 ns	0.1746 ns
LR-6	15.0	274.6	237.1	0.2026

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 4 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.5 Influence of early defoliation on leaf assimilation (P_n), stomatal conductance (g_s), and intracellular partial pressure of CO_2 (C_i) on 21 June 2011.

Treatment[z]	P_n ($\mu molCO_2 m^{-2} s^{-1}$)[y]		g_s ($\mu mol\ CO_2 m^{-2} s^{-1}$)		C_i ($\mu mol\ CO_2 mol^{-1}$)		Pn/leaf	
LR-0	9.7[x]	b	241.7	b	262.0	ns	0.0988	b
LR-6	10.9	b	284.2	a	262.4		0.1092	b
LR-10	13.8	a	301.6	a	252.0		0.1643	a

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 4 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.6 Influence of early defoliation on leaf assimilation (P_n), stomatal conductance (g_s), and intracellular partial pressure of CO_2 (C_i) on 25 June 2011.

Treatment[z]	P_n ($\mu mol\ CO_2 m^{-2} s^{-1}$)[y]		g_s ($\mu mol\ CO_2 m^{-2} s^{-1}$)		C_i ($\mu mol\ CO_2 mol^{-1}$)		Pn/leaf	
LR-0	11.6[x]	ns	177.5	b	214.1	ns	0.1543	ns
LR-6	12.7		217.9	a	224.0		0.1658	

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 4 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.7 Influence of early defoliation on leaf assimilation (P_n), stomatal conductance (g_s), and intracellular partial pressure of CO_2 (C_i) on 25 June 2011.

Treatment[z]	P_n ($\mu molCO_2 m^{-2} s^{-1}$)[y]		g_s ($\mu molCO_2 m^{-2} s^{-1}$)		C_i ($\mu mol\ CO_2 mol^{-1}$)		Pn/leaf	
LR-0	8.4[x]	b	127.1	c	219.9	ns	0.0944	b
LR-6	10.0	b	164.8	b	225.9		0.1162	b
LR-10	12.3	a	214.4	a	225.6		0.1523	a

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Means were based on 4 reps.

[x] Means within the column followed by the same letter are not significantly different at $p = 0.05$ by t-test; ns = no significant difference.

Table 4.8 Influence of early defoliation on leaf respiration point, maximum assimilation, CO_2 compensation point, and carboxylation efficiency on 26 June 2011.

Treatment[z]	Respiration rate ($\mu mol\ CO_2 m^{-2}\ s^{-1}$)	Amax[x] ($\mu mol\ CO_2 m^{-2}\ s^{-1}$)	CO_2 compensation point ($\mu mol\ CO_2 mol^{-1}$)	Carboxylation efficiency ($mol\ CO_2 m^{-2}\ s^{-1}$)
LR-0	-5.32[y]	21.6	57.6	0.070
LR-6	-5.54	23.3	63.7	0.069

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes at bloom.

[y] Measurements taken on the seventh node leaf.

[x] Maximum assimilation.

Table 4.9 Influence of early defoliation on leaf respiration point, maximum assimilation, CO_2 compensation point, and carboxylation efficiency on 26 June 2011.

Treatment[z]	Respiration rate ($\mu molCO_2 m^{-2}\ s^{-1}$)	Amax[x] ($\mu molCO_2 m^{-2}\ s^{-1}$)	CO_2 compensation point ($\mu molCO_2 mol^{-1}$)	Carboxylation efficiency ($molCO_2 m^{-2}\ s^{-1}$)
LR-0	-5.30[y]	19.4	73.4	0.067
LR-6	-5.34	17.8	78.6	0.061
LR-10	-6.35	22.9	62.5	0.075

[z] LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

[y] Measurements taken on the eleventh node leaf.

[x] Maximum assimilation.

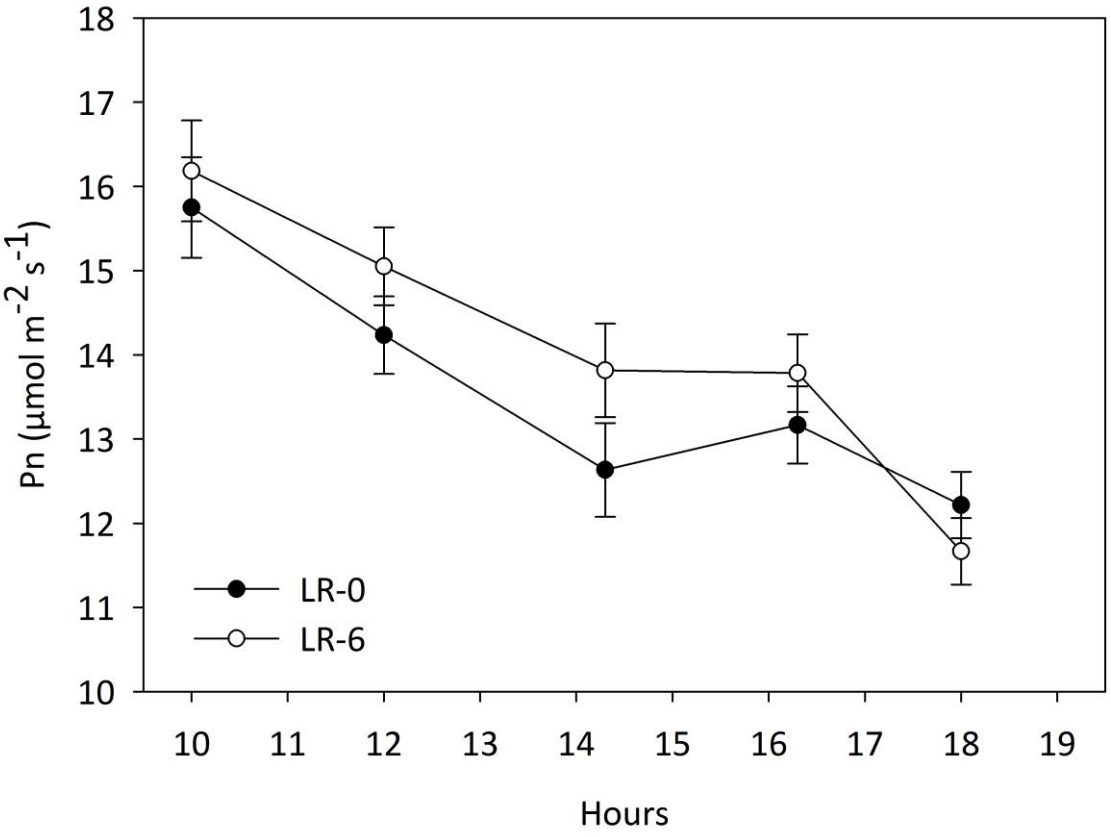

Figure 4.6 Daily leaf assimilation (P_n) of leaf positioned on seventh node on 6 July 2011. Means were based on 3 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes at bloom.

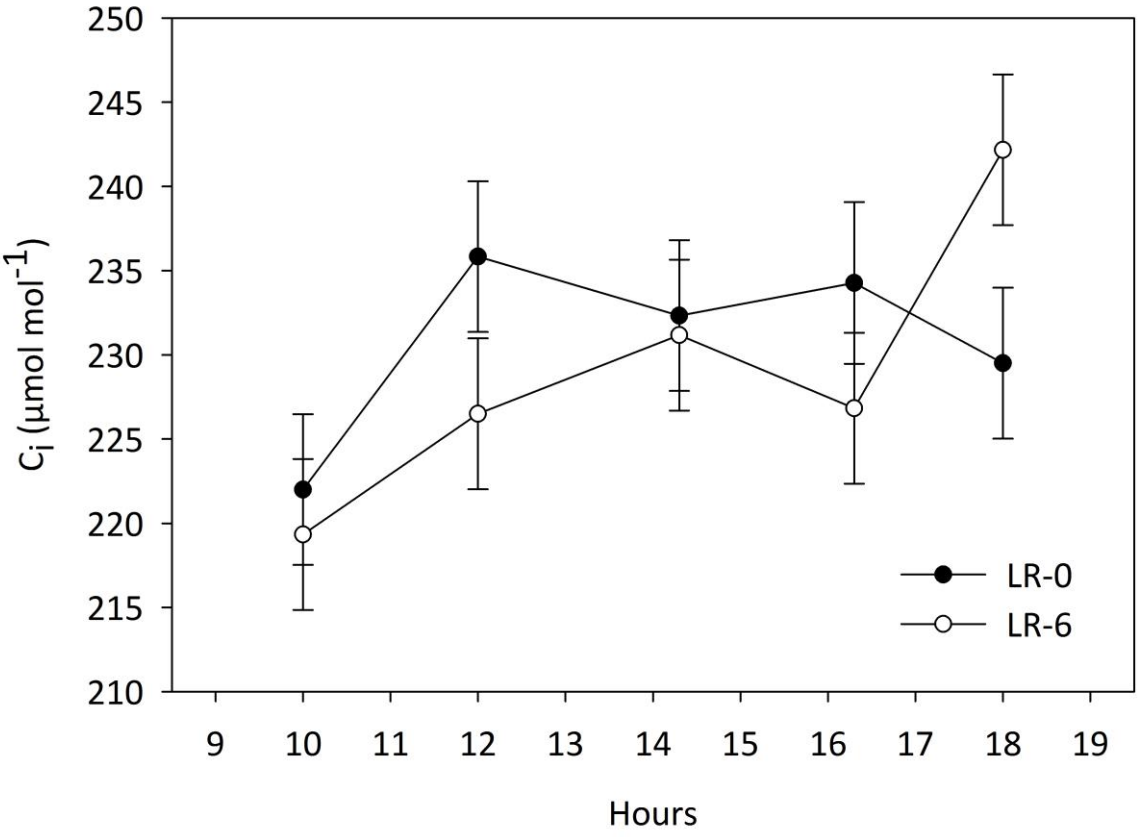

Figure 4.7 Daily values of intracellular partial pressure of CO_2 (C_i) taken on leaf positioned on seventh node on 6 July 2011. Means were based on 3 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes at bloom.

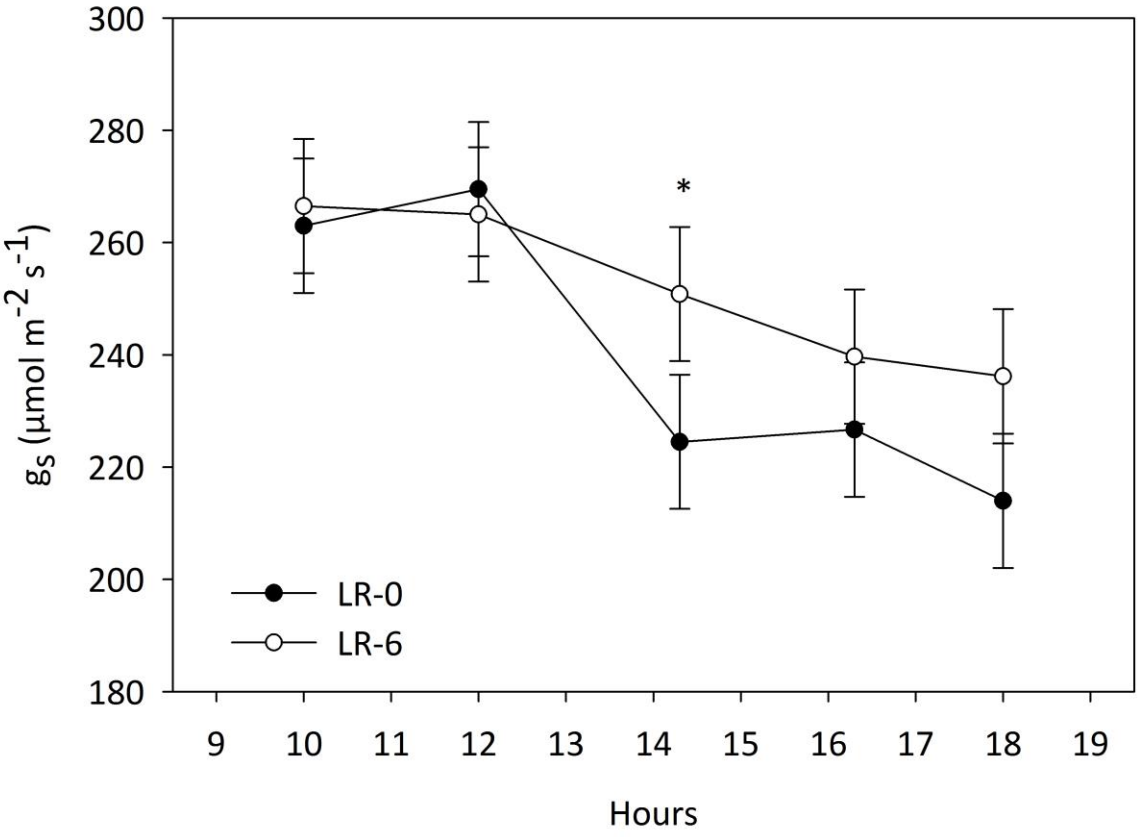

Figure 4.8 Daily stomatal conductance (g_S) taken on leaf positioned on seventh node on 6 July 2011. Means were based on 3 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes at bloom.

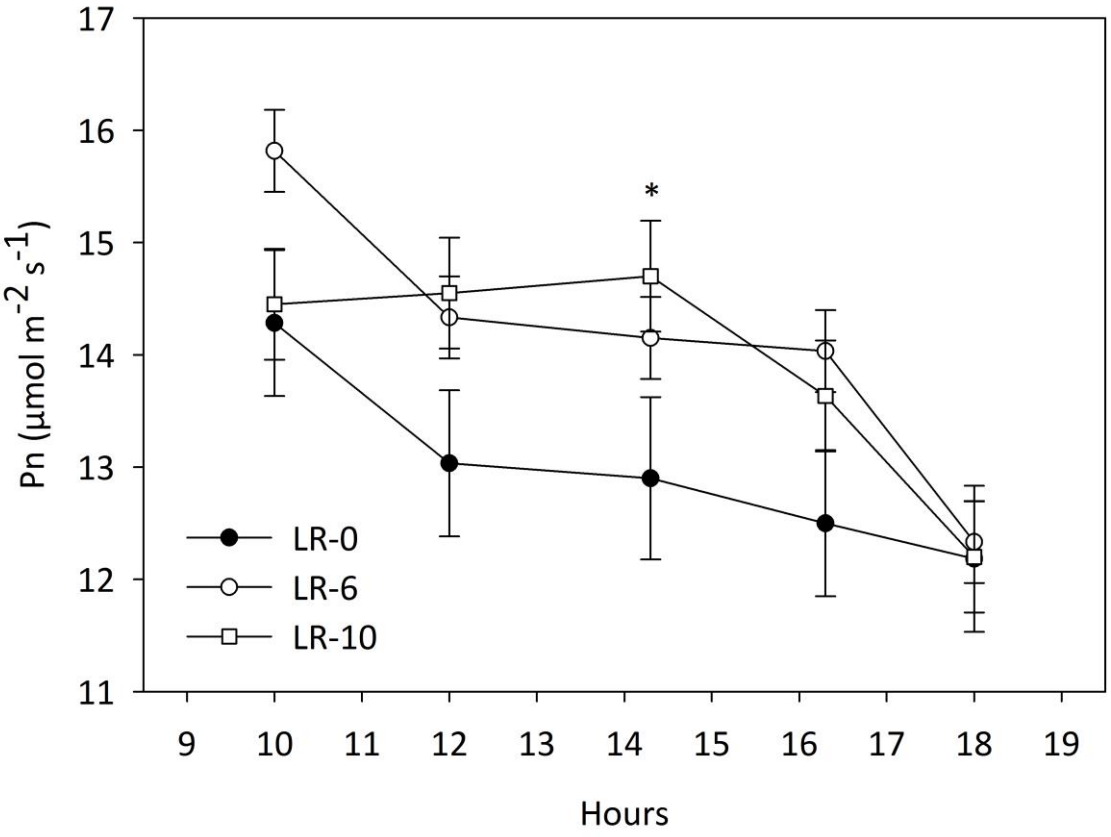

Figure 4.9 Daily leaf assimilation (P_n) of leaf positioned on eleventh node on 6 July 2011. Means were based on 3 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

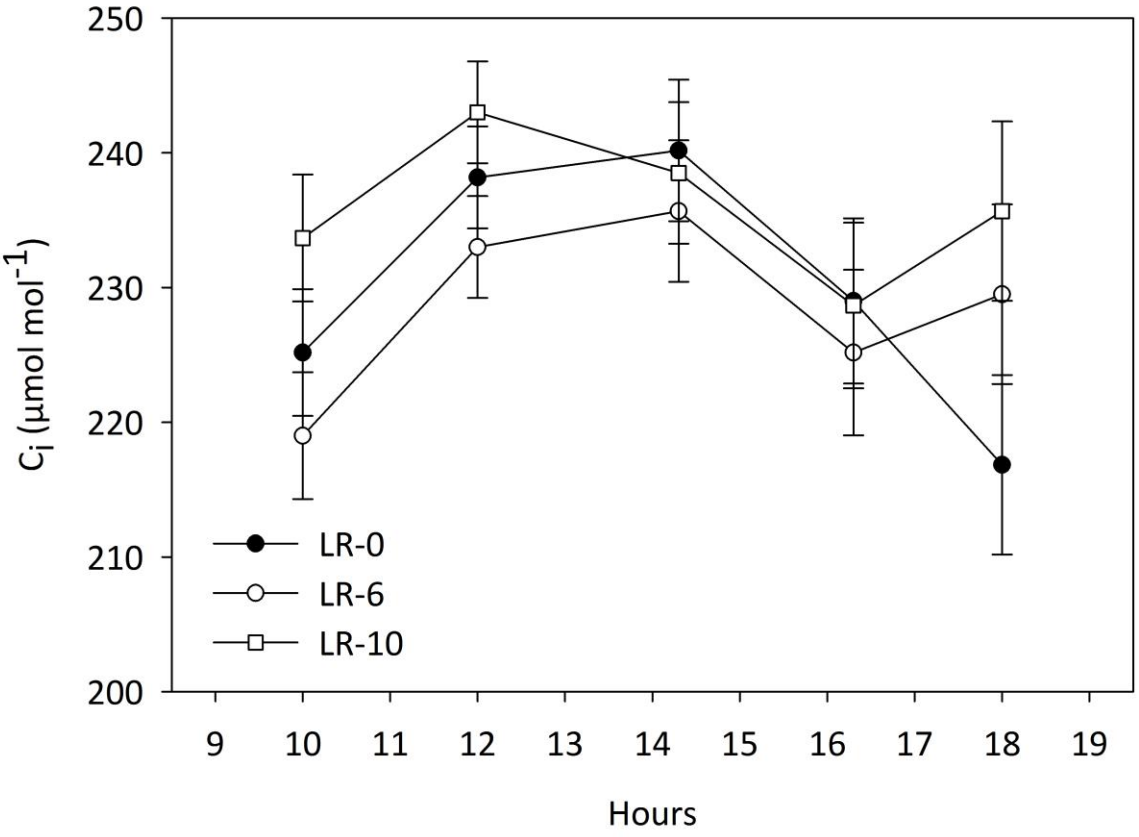

Figure 4.10 Daily variation of intracellular partial pressure of CO_2 (C_i) taken on leaf positioned on eleventh node on 6 July 2011. Means were based on 3 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

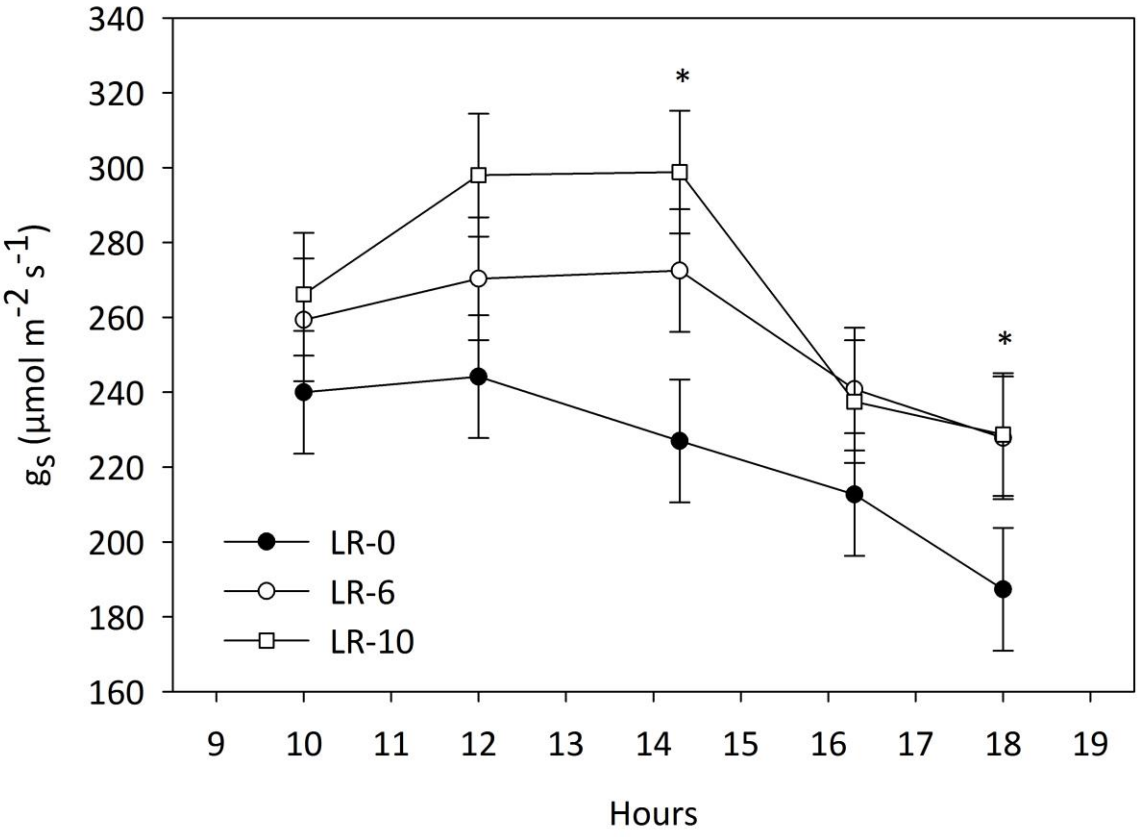

Figure 4.11 Diurnal measurements of stomatal conductance (g_S) taken on leaf positioned on seventh node on 6 July 2011. Means were based on 3 replicates. Significant effects of the treatment at time points were notified by an asterisk, $p = 0.05$. Error bars represent standard error of the mean (SE). LR-0 = no leaves removed; LR-6 = leaves removed from 6 basal nodes; LR-10 = leaves removed from 10 basal nodes at bloom.

4.4 Discussion

The translocation of photoassimilates in grape shoots can be acropetal, basipetal or bidirectional with the direction determined by developmental stage and the relevant sink-source relationship. Before bloom, the direction of photoassimilate translocation is exclusively acropetal to support fast growing shoots and their sink demand. At bloom only the leaves in the terminal portion of the shoots transport photoassimilate to shoot tips. Several leaves below the shoot tips keep a bidirectional translocation pattern for a few days, after which they switch to exporting photoassimilates toward the parent vine (Hale and Weaver, 1962). After fruit set, leaves between clusters and parent vine also use a bidirectional translocation pattern (Hale and Weaver, 1962). At pea-size, a stage of cluster development, the direction of photoassimilate translocation in the apical, middle and basal leaves is mainly basipetal to support berry demand for carbohydrates (Hunter and Visser, 1988a).

Approximately 2 weeks before bloom, the growth of inflorescence starts to rapidly decrease and inflorescence photosynthesis, which is significant at the beginning of inflorescence development, declines together with chlorophyll content and reaches its minimum at fruit set (Lebon et al., 2005; Lebon et al., 2008). The decrease in the rate of photosynthetic activity corresponds with the decreased sink strength of the inflorescence as the young developing leaves and the vine itself dominate and work to restore depleted reserves (Candolfi-Vasconcelos et al., 1994). Hale and Weaver (1962) found no translocation of photoassimilates into the inflorescence from surrounding leaves up to 10 days before bloom. Decreased sink strength of the inflorescence could also be attributed to its relatively small requirement for photoassimilates at pre-bloom.

Shoot basal leaves are the highest contributors of photoassimilates to the clusters at all stages of development (Hunter and Visser, 1988a). One week before bloom to 2 weeks after bloom, leaves that were located on the same side right below and above the cluster distributed the highest amount of ^{14}C-assimilated to the cluster of the total ^{14}C-assimilated found (Motomura, 1990). Three weeks after bloom, the contribution of more distant leaves situated on the cluster side increased as well (Motomura, 1990).

In our work, we found that 1 hour after the shoots had been fed with $^{13}CO_2$, ^{13}C atom excess% was the highest in mature leaves, then in the apical leaves and shoot tips, while clusters, although directly exposed to the $^{13}CO_2$, did not show a notable increase of ^{13}C atom% compared to the natural abundance of ^{13}C found in plant tissues (Figures 4.2 to 4.4). Mature leaves had 84 to 90% of the distribution of the shoot total amount of ^{13}C one hour after pulsing, while shoot tips had 10 to 16% and clusters less than 1%, respectively (Figure 4.5).

Leaf net assimilation, measured 24 hours before defoliation, was similar in all vines for the same node position (Tables 4.1 and 4.2). The values for 11^{th} leaf P_n were notably low due to the fact that these leaves were still young and intensively growing (Table 4.2). They soon reached their final size, which corresponded with the increase in P_n. However, this increase in P_n was significantly higher in LR-10, 7 and 10 days after treatment application (Tables 4.4 and 4.6). This increase in the photosynthesis of the 11^{th} leaf corresponds with data obtained by Candolfi-Vasconcelos and Koblet (1991), where defoliation of basal leaves, a week after bloom,

stimulated CO_2 assimilation in the 11th leaf and the P_n increase was more pronounced as more leaves were removed. Hunter and Visser (1988b) also found that on partially defoliated Cabernet Sauvignon vines, where the first leaf of every three or the first two leaves of every three were removed, photosynthetic activity of all the remaining leaves increased with increased defoliation level.

The drastic decline in source to sink ratio caused by ten leaves removed in our experiment was a signal strong enough to induce the Pinot noir leaf to respond with a higher assimilation rate. In contrast, six leaves removed showed a negligible increase of assimilation rate of leaves positioned on the 7th and 11th nodes compared to the non-defoliated control (Tables 4.4 to 4.7). Our results agree with those of Palliotti et al. (2011), where pre-bloom defoliation of Sangiovese vines (leaves removed on basal nodes up to two nodes above distal cluster) did not trigger a significant change of leaf P_n, probably due to the insufficient defoliation pressure. However, when the whole-canopy gas exchange was measured in Sangiovese, six leaves removed from the base of the shoots at pre-bloom stage reduced net carbon exchange rate (NCER) per vine (Poni et al., 2008). Approximately 2 weeks later (fruit set), NCER per vine was found to be equal to the non-defoliated control. Moreover, NCER per leaf area unit was only temporarily reduced by defoliation and 3 days after the defoliation was significantly higher than the control and reached the highest level at fruit set (Poni et al., 2008).

This abrupt decrease of NCER per vine caused by pre-bloom defoliation of six leaves in Sangiovese was effective in reducing berry number, fruit set, and cluster compactness, but did not change berry size (Poni et al., 2008). In contrast, we found that defoliation of six leaves at

bloom in Pinot noir did not present a sufficient source limitation to cause significant reduction of fruit set nor cluster compactness (See Chapter 2, Tables 2.7 and 2.9). Removing 8 or more leaves in Pinot noir was instead necessary to decrease the fruit set and cluster compactness of this cultivar though, again, berry size and total anthocyanins were not modified by the leaf removal treatment (See Chapter 2, Tables 2.7, 2.9 and 2.10). Moreover, when the same levels of defoliation were applied in the following year, vines responded to the removal of eight leaves with only decreased berry number (See Chapter 3, Table 3.11), but fruit set was significantly changed with ten leaves removed compared to the control (See Chapter 3, Table 3.7). The second year's defoliation, however, did impact berry size also (See Chapter 3, Table 3.9). Candolfi-Vasconcelos et al. (1994) found a lower photosynthetic rate in Pinot noir vines defoliated in the previous season due to their weak initial spring growth. Depleted vine reserves and, possibly, a low assimilation rate on top of leaf removal in the second year were used to explain the reduction in the berry size in our study.

The increased leaf assimilation found in the most developed leaf of LR-10 (11^{th} leaf) did not result in increased ^{13}C atom excess%, hence ^{13}C atom excess% located in LR-10 leaves did not significantly differ from LR-0 and LR-6 (Figure 4.1). ^{13}C atom excess% in leaves of LR-0, although not significant, was lower compared to LR-6 and LR-10, most likely due to the larger leaf area that was present in LR-0 (34 and 69% more than LR-6 and LR-10, respectively) and a dilution effect that the large leaf area had on total amount of ^{13}C found in the control leaves. The day after pulsing, ^{13}C atom excess% rapidly decreased in the mature leaves and increased

in the clusters, while it stayed steady in the shoot tips for all treatments. For the same period, percentage of distribution of the total amount of ^{13}C found in the whole shoot did not differ among treatments for the same plant part and it was in the range between 51 to 60% for the leaves, 22 to 31% for the shoot tips and 9 to 19% for the clusters (Figure 4.5).

Due to the fact that the feeding with labeled $^{13}CO_2$ was performed only once, the highest amount of ^{13}C released from the mature leaves to supply the sink on the shoots occurred mainly during the period 24 hours after the application (Figure 4.2). Parallel with that, clusters received the highest amount ^{13}C 24 hours after the pulsing (Figure 4.4), after which ^{13}C atom excess% was declining due to the respiration loss, though it is also possible that translocation of ^{13}C to other vine parts could have occurred. ^{13}C atom excess% increase was remarkably lower in clusters of LR-10 one day later, and this difference in ^{13}C atom excess% between LR-0 and LR-10 was detected 5 days after pulsing as well. It is possible that the remaining portion of mature leaves in LR-10 could not sufficiently supply the clusters either because their reduced size or their distance from the cluster, or both, degraded their ability to partition and redirect assimilates toward the shoot tips (Motomura, 1990).

Three days after the labeling, ^{13}C atom excess% in the shoot tips of LR-10 was still at the high level (1.0%) it was after 1 and 24 hours, while a slight decrease (from 0.8 to 0.6%) was noticed in LR-6 and LR-10. This greater amount of carbon, registered in LR-10 shoot tips, could be attributed to the slow release of ^{13}C and its translocation from the developed leaves. For

the next 4 days ^{13}C atom excess% gradually decreased in all treatments due to either respiration loss or/and translocation, but the level of ^{13}C stayed continuously higher in the shoot tips of LR-10 compared to those of LR-6 and LR-10.

Seven days after the labeling, ^{13}C atom excess% reached a stable level of approximately 0.2% in the clusters of all treatments, meaning that 0.2% of ^{13}C atom excess was used for the building of complex C-molecules that would stay incorporated in the clusters tissues for a longer period rather than being lost as CO_2 via respiration. Although the same ^{13}C atom excess% was present in the clusters of all treatments, LR-10 had notably smaller clusters (65% compared to the control), indicating that a smaller amount of carbon was integrated into them.

The carbon partitioning in LR-10 directed toward the shoot tips at the expense of cluster size was clearly seen in Figure 4.5. Firstly, note that the percentage of ^{13}C distribution in the leaves 1 day after pulsing was in the range of 51 to 59% for all treatments. In the control, the other 42% of total ^{13}C found in the shoots was nearly equally distributed between the shoot tips (22%) and clusters (19%). In LR-6, however, the shoot balance of total ^{13}C found was more heavily directed toward the shoot tips (30%) as opposed to clusters (18%). Finally, In LR-10 the remaining portion of total ^{13}C was also located mostly in the shoot tips (31%), but, interestingly and in contrast, the cluster amount decreased significantly to 9% having given up more to the leaves (60%). Secondly, ^{13}C atom excess% in apical leaves and shoot tips were remarkably higher in LR-10 compared to LR-6 and LR-0 both 3 and 7 days after pulsing (Figure 4.3), while

^{13}C atom excess% in LR-10 clusters was notably lower 1 and 3 days after pulsing (Figure 4.4). This reduced ^{13}C atom excess% found early in clusters of LR-10 corresponds to the decrease in fruit set obtained by the removal of ten leaves (See Chapter 2, Table 2.6). On the other hand, the similar values for ^{13}C atom excess% found in clusters of LR-6 and LR-10 reflects the same level of fruit set achieved by these two treatments (See Chapter 2, Table 2.6). Furthermore, the difference in fruit set obtained by early leaf removal on ten nodes led to reduced cluster size and compactness index (CI) in LR-10 compared to the two other treatments (See Chapter 2, Tables 2.8 and 2.9).

Diurnal measurements of photosynthesis parameters taken on 6 July showed that stomatal conduction in LR-0 was lower at 1430 hr regardless of leaf position and it could be attributed to the fact that LR-0 had the highest leaf surface and, presumably, the highest transpiration rate compared to LR-6 and LR-10 under the same conditions with regard to the size of the roots and water supply. However, this reduction in stomatal conductance would not be sufficient to cause a decrease in assimilation rate in LR-0, rather it should be attributed to the feedback inhibition effect (Figures 4.6 to 4.11). Photosynthetic parameters derived from CO_2 response curves, which were constructed upon readings taken on 7^{th} and 11^{th} leaves, fall in a range found for normal healthy leaves (During, 1994; Schultz HR et al., 1996).

4.5 Conclusion

The objective of this study was to investigate the influence of early defoliation on leaf assimilate translocation along the shoots and on partitioning patterns among the sinks: clusters and shoot tips. The experiment showed that the carbon partitioning in LR-10 was directed toward the shoot tips at the expense of cluster. The percentage of ^{13}C distribution in the leaves 1 day after pulsing was in the range of 51 to 59% for all treatments. In the control, the other 42% of total ^{13}C found in the shoots was nearly equally distributed between the shoot tips (22%) and clusters (19%). In LR-6, however, the shoot balance of total ^{13}C found was more heavily directed toward the shoot tips (30%) as opposed to clusters (18%). Finally, In LR-10 the remaining portion of total ^{13}C was also located mostly in the shoot tips (31%), but, interestingly and in contrast, the cluster amount decreased significantly to 9% having given up more to the leaves (60%).

The severe defoliation in LR-10 lead to a higher assimilation rate of 11^{th} leaf. In contrast, six leaves removed showed a negligible increase of assimilation rate of leaves positioned on the 7^{th} and 11^{th} nodes compared to the non-defoliated control. However, the higher assimilation rate of the fully developed leaves in LR-10 could not compensate the loss in the total leaf area, and together with assimilates partitioning, which was preferably directed toward the shoot tips, caused a reduction in fruit set in LR-10. On the other hand, the similar assimilates distribution between clusters and shoot tips found in LR-0 and LR-6 reflects the same level of fruit set achieved by these two treatments.

5 FRUIT SET AND CLUSTER MORPHOLOGY AS A FUNCTION OF SOURCE AVAILABILITY AND SINK REQUIREMENT OF PINOT NOIR CLONE UCD29

5.1 Introduction

During bloom and fruit set, nutrition of flower ovaries in grapevine is conditioned by photoassimilates and stored carbohydrate reserves (Lebon et al., 2008). The suboptimal availability of carbohydrates leads to compromised embryo development and abortion resulting in reduced fruit set (Lebon et al., 2008). Instead, developing clusters may attract assimilates from their shoot of origin, and from adjacent shoots, roots, and trunk when carbohydrate resources are restricted (Mansfield and Howell, 1981; Hunter and Visser, 1988a; Candolfi-Vasconcelos et al., 1994). However, inflorescences are a weak sink and their capability to attract nutrients is considerably reduced making them sensitive to low source availability and, consequently, leading to poor fruit set (Hale and Weaver, 1962, Candolfi-Vasconcelos and Koblet, 1990).

Average fruit set for grapevines is about 50%, but subsequent coulure (aka shatter) due to subpar physiological or environmental conditions can reduce fruit set values ever further, below 30% (May, 2004). However, under favorable conditions, Pinot noir can set more berries than other cultivars, up to 65 %, resulting in high cluster compactness (Lebon et al., 2004). Due to the presence of reserves (starch granules) in its ovules and anthers (Lebon et al., 2004), this

cultivar is known for its low sensitivity to the reduced availability of carbohydrates and its high capability to set more berries compared to other sensitive varieties (e.g., Gewurztraminer).

Additionally, carbohydrates stored in permanent vine components play an important role in berry setting. Reserves, mainly stored in roots in the form of starch and amino acids, are mobilized during early spring shoot growth and used to support the development of inflorescences and other annual organs (Zapata et al., 2004). Depletion of reserves coincides with flowering and it is cultivar specific (Zapata et al., 2004). When reserves are depleted, permanent vine organs become sinks and start competing with inflorescences and growing shoots for available photoassimilates. For Pinot noir that point occurs at early bloom when vine sinks become pre-dominantly supported by carbohydrates assimilated by leaves. In contrast, in Merlot vines, growing organs are dependent on root reserves until pea berry size making this variety more prone to fruitlet abscission under challenging climatic conditions.

One of the methods for fruit set enhancement in sensitive cultivars is trunk girdling. This is a common practice used not only to improve fruit set, but to increase berry size and improve fruit composition by limiting the root's demand for photoassimilates in favor of cluster development (Coombe, 1959; Harrell and Williams, 1987). Similar effects were achieved with shoot girdling by Caspari et al. (1998). For example, shoot girdling in Sauvignon blanc with no leaf removal resulted in 55% fruit set compared to 33% in the non-girdled/non-defoliated control (Caspari et al., 1998). Leaf removal on girdled shoots reduced fruit set from 44% to 0%, depending on the number of defoliated nodes. In contrast, defoliated but non-girdled shoots had fruit set equivalent to the control, suggesting that non-girdled shoots used reserves from the vine and translocation of carbohydrates from permanent structures may occur.

To summarize, greater berry setting in Pinot noir and its relative tolerance to the decrease in source to sink ratio during fruit set could be attributed to three sources: the presence of starch granules in the ovules and anthers (Lebon et al., 2004), the early transition from heterotrophic to autotrophic allocation of nutrients (Zapata et al., 2004), and, possibly, significant translocation of stored nutrients in permanent vine parts to the cluster at berry set stage. Contributions of assimilates stored in roots, canes and trunk or produced by leaves on distant shoots to the inflorescences in bloom and their influence on fruit set in Pinot noir were not sufficiently explored by the literature. To fill that gap, we aimed to test the following hypothesis: during fruit set inflorescences located on the defoliated side of the cordon could attract available nutrients from the other foliated side of the cordon and use it to set berries. We designed a study in which we used Pinot noir vines trained to VSP with bilateral cordons. On one half of the vine, i.e., one cordon side, we manipulated the sinks, while on the other cordon side we manipulated the sources (Figures 5.1 to 5.2). Thus, the influence of carbohydrates reserves on cluster development after bloom was evaluated in an indirect manner via a comparison of the percentage of fruit set.

5.2 Material and Methods

5.2.1 Plant Material and Experimental Design

Twenty-year old *V. vinifera* vines cv. Pinot noir (clone UCD29) grafted on C3309 rootstock were used in this study during 2011 and 2012. The vineyard was located at the Southwest Michigan Research and Extension Center (SWMREC; lat. 40°09′ N; long. 86°36′ W; elevation 220 m) near Benton Harbor, Michigan. Vines were planted in Spinks loamy fine soil (U.S. Department of Agriculture, Soil Conservation Service, 1957), with a spacing of 1.8 m between vines and 3.0 m between rows, and trained to a vertical shoot positioning system (VSP). Vines were spur-pruned during the winter leaving approximately sixty buds per vine. Recommended crop protection practices were followed and the pest management program was based on scouting, experience and weather conditions. During bloom, to avoid potential mechanical damage to flowers by the sprayer, no application of fungicide or insecticide was performed. Relevant meteorological data were recorded during the experiment by an automated weather station from the Michigan Automated Weather Network (MAWN) located on the site at 120 m from the experimental vineyard. Total monthly precipitation, daily precipitation, daily minimum, maximum, and average temperature and Growing Degree Days (GDD) calculated with the Baskerville-Emin method using a base temperature of 10°C (Baskerville and Emin, 1969). No irrigation was used and standard summer vineyard practices were applied.

The experiment was arranged as a randomized complete block design with two categorical factors, leaf removal (L) and sink removal (S). Leaf removal consisted of two levels of

defoliation: no leaves removed (LNO) and leaves removed from 10 basal nodes (LYES). Leaves were removed only from the shoots on one cordon side, which we call the source side. The second factor, sink removal, also had two levels: sinks present (SNO) and sinks removed (SYES). Sinks encompassed all clusters and shoot tips found on the side of the cordon positioned opposite the source side, which is named the sink side. At the time of the application of treatments, leaves were accordingly removed or retained on the source side and inflorescences and shoot tips were removed or retained on the sink side, respectively (see Figures 5.1 and 5.2). Approximately 3 weeks before bloom, the number of inflorescences was adjusted to be equal on each side of the cordon and vines were organized in blocks by the total number of inflorescences per vine. After blocking, vines were tagged, and then each treatment combination was randomly assigned to three vines in 2011 and to four vines in 2012. Additionally, a sub-sample of three shoots per source side of each vine was randomly chosen and tagged for the taking of detailed measurements of shoot length, degree of fruit set, cluster parameters, and fruit chemistry. Treatments were applied at full bloom, developmental stage 23 after Eichhorn and Lorenz (1977). Vines were trimmed on July 25, Day 206 of the year (DOY) in both 2011 and 2012. Timing of budburst, bloom, pea size berries, and harvest were also recorded (Table 5.1) and the number of inflorescences and shoots per vine before treatment application were counted (Table 5.3).

5.2.2 Estimation of Leaf Area

Shoot length was measured weekly over a period of 40 days, starting from June 7 in 2011 and from June 3 in 2012, which corresponded to approximately one week before bloom,

up to July 13 (in both years), one month after bloom. A sample of ten or more shoots, collected weekly from guard vines, was used for estimation of the total leaf area (LA) per shoot. Leaves removed using each defoliation level were collected in ziplock bags and transported to campus. In the laboratory, total LA was determined by measuring the single LA with a leaf area meter (LI-3050AHS, Lambda Instruments Corporation, Nebraska). A linear relationships between the LA (y) and shoot length (x): $y = 19.2x - 455.1$, $R^2 = 0.91$ (for 2011) and $y = 17.2x - 218.1$, $R^2 = 0.64$ (for 2012) was used for estimation of total LA (Figures 5.3 and 5.4). After defoliation, LA removed per shoot was measured and subtracted from total LA for calculation of retained LA.

5.2.3 Estimation of Fruit Set

At developmental stage 20 (onset of bloom), after Eichhorn and Lorenz (1977), basal clusters were photographed against a dark background. The same procedure was repeated at developmental stage 31 (berries pea size). Samples of twenty clusters at developmental stage 20 and twenty clusters at stage 31 from the guard vines were also photographed against a dark background and then separately collected in ziplock bags and transported to the laboratory. Using the same methodology described by Poni et al. (2006), the actual number of florets and berries were destructively counted. The number of florets and berries visible in the photos were counted using Microsoft Office Paint (Windows XP). The linear relationships between the actual number of florets (y) and the counted florets (x): $y = 1.85x$, $R^2 = 0.86$ (for 2011) and $y = 1.49x$, $R^2 = 0.87$ (for 2012, Figures 5.5 and 5.6); and actual number of berries (y) and counted berries (x) in the photos: $y = 1.58x$, $R^2 = 0.90$ (for 2011) and $y = 1.30x$, $R^2 = 0.94$ (for 2012,

Figures 5.7 to 5.8) were used to estimate the initial number of florets and set berries of each basal cluster per tagged shoot.

The percentage of fruit set was expressed in two ways: percentage of fruit set at developmental stage 31 (FS-31) and percentage of fruit set at developmental stage 38, harvest (FS-38). FS-31 was calculated as the ratio between the estimated number of set berries three weeks after bloom and the estimated number of florets. FS-38 was calculated as a ratio between the number of berries at harvest and the estimated number of florets.

5.2.4 Cluster Parameters and Morphology

At harvest, basal clusters on tagged shoots were individually collected in ziplock bags then taken to the laboratory and weighed. Berries were separated from the rachis and total berry numbers, total berry weights, and rachis weights were recorded. Rachis length was calculated as the sum of the central axis length (inner arm), lateral wing or shoulder length (outer arm), and secondary branch length (if they were longer than 5 mm). The number of secondary branches on the inner and outer was also recorded. Cluster compactness was expressed in two different ways: as the ratio between the number of berries and rachis length (compactness index, CI) and as the ratio between the number of branches and rachis length.

5.2.5 Basic Fruit Chemistry and Color Analysis

Basic fruit chemistry and color analysis were analyzed as described in Iland et al. (2004). Approximately 20 mL of juice was collected for soluble solids (^{O}Brix) analysis using an Atago PAL-1 refractometer (Kirkland, WA) and pH measurement with a Thermo Scientific Orion 370

pH meter (Beverly, MA). For total acidity (TA) determination, 10 mL of juice was titrated against a standardized 0.1*N* sodium hydroxide solution in an automatic titrator coupled to an auto sampler and control unit (Titroline 96, Schott, Germany) and expressed as g/L of tartaric acid equivalents. The remaining portions of the berries were briefly frozen and subsequently cold-ground with a tissue grinder (Model PT 10/35, Brinkmann Instruments Co, Switzerland). Approximately 1 g of each sample was transferred to a 15 mL centrifuge tube. Anthocyanins and total phenolics of the berries were extracted in a 50% ethanol solution, pH 2, for 1 hr and then centrifuged at 20,000 rpm. One mL of the resulting supernatant was diluted into 10 mL of 1*M* HCl and stabilized for 3 hr. The absorbance of extracts were read at 520 and 280 nm on a spectrophotometer (UV-1800, Shimadzu, Japan) and expressed as mg of anthocyanins per gram of berry weight and absorbance units of phenolics per gram of fresh berry weight.

5.2.6 Statistical Analysis

Data were analyzed using two-way ANOVA in PROC MIXED procedure, SAS 9.3. The effect of a year was ignored so the three random blocks in the 2011 and four random blocks in 2012 were combined and the experiment was treated as a RCBD with seven blocks. Only measurements of shoot length and retained leaf area were analyzed separately for each year using the REPEATED statement function in PROC MIXED because the intervals between two measurements were not identical in 2011 and 2012.

Normality of the residuals was assessed by visual inspection of the normal probability plot and Kolmogorov test. Whenever the distribution of the residuals was found to significantly diverge from the normal distribution, data were subjected to either logarithmic or square root

transformation. Homogeneity of variances was checked using the side-by-side box plot and Levene's test. Models with equal and unequal variances as well as models with different variance - covariance structures in repeated measurements were compared using the goodness of fit indicators. The model that showed the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) was used for further analysis. When the treatment interaction was found to be statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the treatment combinations were conducted using the t-test. When the treatment interaction was not statistically significant at $\alpha = 0.05$, all-pairwise comparisons among the treatment combinations were conducted using Tukey's HSD and significant effects of the single factors were also reported. Significant three-way interaction at $\alpha = 0.05$ in the repeated measurements was sliced by day and all-pairwise comparisons among the treatment combinations were conducted using the t-test. Only when three-way interaction was not significant were all-pairwise comparisons among the treatment combinations then conducted using Tukey's HSD.

Table 5.1 Timing of developmental stage (bud burst, bloom, fruit set and harvest) in 2011 and 2012, showing calendar date and day of the year.

Year	**2011**		**2012**	
Developmental stage	Date	DOY[z]	Date	DOY
Bud-burst	May 10	130	March 30	89
Bloom	June 15	166	June 6	157
Pea size berry	July 7	188	June 27	178
Harvest	September 23	266	September 6	249

[z] Day of the year

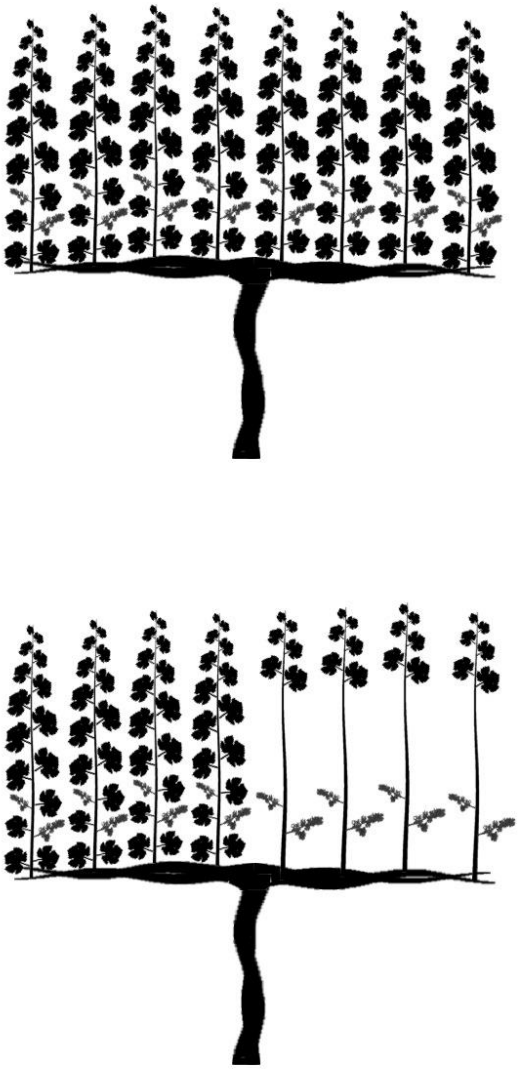

Figure 5.1 Above: the schematic presentation of a control vine with no leaves removed on the source side of the cordon and all shoot tips and inflorescences present on the sink side (LNO+SNO). Below: the schematic presentation of a vine with ten leaves removed on the source side of the cordon; no change to the sink side (LYES+SNO).

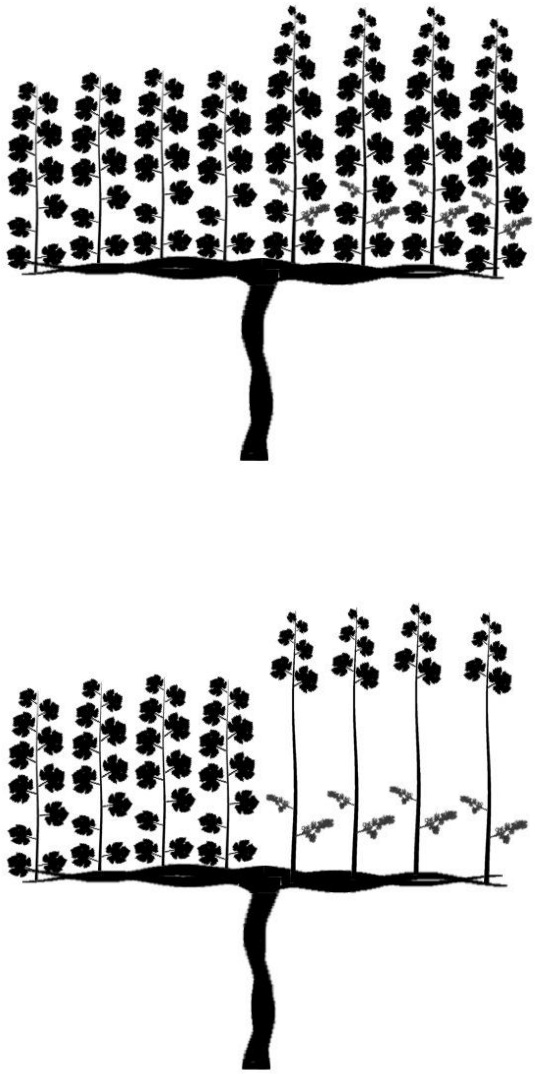

Figure 5.2 Above the schematic presentation of a vine with no leaves removed on the source side of the cordon and shoot tips and inflorescences absent on the sink side (LNO+SYES). Below the schematic presentation of a vine with ten leaves removed on the source side of the cordon and shoot tips and inflorescences absent on the sink (LYES+SYES).

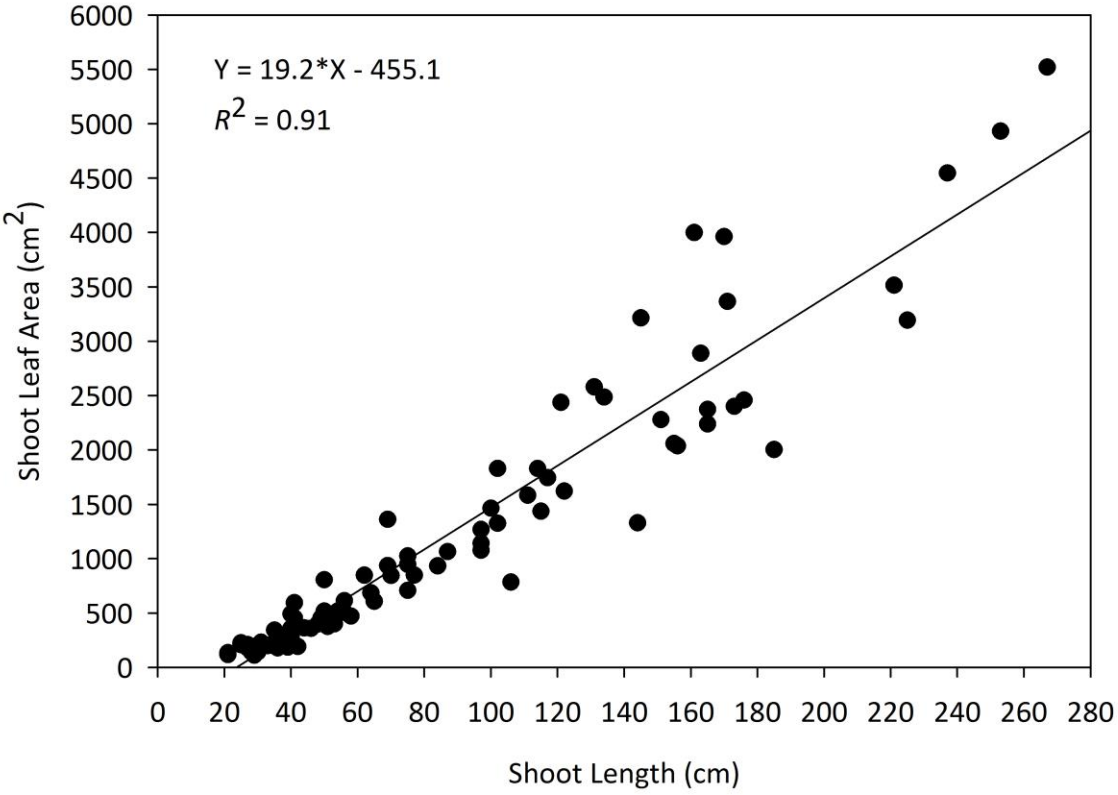

Figure 5.3 Linear correlation between shoot length (cm) and shoot leaf area (cm^2) established on eighty shoots, collected from May 31 to July 14 in 2011.

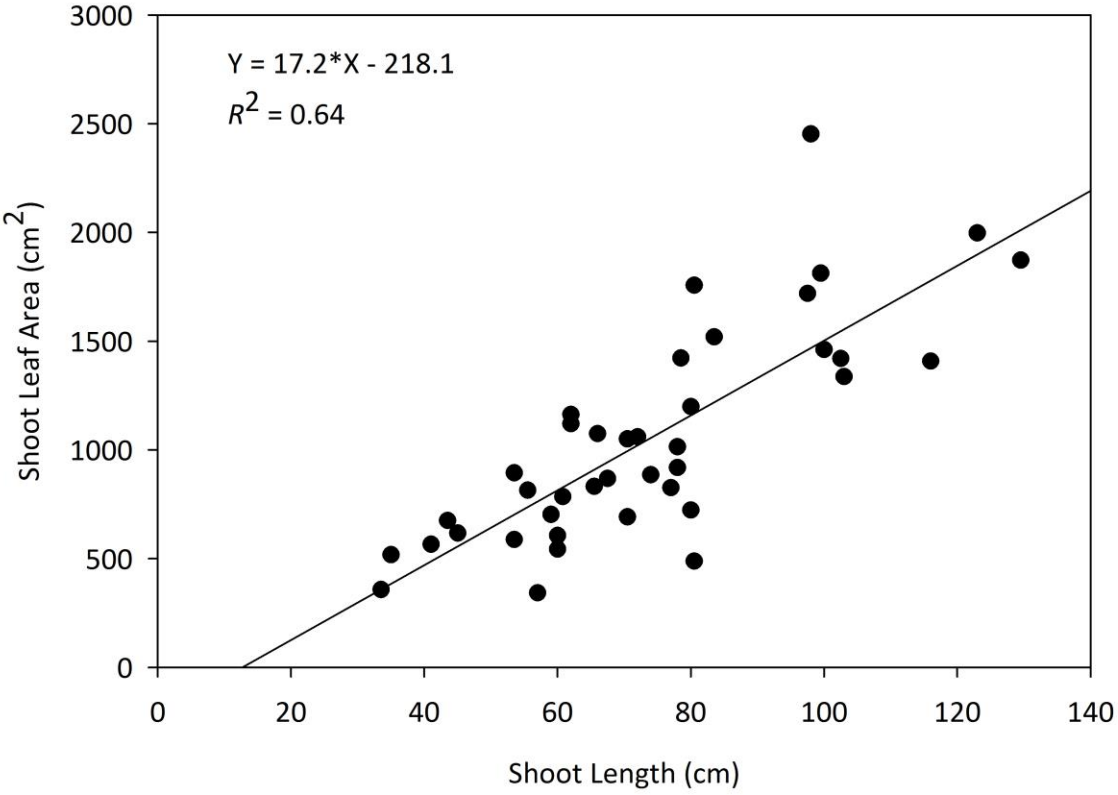

Figure 5.4 Linear correlation between shoot length (cm) and shoot leaf area (cm^2) established on forty shoots, collected from June 3 to June 19 in 2012.

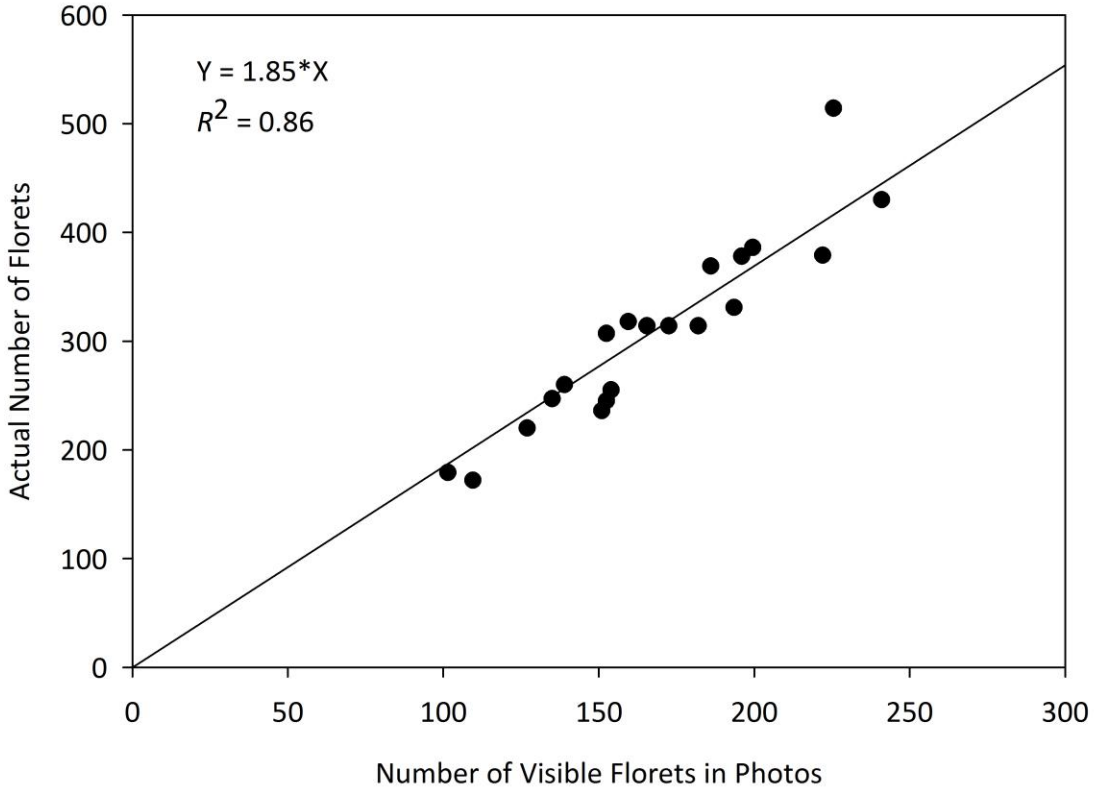

Figure 5.5 Linear correlation between number of visible florets in photos and number of actual florets per cluster based on the sample of twenty inflorescences collected at developmental stage 20 in 2011, after Eichhorn and Lorenz (1977).

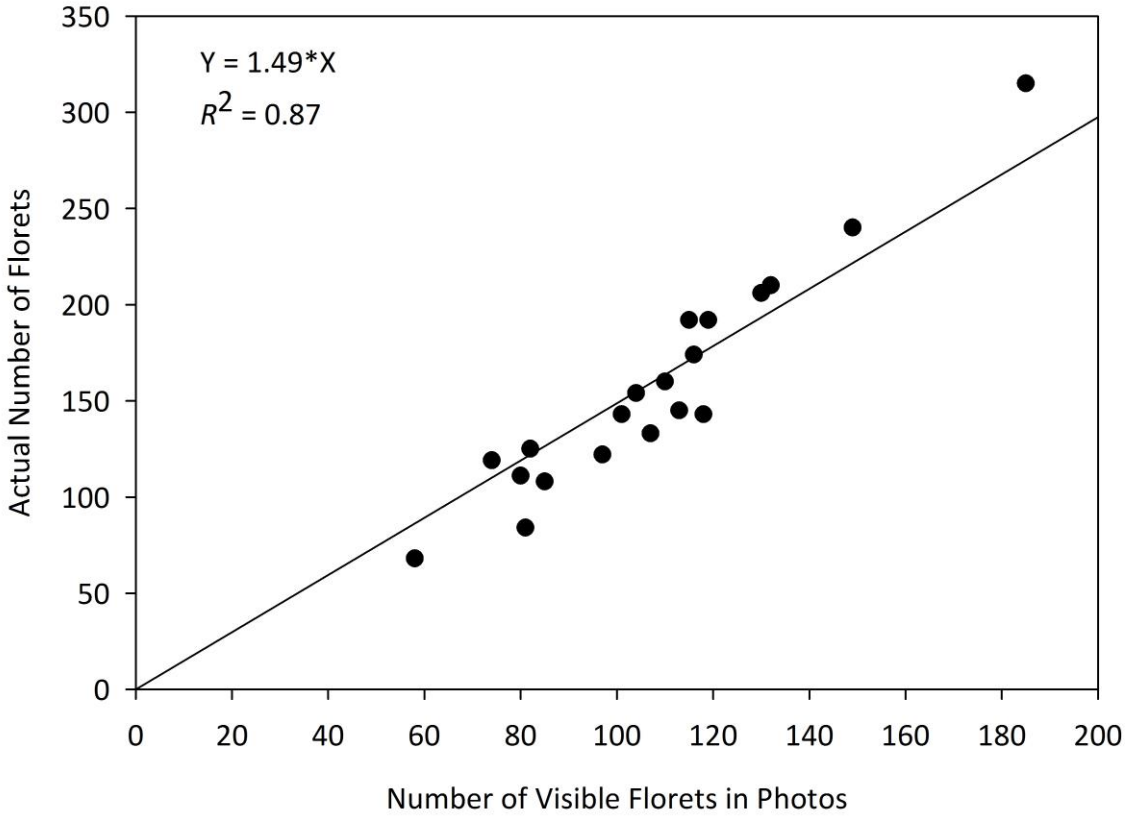

Figure 5.6 Linear correlation between number of visible florets in photos and number of actual florets per cluster based on the sample of twenty inflorescences. Inflorescences were collected at developmental stage 20 in 2012, after Eichhorn and Lorenz (1977).

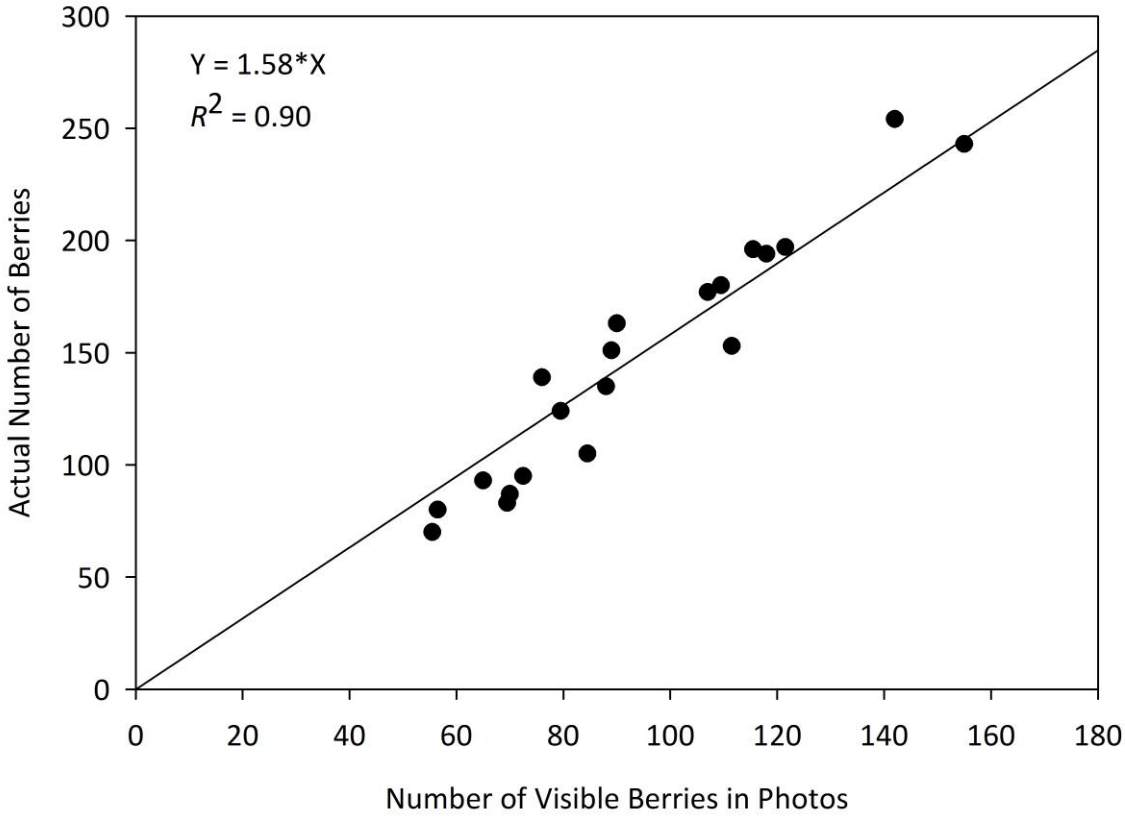

Figure 5.7 Linear correlation between number of visible berries in photos and number of actual berries per cluster based on the sample of twenty clusters. Clusters were collected at developmental stage 31 in 2012, after Eichhorn and Lorenz (1977).

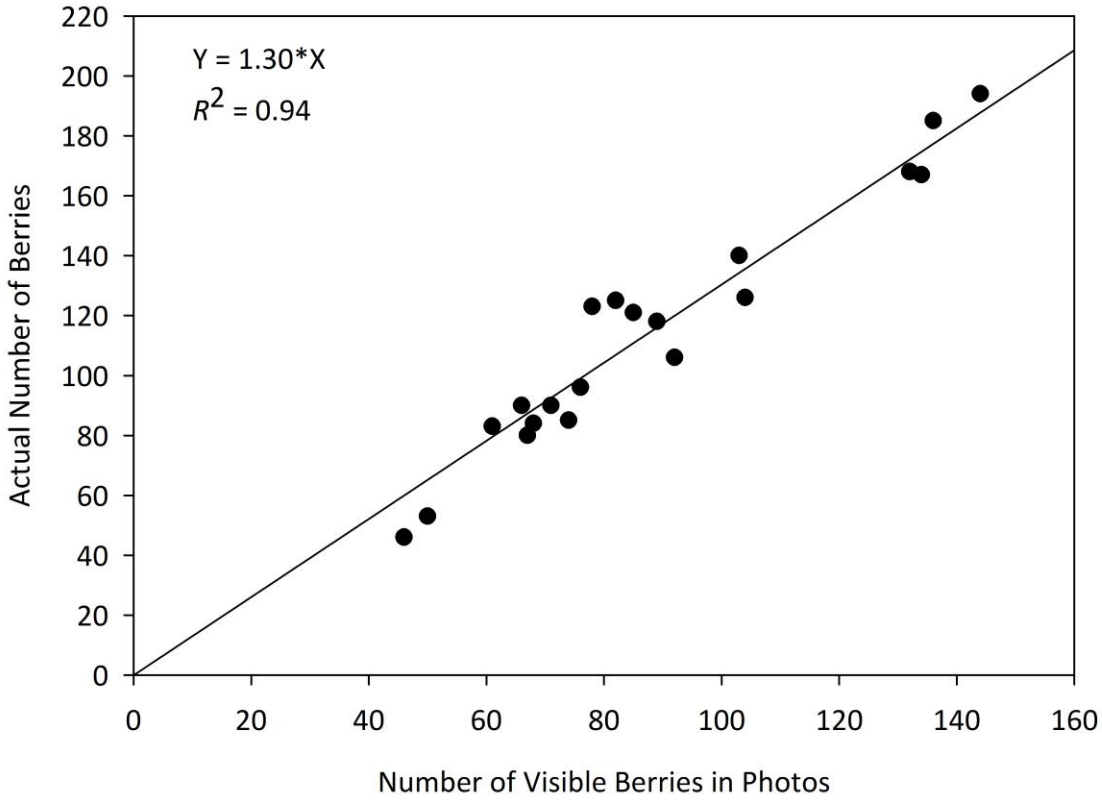

Figure 5.8 Linear correlation between number of visible berries in photos and number of actual berries per cluster based on the sample of twenty clusters. Clusters were collected at developmental stage 31 in 2012, after Eichhorn and Lorenz (1977).

5.3 Results

5.3.1 Treatments Impacts on Shoot Growth and Retained Leaf Area

One week before leaf and sink removal, shoot lengths for all vines were in the range from 50 to 55 cm in 2011 and from 62 to 69 cm in 2012. With defoliation, the amount of removed leaf area (LA) was 692 and 699 cm^2 in 2011 and 824 and 1040 cm^2 in 2012 for treatments LYES+SYES and LYES+SNO, respectively (Table 5.2). Although after the application of treatments, the retained LA of defoliated vines was 82% less in 2011 and 90% less in compared to the non-defoliated ones (Tables 5.3 and 5.5) with no significant differences observed related to shoot length. Also, the source removal treatment had no effect on vegetative growth nor was the interaction between treatments significant. Therefore, 1 month after vines had been subjected to leaf removal and sink removal, shoot lengths were between 136 and 168 cm in 2011 and 124 and 152 cm in 2012 (Tables 5.2 and 5.4), with no statistical differences between treatments.

However, manipulation with sinks and LA in 2011 resulted in lower LA per shoot for LYES after treatment application and this difference was present up to the last measurement day. A month after sink removal, SNO showed significantly lower retained LA compared to SYES, so by July 13 2011, LNO, LYES, SYES and SNO had 2616, 1679, 2428 and 1867 cm^2 of retained LA, respectively (Table 5.4). In 2012, the interaction of leaf removal times sink removal times day was significant together with leaf removal, but sink removal had no effect on retained LA (Table 5.6). With fast developing leaves, LYES reached 1302 cm^2 of LA by 13th July 2012, while LNO

had 2323 cm^2 of LA. Contrarily, SYES and SNO had similar LA of 1957 and 1669 cm^2, respectively 1 month after the vines were subjected to the treatments.

5.3.2 Impact of Defoliation on Fruit Set

Over two years of the experiment, the estimated numbers of florets per cluster fluctuated between 256 and 282 among all vines (Table 5.7). Although the vines were subjected to leaf removal treatments on the source side of the cordon, and shoot tips and cluster removal on the sink side of the cordon, only leaf removal had a significant effect on the estimated and actual number of berries per cluster. They were reduced in defoliated vines to the level of 92 and 82 berries per cluster, while non-defoliated ones had 109 and 101 berries per cluster, respectively (Tables 5.6 and 5.7). Vines with sinks removed had 105 and 97 berries per cluster and those with sinks present 96 and 85 berries per cluster, respectively (Tables 5.6 and 5.7). Leaf removal had a significant effect on fruit set at developmental stages 31 and 38 (FS-31 and FS-38). Thus, LNO had notably higher FS-31: 42% compared to 36% in LYES. An even greater difference between defoliated and non-defoliated vines was achieved later at developmental stage 38, when FS-38 in LNO was 39% compared to 31% of LYES. However, vines on which sinks were removed did not benefit because of the increased source to sink ratio and, consequently, set more berries. Therefore, FS-31 and FS-38 were 39% and 35% for SYES and SNO, respectively. No significant interaction between two factors, leaf removal and sink removal, was present for FS-31 and FS-38 (Table 5.7).

5.3.3 Change in Cluster Components and Morphology Caused by Leaf and Sink Removal

Table 5.8 shows that the total cluster weight was significantly affected only by the sink removal and thus vines, on which shoot tips and clusters were retained on the sink side of the cordon, had reduced cluster weight (87 g) on the source side of the cordon in contrast to the vines on which sinks were removed (115 g). Although not statistically different, clusters on the cordon where ten leaves were removed were lighter (96 g) compared to clusters on the completely foliated cordon (106 g).

Consistent with the significant influence on the estimated number of berries, leaf removal also showed an effect on the actual berry number, which was decreased in defoliated vines to 82 berries per cluster, while clusters on foliated vines had 101 berries (Table 5.8). However, there was no significant difference between SNO and SYES with respect to the actual berry number. Interestingly, both leaf removal and sink removal treatments affected mean berry weight. Removal of ten leaves resulted in an increase in mean berry weight of 1.12 g compared to berry mass of 0.97 g found in foliated vines.

No significant effect of the factors on rachis weight or rachis length was noticed (Table 5.8). Rachis weight was in the range from 4.1 to 4.8 g across the treatments, while rachis length was slightly reduced when leaf and sink were removed, so LNO and LYES had rachis of 12.1 and 13.6 cm in length and rachis of SYES and SNO was 13.3 and 12.4 cm, respectively. Number of branches per cluster was affected by neither leaf removal nor sink removal, thus clusters of all treatments had approximately thirteen branches (Table 5.9).

Without the notable change in rachis length and branch number, all of the treatments have a similar number of branches per rachis length (Table 5.9). However, cluster compactness was changed with leaf removal and LNO had considerably higher compactness index (8.6) compared to LYES (6.6; Table 5.9). The reduction in CI caused by leaf removal corresponded to a decrease in rot severity on the vines exposed to defoliation of 10 nodes; rot severity of LNO and LYES was 30.8 and 11.0%, respectively. Conversely, sink removal resulted in unaffected rot severity for SYES and SNO, which had 17.6 and 24.2% respectively.

5.3.4 Impact of Leaf and Sink Removal on Fruit Chemistry and Color

Unchanged by early defoliation and sink removal, sugar accumulation reached 22°Brix in all treatments (Table 5.10). Unlike soluble solids, both factors and their interaction were significant for pH; thus, LNO, LYES, SYES and SNO had 3.6, 3.8, 3.7 and 3.6, respectively. Total acidity of grape juice was influenced by defoliation and reduced from 7.64 in LNO to 5.38 in LYES. Finally, anthocyanin concentration was not affected by either leaf removal or sink removal and measured approximately 0.21 mg/g. However, the amount of phenolics in the grapes on the source side of the cordon was notably decreased with the absence of shoot tips and clusters on the sink side from 1.01 to 0.83 a.u./g (Table 5.10).

Table 5.2 Removed leaf area (± SE) by treatment application in 2011 and 2012.

Treatment[z]	Removed LA (cm^2)
2011	
LNO+SYES	0.0
LNO+SNO	0.0
LYES+SYES	692.1 (75.1)
LYES+SNO	699.2 (106.0)
2012	
LNO+SYES	0.0
LNO+SNO	0.0
LYES+SYES	824.5 (40.5)
LYES+SNO	1039.7 (118.7)

[z] LNO = no leaves removed; LYES = leaves removed from ten basal nodes at bloom; SYES = sinks removed; and SNO = sinks present.

Table 5.3 Effect of leaf removal and sink removal on shoot length (cm) measured in weekly intervals before and after treatment application in 2011.

Treatment[z]	June 7		June 14[v]		June 21		June 29		July 6		July 13	
Leaf Removal (L)												
LNO	54[x]	ns[w]	71	ns	95	ns	122	ns	140	ns	157	ns
LYES	51		69		91		114		128		147	
Sink Removal (S)												
SNO	50		66		87		109		126		136	
SYES	55	ns	73	ns	99	ns	128	ns	142	ns	168	ns
Significance[y]												
LxSxDay	ns											
L	ns											
S	ns											

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by Tukey's HSD; ns = non-significant differences.

[v] Treatments were performed on June 14 in 2011.

Table 5.4 Effect of leaf removal and sink removal on retained leaf area (cm^2) measured in weekly intervals before and after treatment application in 2011.

Treatment[z]	June 7		June 14[v]		June 21		June 29		July 6		July 13	
Leaf Removal (L)												
LNO	579[x]	ns[w]	909	a	1365	a	1893	a	2170	a	2616	a
LYES	529		167	b	589	b	1046	b	1313	b	1679	b
Sink Removal (S)												
SNO	503		471		862		1291		1560		1867	b
SYES	605	ns	604	ns	1091	ns	1649	ns	1923	ns	2428	a
Significance[y]												
LxSxDay	ns											
L	**											
S	ns											

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by Tukey's HSD; ns = non-significant differences.

[v] Treatments were performed on June 14 in 2011.

Table 5.5 Effect of leaf removal and sink removal on shoot length (cm) measured in weekly intervals before and after treatment application in 2012.

Treatment[z]	June 3		June 6[v]		June 13		June 19		June 29		July 13	
Leaf Removal (L)												
LN	64[x]	ns[w]	68	ns	83	ns	102	ns	123	ns	139	ns
LYES	68		72		91		110		128		138	
Sink Removal (S)												
SNO	62		66		77	b	96		112		124	
SYES	69	ns	74	ns	97	a	115	ns	139	ns	152	ns
Significance[y]												
LxSxDay	ns											
L	ns											
S	*											

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by Tukey's HSD; ns = non-significant differences.

[v] Treatments were performed on June 6 in 2011.

Table 5.6 Effect of leaf removal and sink removal on retained leaf area (cm^2) measured in weekly intervals before and after treatment application in 2012.

Treatment[z]	June 3		June 6[v]		June 13		June 19		June 29		July 13	
Leaf Removal (L)												
LNO	877[x]	ns[w]	944	a	1206	a	1531	a	1975	a	2323	a
LYES	9512		92	b	418	b	735	b	1051	b	1302	b
Sink Removal (S)												
SNO	853		498		699		1016		1376		1669	
SYES	976	ns	537	ns	925	ns	1249	ns	1651	ns	1957	ns
Significance[y]												
LxSxDay	*											
L	**											
S	ns											

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] Means within the column followed by the same letter are not significantly different at *p*-value = 0.05 by Tukey's HSD; ns = non-significant differences.

[v] Treatments were performed on June 6 in 2011.

Table 5.7 Effect of leaf removal and sink removal on number of berries per cluster and percentage of fruit set at developmental stages 31 and 38.

Treatment[z]	Estimated number of florets per cluster[x]	Estimated number of berries per cluster	FS-31 (%)[w]	FS-38 (%)[v]
Leaf Removal (L)				
LNO	257.3	108.7	41.8	38.8
LYES	280.6	92.1	35.7	30.6
Sink Removal (S)				
SNO	256.2	96.2	38.8	34.5
SYES	281.7	104.6	38.7	34.9
Significance[y]				
L	ns	*	*	**
S	ns	ns	ns	ns
LxS	ns	ns	ns	ns

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] Percentage of fruit set, which is derived from berry number at developmental stage 31, after Eichhorn and Lorenz (1977).

[v] Percentage of fruit set, which is derived from berry number at developmental stage 38, after Eichhorn and Lorenz (1977).

Table 5.8 Effect of leaf removal and sink removal on cluster size, berry weight, and rachis weight and length.

Treatment[z]	Cluster weight (g)[x]	Actual berry number	Berry weight (g)	Rachis weight (g)	Rachis length (cm)
Leaf Removal (L)					
LNO	105.7	100.7	0.97	4.6	12.1
LYES	96.2	81.7	1.12	4.3	13.6
Sink Removal (S)					
SNO	86.7	85.1	0.99	4.1	12.4
SYES	115.2	97.3	1.10	4.8	13.3
Significance[y]					
L	ns	*	**	ns	ns
S	*	ns	*	ns	ns
LxS	ns	ns	ns	ns	ns

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

Table 5.9 Effect of leaf removal and sink removal on components of cluster morphology and bunch rot severity.

Treatment[z]	Branch number	CI[w]	Number of branches per rachis length	Rot severity[v] (%)
Leaf Removal (L)				
LNO	12.7	8.6	1.1	30.8
LYES	13.4	6.6	1.0	11.0
Sink Removal (S)				
SNO	12.5	7.3	1.0	24.2
SYES	13.6	7.9	1.0	17.6
Significance[y]				
L	ns	**	ns	**
S	ns	ns	ns	ns
LxS	ns	ns	ns	ns

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] CI = Compactness Index expressed as number of berries per 1 cm of rachis length.

[v] Rot severity was calculated as a percentage of affected berries per tagged cluster.

Table 5.10 Effect of leaf removal and sink removal on fruit chemistry and color content.

Treatment[z]	Soluble solids (°Brix)[x]	pH	TA[w] (g/L)	Anthocyanin (mg/g)	Phenolics (a.u./g)
Leaf Removal (L)					
LNO	22.3	3.64	7.64	0.20	0.94
LYES	21.9	3.76	5.38	0.22	0.90
Sink Removal (S)					
SNO	22.0	3.65	6.72	0.21	1.01
SYES	22.2	3.75	6.30	0.21	0.83
Significance[y]					
L	ns	**	**	ns	ns
S	ns	**	ns	ns	**
LxS	ns	*	ns	ns	ns

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Results of analysis of variances for the main effects and interaction: *,**, and ns indicate significance at *p*-value ≤ 0.05, ≤ 0.01, and not significant, respectively.

[x] Means were based on seven replicates.

[w] TA = Total acidity.

5.4 Discussion

Shoot length increased from the period the treatments were applied to one month after it (Tables 5.2 and 5.4), however none of the factors considerably affected growth of the shoots in both years. Since LA of the vines was manipulated by defoliation, leaf removal treatment had a significant effect on the amount of retained leaf area and the interaction L times day was also significant in both years of the experiment, showing 82 and 90% LA loss in LYES in 2011 and 2012, respectively, immediately after application of treatments (Tables 5.3 and 5.5). However, during the following month, new growing foliage reduced the difference between retained leaf area of defoliated and foliated vines. Therefore, LY resulted in only 36 and 44% less leaf area in 2011 and 2012, respectively, compared to LNO. Although, sink removal treatment had no influence on retained leaf area in any year, the interaction S times day was significant in 2011, indicating that the absence of clusters and shoot tips over time promoted the development of new leaves, which contributed to a substantial LA enlargement of 30 % in SYES compared to SNO a month later. The investment in vegetative growth is a classic response to under-cropped or sink limited conditions (Keller, 2010).

The results indicate that only the leaf removal treatment had a significant impact on fruit set and, consequently, on the number of berries per cluster suggesting the importance of local photoassimilate availability for the inflorescences during bloom (Tables 5.6 and 5.7). Source availability of a single shoot, was of greater significance for fruit set than the sink demand of a whole vine during the bloom. Therefore, the vines, on which ten leaves were removed, had FS-31 and FS-38 reduced by 15 and 21%, respectively, and actual berry numbers per cluster decreased by 19%. Restriction of carbohydrates at bloom by defoliation has been

confirmed as an efficient tool to reduce fruit set (Candolfi-Vasconcelos and Koblet, 1990; Poni et al., 2006; Intrieri et al., 2008). Previous studies suggested the elimination of strongly competitive shoot tips by pinching and topping as measures for improvement of fruit set (Coombe, 1959; May, 1972). There are also reports that halving the crop level in Pinot noir by removing every distal cluster on shoots at bloom increased the number of berries per cluster in 2 out of 4 years of experimentation (Reynolds et al., 1994). However, in those experiments with pinching and/or topping and cluster thinning the treatments were equally applied on the vines, creating a condition for the sinks to enter into direct competition with each other over the available sources within a shoot. In our experiment, the bilateral cordon with shoot tips and cluster removal on the "sink side" and retained sinks on the "source side" presented a spatial barrier buffering the direct beneficial effect of shoot pinching or cluster thinning on fruit set.

It is worth noting that leaf removal targeted a specific time frame, which was important for determination of the final berry number. However, the temporary restriction of the photoassimilates becomes less important once the shoots developed new foliage and compensated for the loss of leaf area. Unlike the leaf removal treatment, the elimination of the clusters and growing tips was the factor, which, raising the source to sink ratio of the whole vine for a longer period, evidently influenced cluster development mainly through the alteration of mean berry weight. That was the reason why sink removal rather than source (leaf) removal had a significant effect on cluster weight, increased by 33 % compared to vines on which sinks remained untouched. Our finding is in agreement with those of Reynolds et al. (1994) where reduction in crop level by half increased the berry weight and, consequently, the cluster weight of Pinot noir.

Both leaf and sink removal had an effect on berry development and the final berry weight (Table 5.8). Defoliation performed at bloom reduced the berry weight while sink removal increased it. Firstly, the increase in berry weight caused by defoliation is related to the early leaf removal impact on berry number per cluster; fewer berries per cluster stimulates berry growth in relation to the increased availability of nutrients necessary for their development. Contrarily, foliated vines had more berries per cluster making them compete for the limited amount of photoassimilates at the cluster level and this, in turn, resulted in reduced berry size. Compensation in berry growth, triggered by the reduced number of total berries per cluster, has been reported for other varieties (Poni et al., 2009; Tardaguila et al., 2010; Tardaguila et al., 2012). However, the literature also concluded that the source limitation at bloom could lead to a decrease in berry size (Poni et al., 2006; Intrieri et al., 2008; Lohitnavy et al., 2010), not always reported in Pinot noir (Candolfi-Vasconcelos and Koblet, 1990; Lee and Skinkis, 2013). Secondly, the total absence of clusters and shoot tips on the sink side of the cordon had a positive long-term effect on the development of berries on the source side, enlarging their mass by 11%. Our results are consistent with previous findings that a decrease in crop load performed early during the first period of berry development resulted in increased berry weight (Dokoozlian and Hirschfelt, 1995; Tardaguila et al., 2012). Coombe (1959) also reported that shoot pinching at bloom slightly increased berry weight.

Table 5.8 shows that leaf removal and sink removal treatments did not considerably change either the rachis weight or the rachis length. Moreover, the treatments' means for the number of branches per cluster were similar (Table 5.9). However, the leaf removal treatment, reducing the number of berries per cluster but not rachis length, decreased the compactness

index from 8.6 to 6.6 and thus changed the cluster morphology. This reduction in cluster compactness correlated with a decrease in rot severity from 30.8% in non-defoliated vines to 11.0% in defoliated (Table 5.9). Previous experiments also had demonstrated that early leaf removal helped in reducing bunch rot incidence by reducing the cluster compactness and improving the cluster microclimate (Poni et al., 2006; Sabbatini and Howell, 2010; Tardaguila et al., 2010; Palliotti et al., 2012).

With sink removal vines were subjected to cluster thinning, which resulted in reduction of half of the inflorescences/clusters per vine. Interestingly enough, the manipulation and decrease of cluster number and thus a decrease of yield per vine did not lead to improvement in soluble solids. Likewise, leaf removal treatment did not change sugar accumulation and all the vines reached 22°Brix at harvest (Table 5.9). In contrast, the results imply that fruit maturity, as demonstrated by the increase in pH, was affected by both factors and even their interaction was significant. However, only the leaf removal significantly decreased total acidity of the juice (Table 5.9). Disagreeing with our results, Reynolds et al. (1994) obtained advanced fruit maturity characterized with both higher °Brix and pH with crop level reduction in a three-year experiment on Pinot noir. There could be two possible reasons for a lack of effect from leaf removal on sugar accumulation in our experiment. Firstly, the intensive development of new foliage and the resulting compensation via substantially increased leaf area on vines, coupled with the likely increase in photosynthesis, was sufficient enough to provide fully ripening of the clusters. Secondly, a proportion of the sugar found in clusters on the defoliated cordon came from other parts of the vine (May et al., 1969; Mansfield and Howell, 1981; Candolfi-

Vasconcelos et al., 1994). The option that the foliated vines were over-cropped, which would cause their slow sugar accumulation and thus make their mean for soluble solids similar to defoliated ones was ruled out (Table C.1).

No effect on total anthocyanins was observed in our experiment and this was opposite to the effect, described by Reynolds et al. (1994), of cluster thinning on Pinot noir. Only sink removal had a significant impact on total phenolics, decreasing them (Table 5.9). Mazza et al. (1999) reported that cluster thinning at bloom on Pinot noir significantly reduced skin phenolics and anthocyanins, while leaf removal increased them in one year of a two-year experiment. In juxtaposition, King et al. (2012) found no benefit to phenolic levels from leaf removal and fruit exposure to light at stage 33, after Eichhorn and Lorenz. However, the same authors stated that reducing Merlot crop load through crop removal at veraison increased total phenolics in wine.

5.5 Conclusion

The goal of this experiment was to investigate whether a long distance translocation of the nutrients occurred during the fruit set if the half of the vine was defoliated. The results showed that only the leaf removal treatment had a significant impact on fruit set and the number of berries per cluster suggesting the importance of local photoassimilate availability for the inflorescences during bloom. In the other words, source availability of a single shoot, was of greater significance for fruit set than the sink demand of a whole vine during the bloom. On the other hand, the increased source to sink ratio caused by sink removal lasted for the whole season and influenced cluster development mainly through the alteration of mean berry weight and increased cluster weight by 33 % compared to vines on which sinks remained untouched. Interestingly, the results also showed that a reduced number of clusters per vine and thus a decrease of yield per vine did not lead to improvement in soluble solids.

APPENDICES

APPENDIX A - IMPACT OF EARLY DEFOLIATION ON FRUIT SET, CLUSTER MORPHOLOGY, BUNCH ROT AND FRUIT QUALITY OF PINOT NOIR CLONE 777 IN 2011

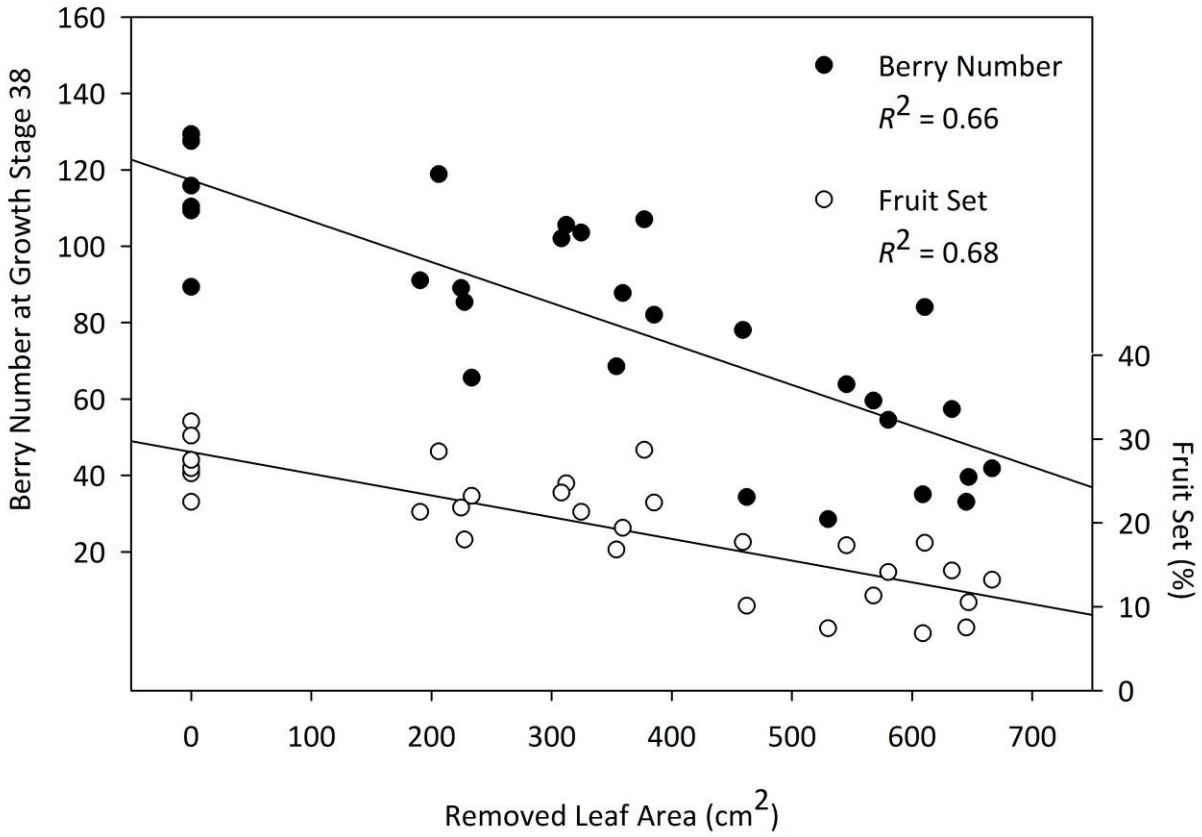

Figure A.1 Correlation between the removed leaf area and actual berry number (r = 0.81; $p < 0.001$) and percentage of FS-38 (r = 0.83; $p < 0.001$).

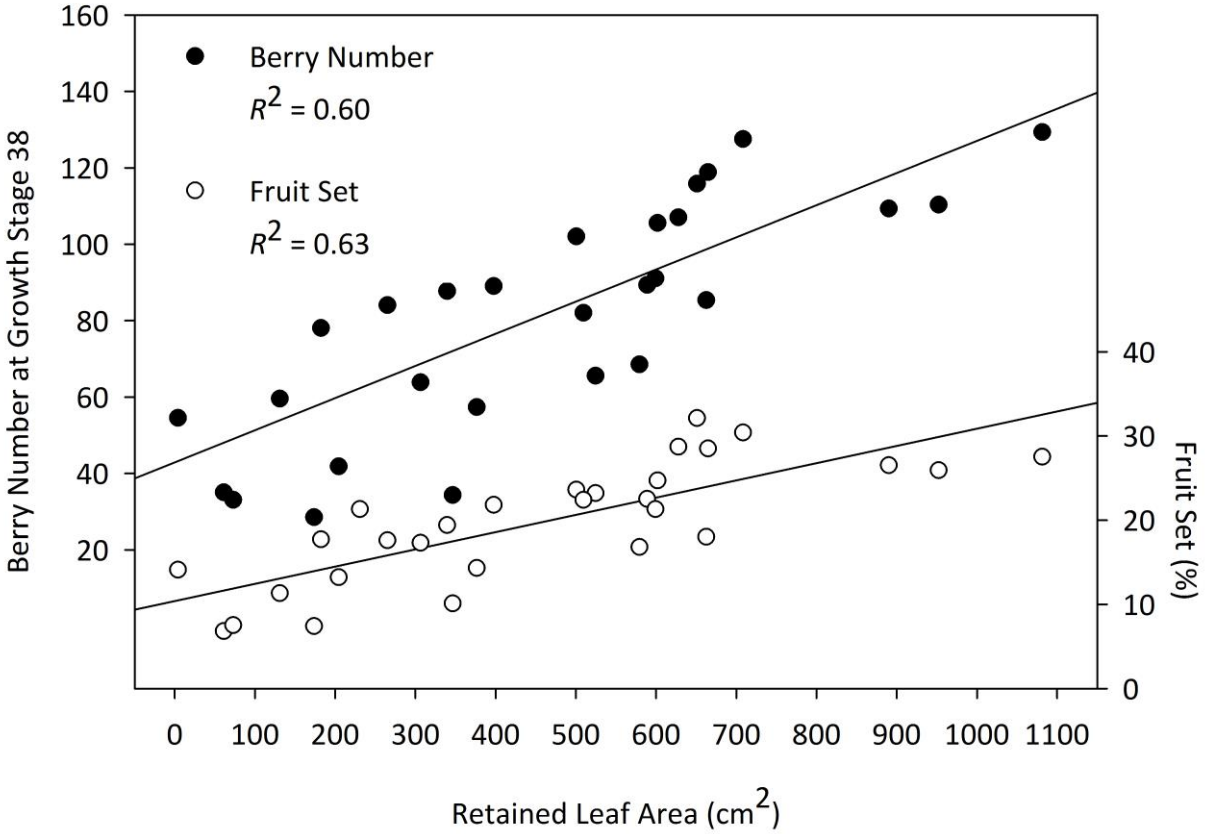

Figure A.2 Correlation between the retained leaf area (n = 30) and actual berry number (r = 0.79; $p < 0.001$) and percentage of FS-38 (r = 0.80; $p < 0.001$).

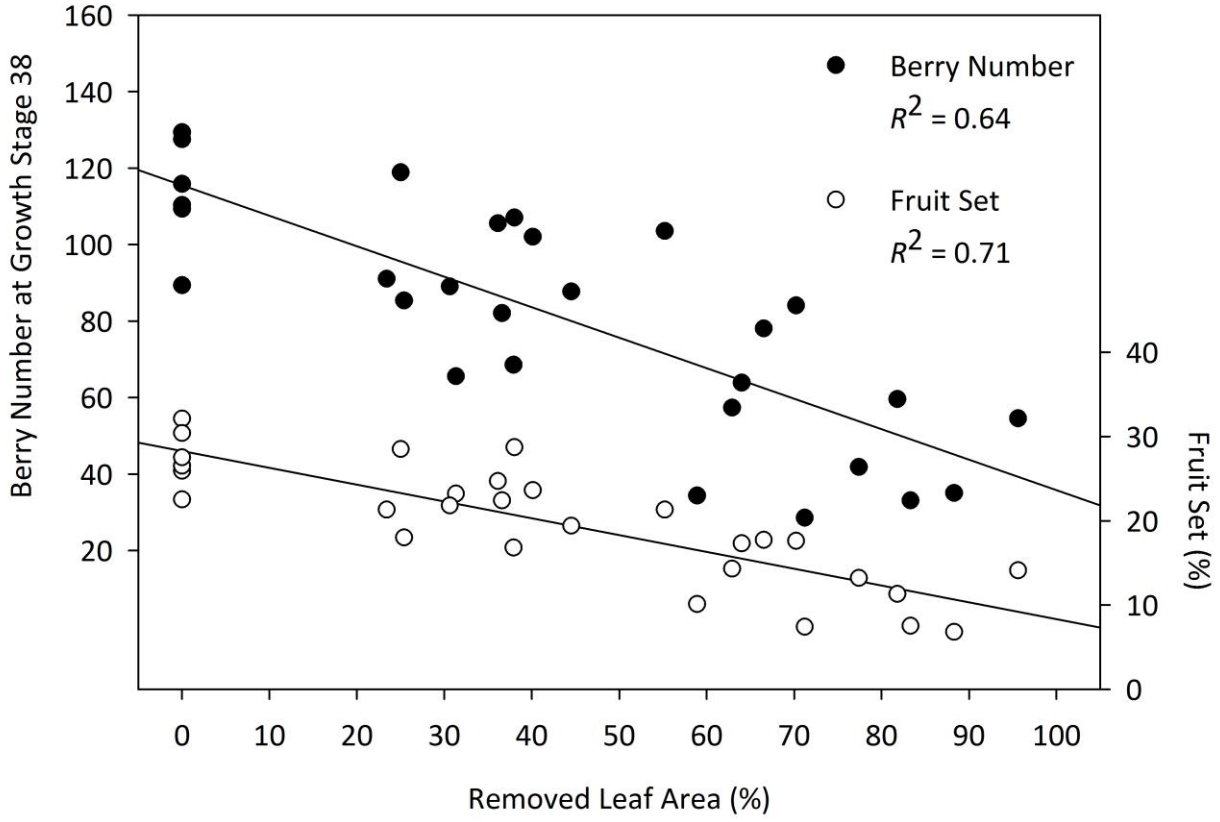

Figure A.3 Correlation between the percentage of removed leaf area and actual berry number (r= 0.80; $p < 0.001$) and percentage of FS-38 (r = 0.84; $p < 0.001$).

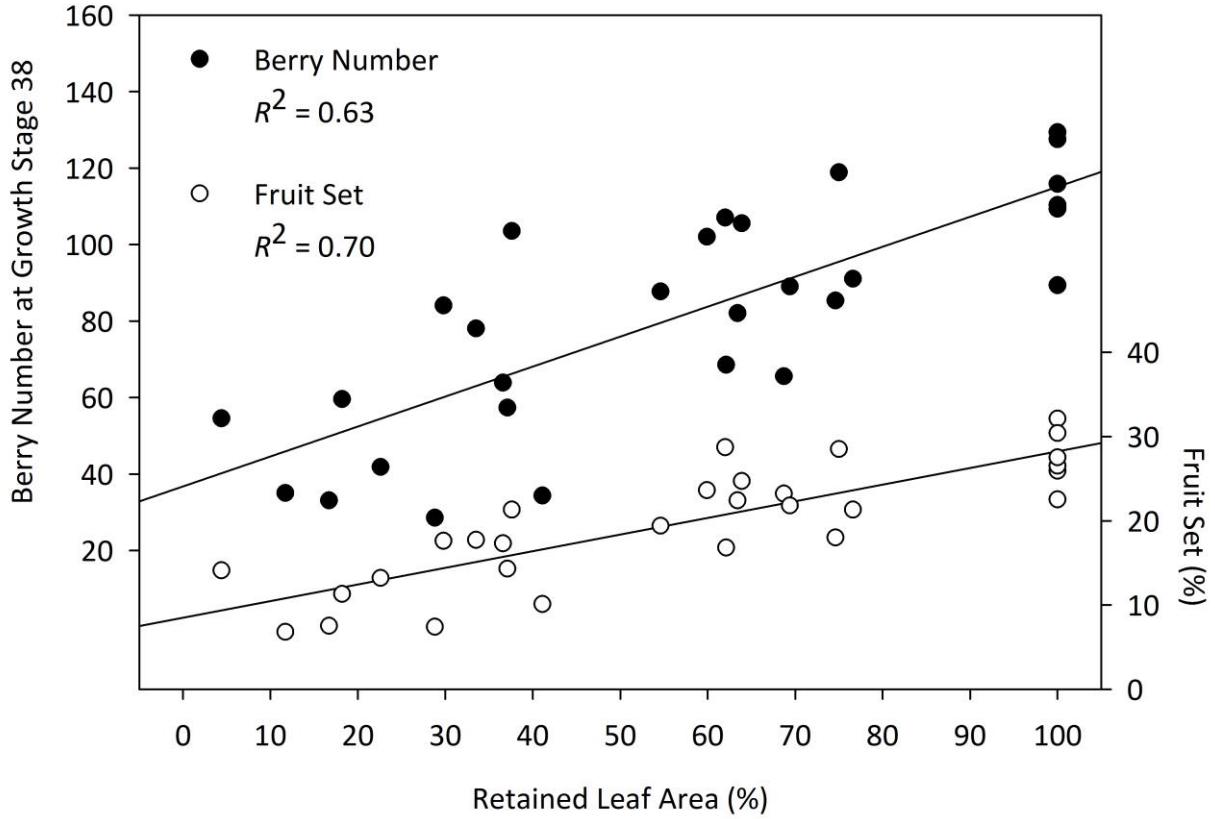

Figure A.4 Correlation between the percentage of retained leaf area and actual berry number (r = 0.79; $p < 0.001$) and percentage of FS-38 (r = 0.84; $p < 0.001$).

Table A.1 The difference between estimated number of berries at growth stage 31 and actual number of berries at growth stage 38 in 2011.

Treatment[z]	Difference between estimated and actual number of berries[y]	Percentage decrease between estimated and actual number of berries (%)
LR-0	2.2	2.0
LR-4	13.6	12.8
LR-6	14.2	13.4
LR-8	18.9	24.8
LR-10	29.1	39.8

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes.

[y] Means were based on 6 replicates.

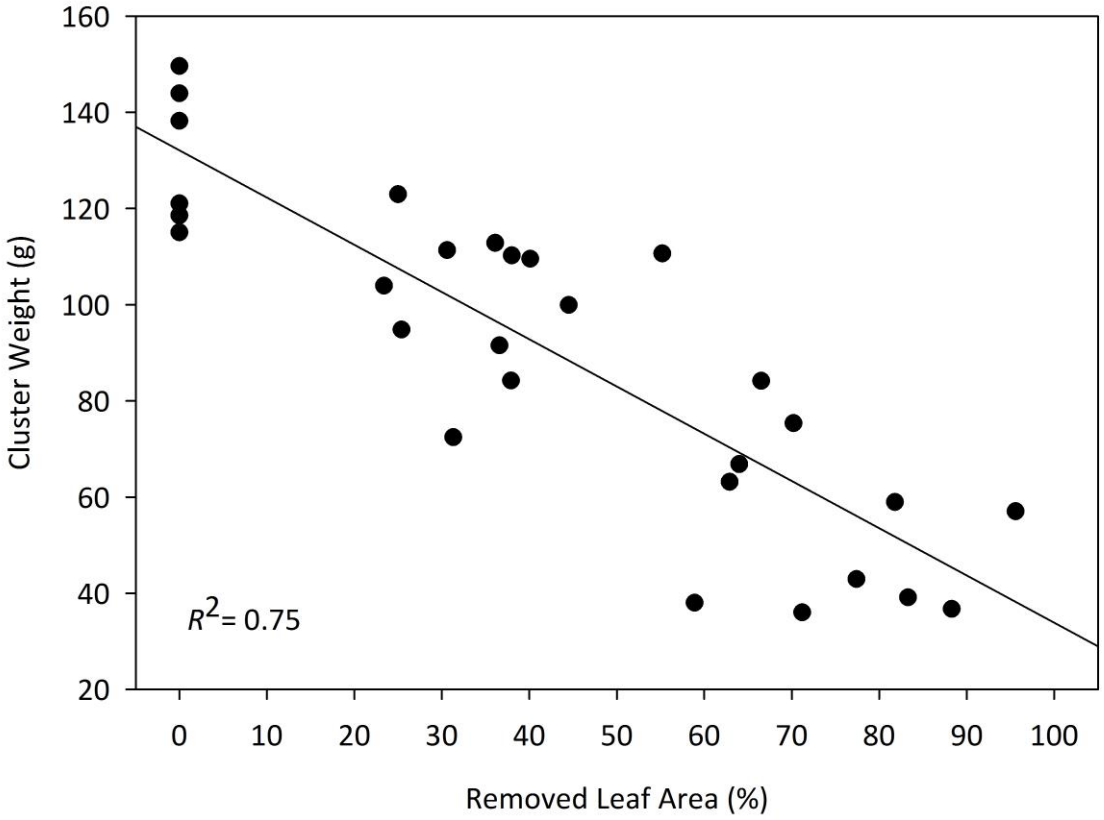

Figure A.5 Correlation between percentage of removed leaf area and cluster weight (r = 0.87; $p < 0.001$).

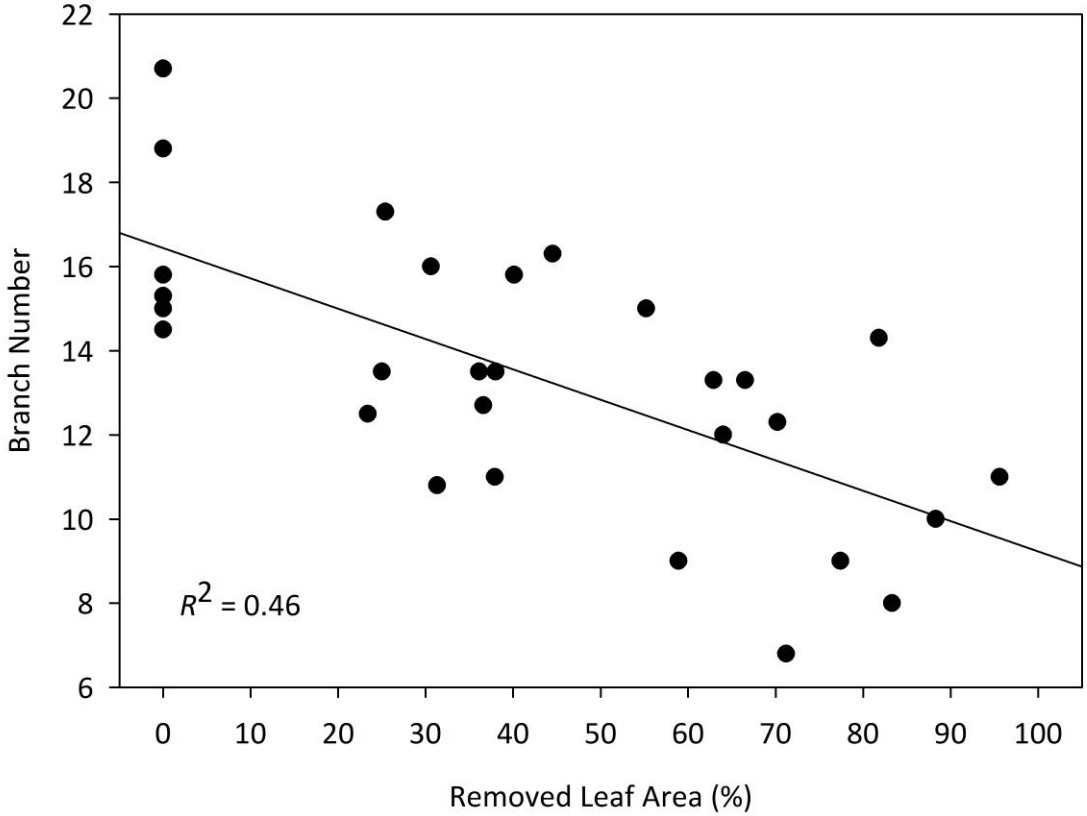

Figure A.6 Correlation between percentage of removed leaf area and branch number per cluster (r = 0.68; $p < 0.001$).

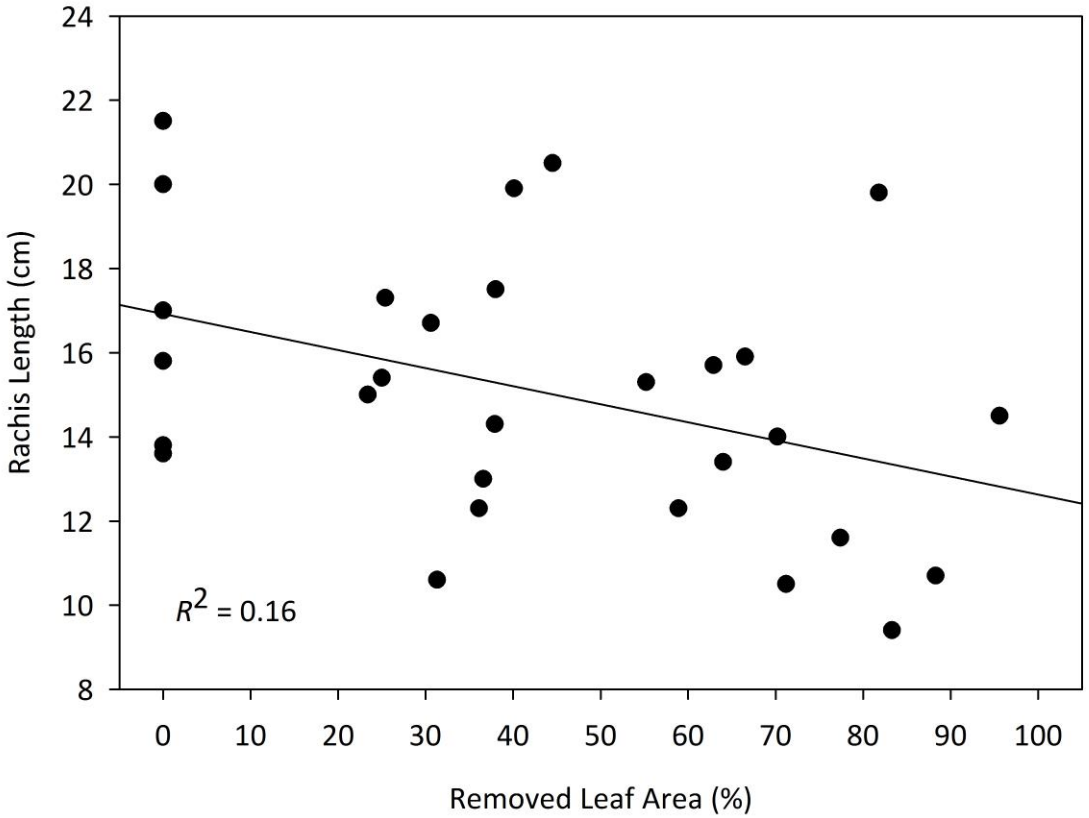

Figure A.7 Correlation between percentage of removed leaf area and rachis length (r = 0.40; *p* = 0.033).

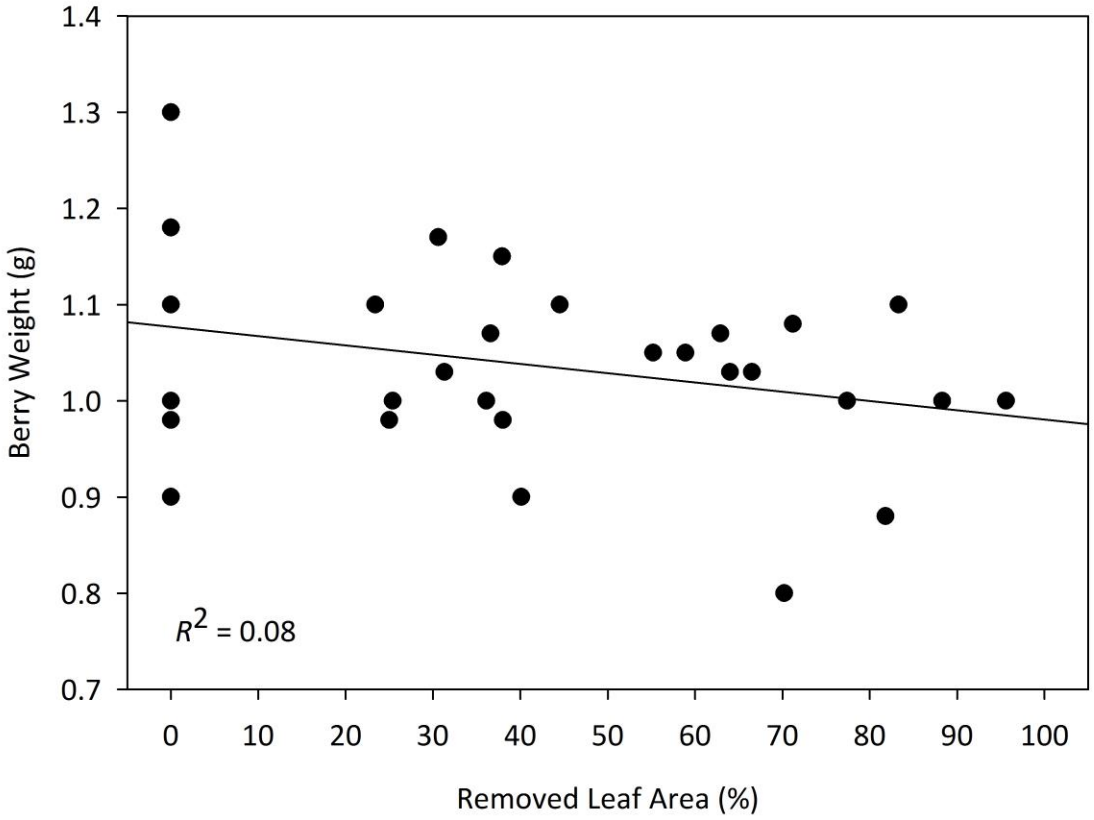

Figure A.8 Correlation between percentage of removed leaf area and berry weight (r = 0.29; *p* = 0.128).

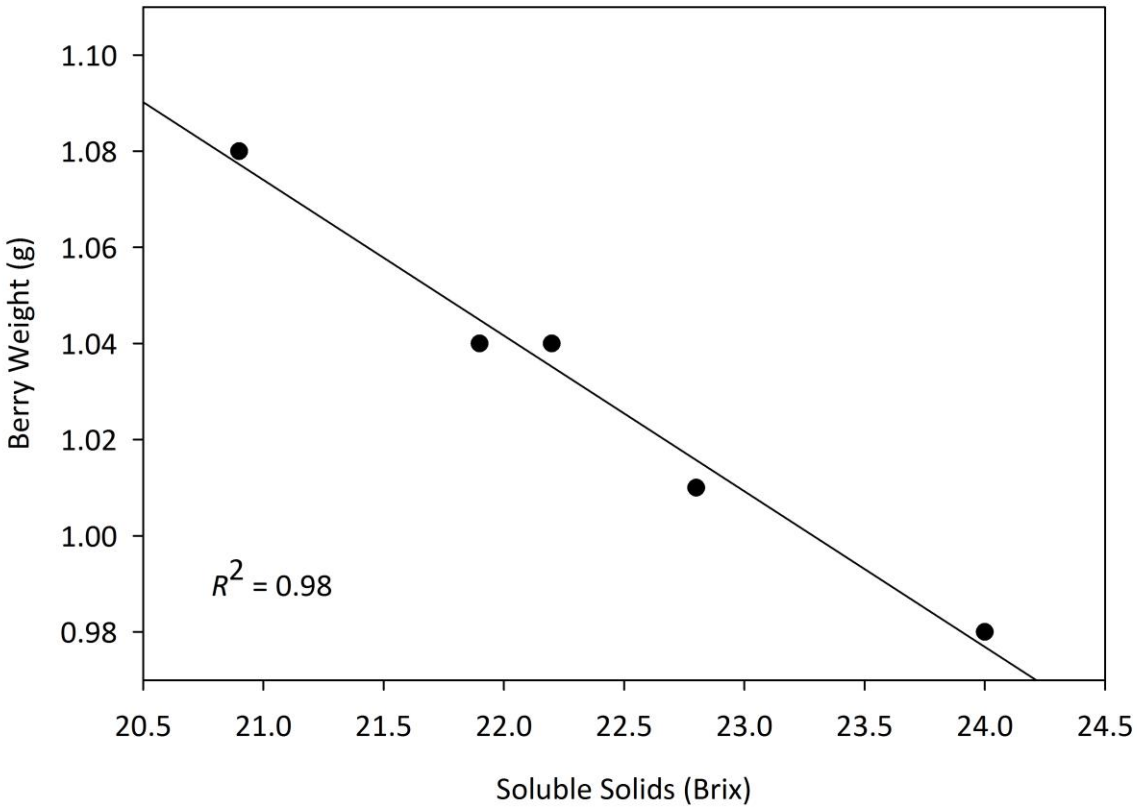

Figure A.9 Correlation between soluble solids and berry weight (r = 0.99; $p < 0.001$).

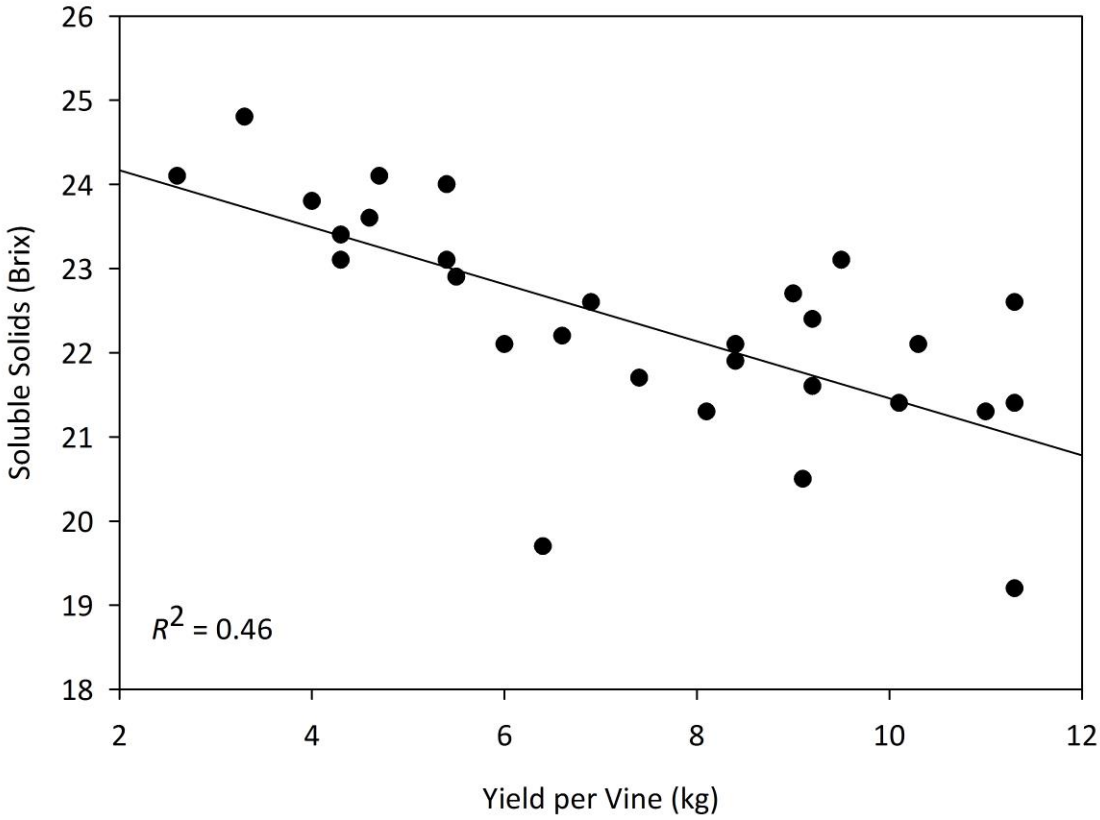

Figure A.10 Effect of vine yield on soluble solids in grape juice (r = 0.68; $p < 0.001$).

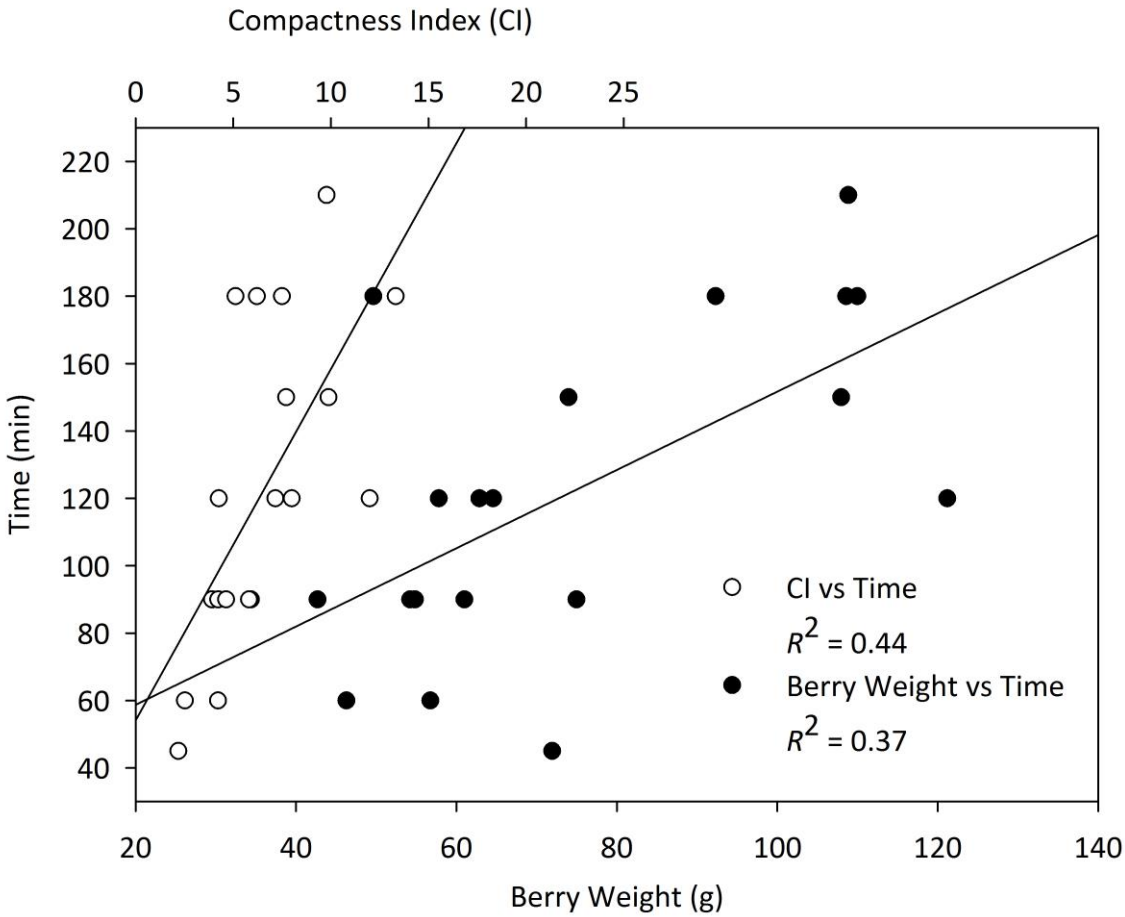

Figure A.11 Correlation between drying time (n = 20) and berry weight (r = 0.61; *p* = 0.006) and between drying time and compactness index (r = 0.66; *p* = 0.002).

APPENDIX B - SUSTAINABILITY OF EARLY DEFOLIATION ON

PINOT NOIR CLONE 777 IN 2012

Table B.1 Linear correlations between number of florets per inflorescence, inflorescence number, and shoot number in 2012 and retained and removed leaf area per shoot in 2011.

	Retained LA at stage 31 (cm^2)	Retained LA at stage 31 (%)	Removed LA at stage 31 (cm^2)	Removed LA at stage 31 (%)
Inflorescence number	r = 0.365 *p* = 0.052	r = 0.408 *p* = 0.028	r = 0.409 *p* = 0.025	r = 0.434 *p* = 0.019
Floret number	r = 0.557 *p* = 0.002	r = 0.544 *p* = 0.002	r = 0.548 *p* = 0.002	r = 0.557 *p* = 0.002
Shoot number	r = 0.138 *p* = 0.474	r = 0.019 *p* = 0.923	r = 0.068 *p* = 0.721	r = 0.036 *p* = 0.851

Table B.2 Linear correlations between berry number and fruit set at two developmental stages, 31 and 38, and removed and retained leaf area per shoot in 2012, n = 30.

	Retained LA at stage 31 (cm^2)	Retained LA at stage 31 (%)	Removed LA at stage 31 (cm^2)	Removed LA at stage 31 (%)
Estimated number of berries	r = 0.667 *p* < 0.001	r = 0.685 *p* < 0.001	r = 0.641 *p* <0.001	r = 0.685 *p* < 0.001
Actual number of berries	r = 0.657 *p* < 0.001	r = 0.661 *p* < 0.001	r = 0.604 *p* < 0.001	r = 0.661 *p* < 0.001
FS-31	r = 0.347 *p* = 0.060	r = 0.436 *p* = 0.016	r = 0.454 *p* = 0.012	r = 0.436 *p* = 0.016
FS-38	r = 0.473 *p* = 0.008	r = 0.532 *p* = 0.002	r = 0.522 *p* = 0.003	r = 0.532 *p* = 0.002

Table B.3 The Difference between estimated number of berries at growth stage 31 and actual number of berries at growth stage 38 in 2012.

Treatment[z]	Difference between estimated and actual number of berries[y]	Percentage decrease between estimated and actual number of berries (%)
LR-0	9.4	12.3
LR-4	6.9	8.2
LR-6	7.4	11.0
LR-8	10.8	19.3
LR-10	11.0	26.7

[z] LR-0 = no leaves removed; LR-4 = leaves removed from 4 basal nodes; LR-6 = leaves removed from 6 basal nodes; LR-8 = leaves removed from 8 basal nodes; LR-10 = leaves removed from 10 basal nodes.

[y] Means calculated on sample size of n = 24.

Table B.4 Linear correlations between berry weight, cluster weight, rachis length, rachis weight, and branch number, and removed and retained leaf area at two developmental stages, 31 and 38, in 2012.

	Retained LA at stage 31 (cm^2)	Retained LA at stage 31 (%)	Removed LA at stage 31 (cm^2)	Removed LA at stage 31 (%)
Berry weight	r = 0.670 $p < 0.001$	r = 0.596 $p < 0.001$	r = 0.439 $p = 0.015$	r = 0.596 $p < 0.001$
Cluster weight	r = 0.788 $p < 0.001$	r = 0.760 $p < 0.001$	r = 0.672 $p < 0.001$	r = 0.760 $p < 0.001$
Rachis length	r = 0.547 $p = 0.002$	r = 0.496 $p = 0.005$	r = 0.466 $p = 0.010$	r = 0.496 $p = 0.005$
Rachis weight	r = 0.686 $p < 0.001$	r = 0.651 $p < 0.001$	r = 0.576 $p < 0.001$	r = 0.651 $p < 0.001$
Branch number	r = 0.568 $p = 0.001$	r = 0.600 $p < 0.001$	r = 0.555 $p = 0.001$	r = 0.600 $p < 0.001$

Table B.5 Linear correlations between berry weight, skin to pulp ratio, skin ratio, seed ratio, and pulp ratio in 2012.

	Skin to berry ratio	Pulp to berry ratio	Seed to berry ratio	Skin to pulp ratio
Berry weight	r = 0.658 $p < 0.001$	r = 0.780 $p < 0.001$	r = 0.628 $p < 0.001$	r = 0.711 $p < 0.001$

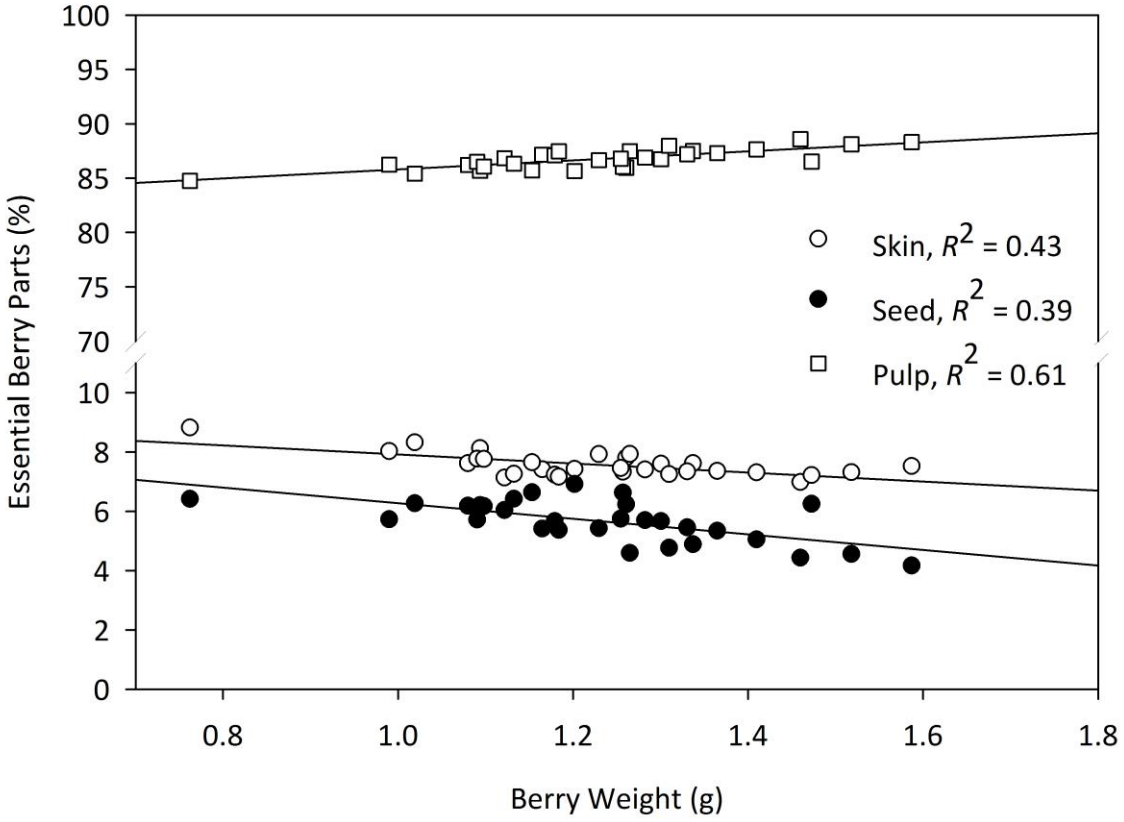

Figure B.1 Linear regressions between berry weight, skin to berry ratio, seed to berry ratio, and pulp to berry ratio in 2012.

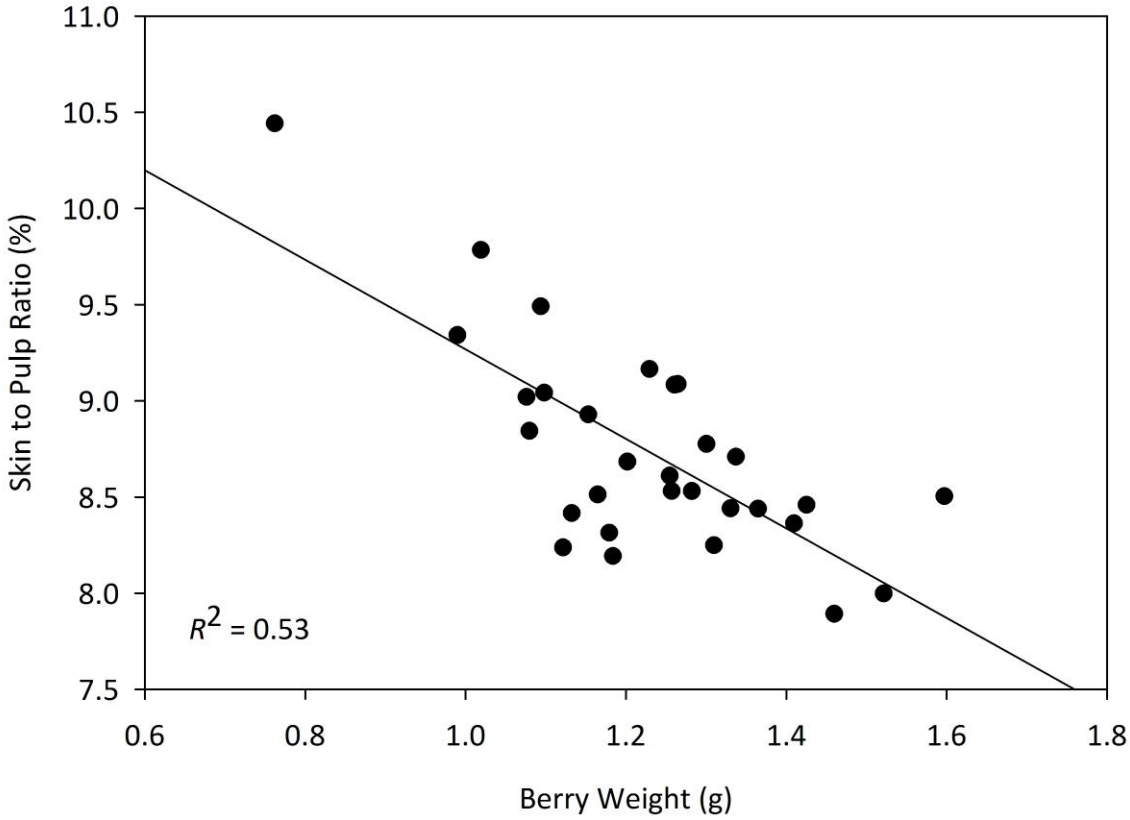

Figure B.2 Linear regressions between berry weight and skin to pulp ratio in 2012 (r = 0.73; $p < 0.001$).

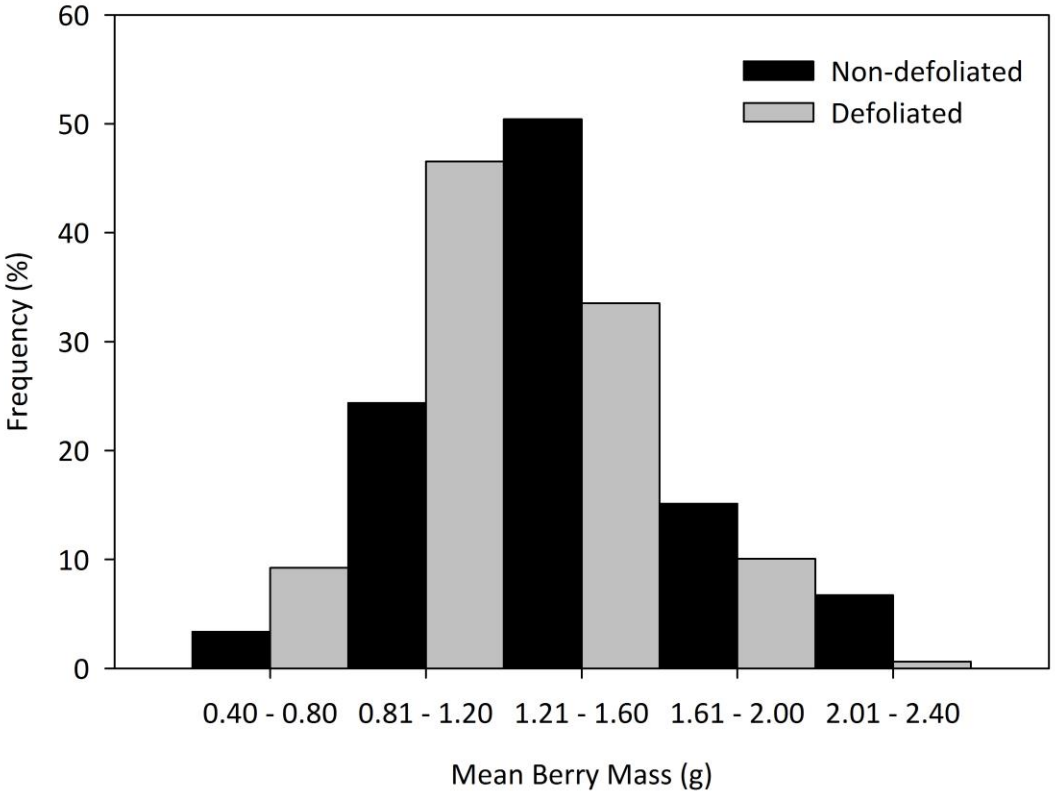

Figure B.3 Frequency of distribution (% of sample population) for berry mass in clusters from non-defoliated and defoliated vines. Berry size categories were: 0.40-0.80 g; 0.81-1.20 g; 1.21-1.60 g; 1.61-2.00 g; 2.01-2.40 g. Sample sizes of non-defoliated and defoliated vines were 199 and 477 berries, respectively.

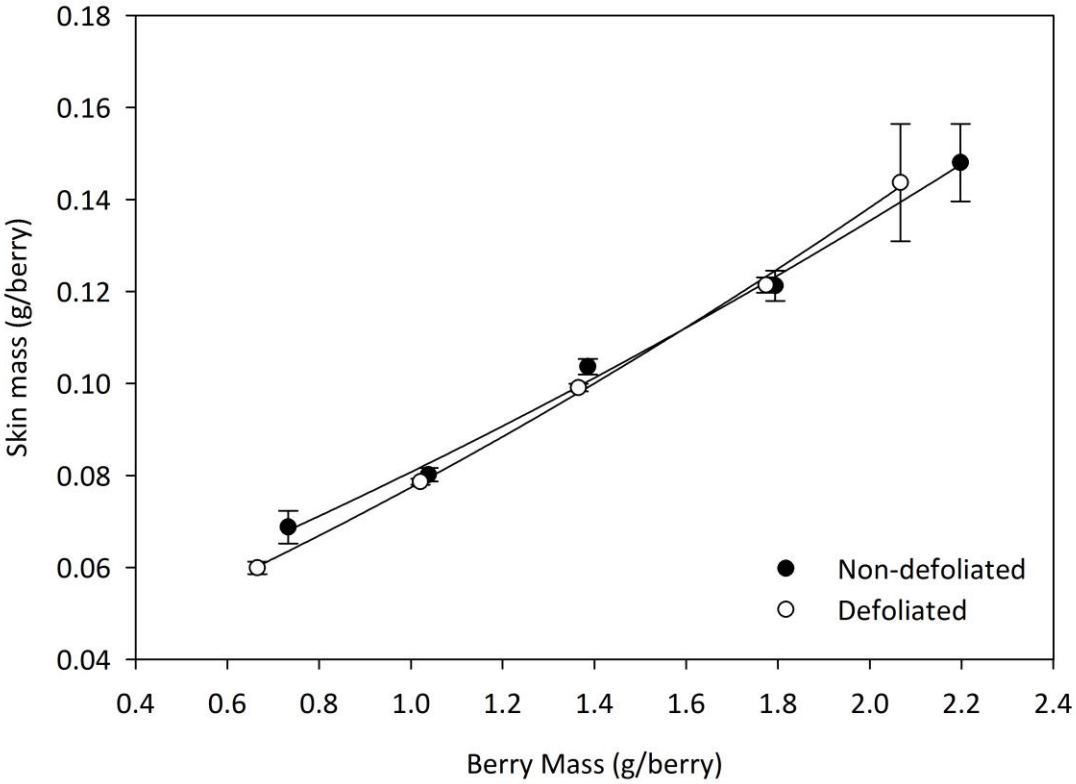

Figure B.4 Skin mass per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent the means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

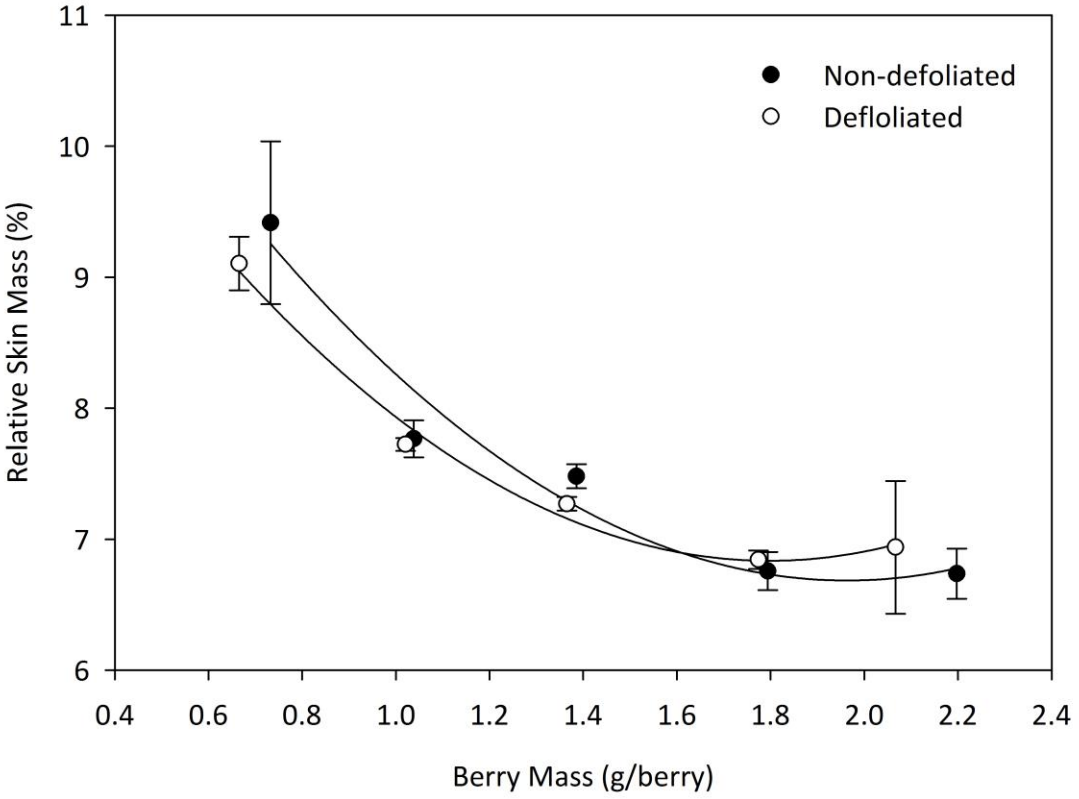

Figure B.5 Relative skin mass per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

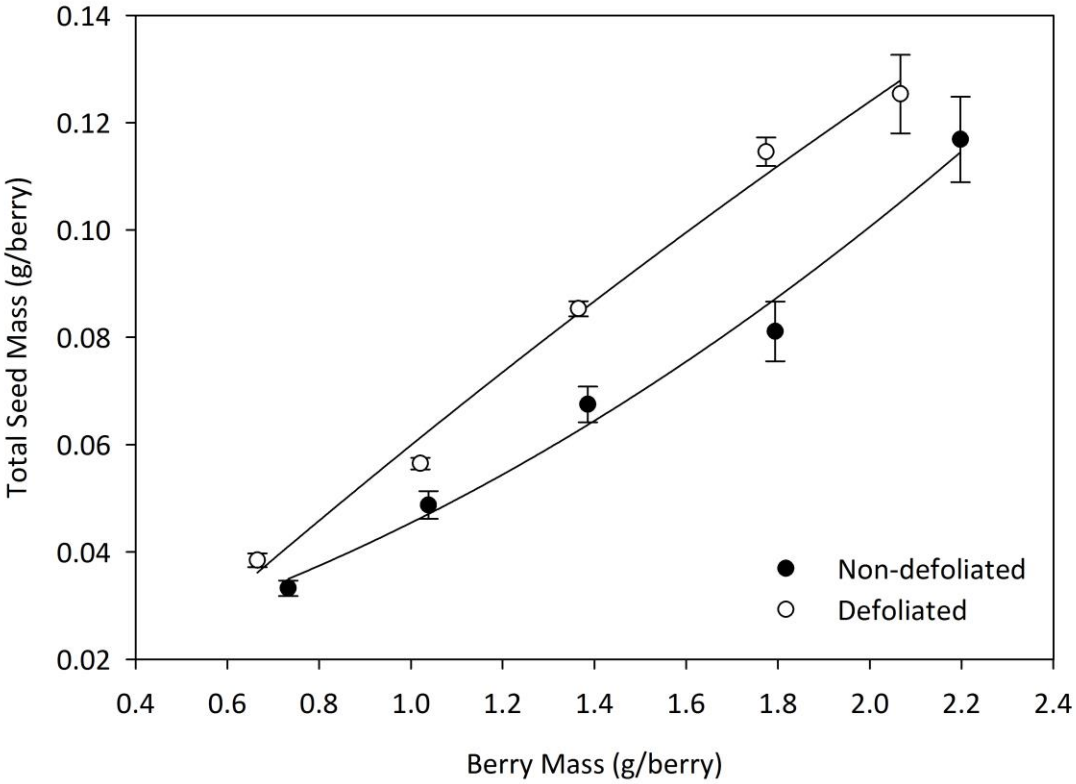

Figure B.6 Total seed mass per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

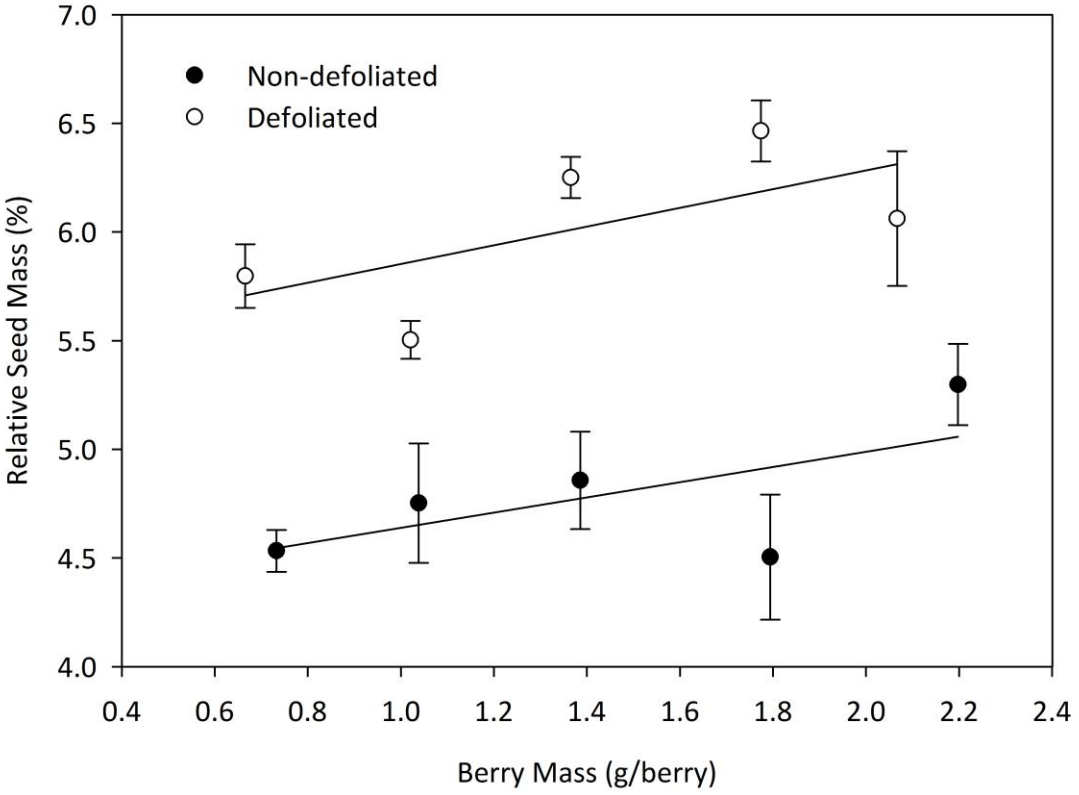

Figure B.7 Relative seed mass per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

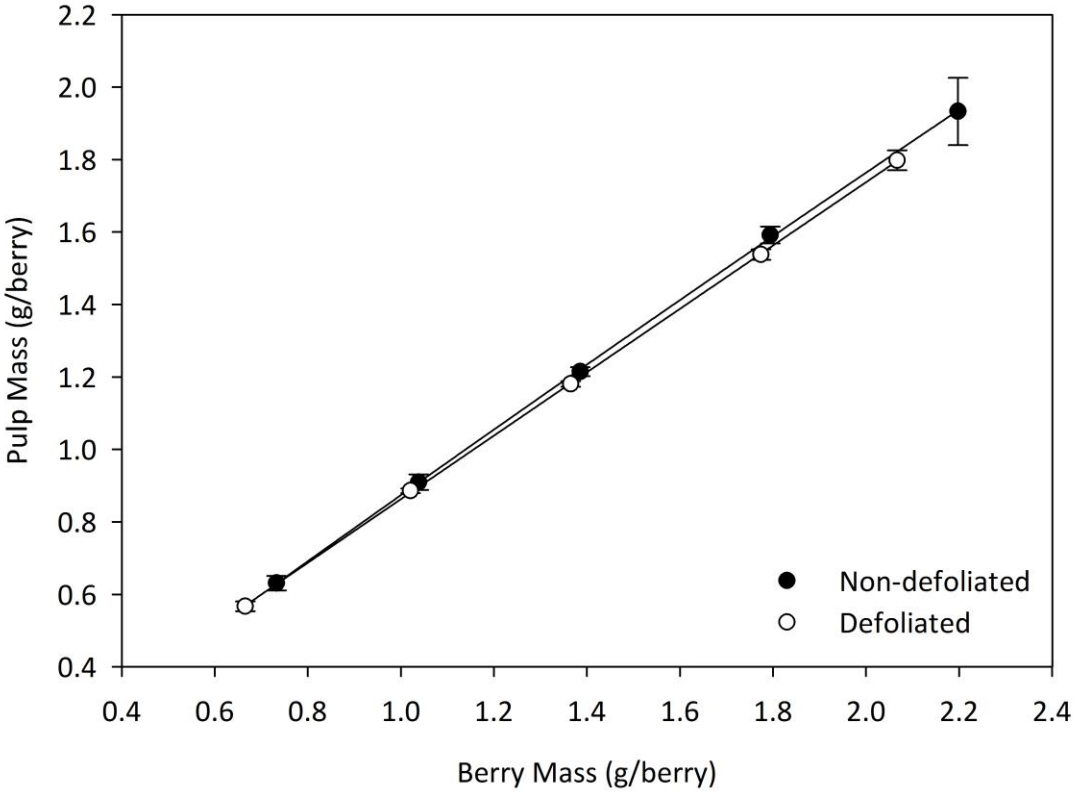

Figure B.8 Pulp mass per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

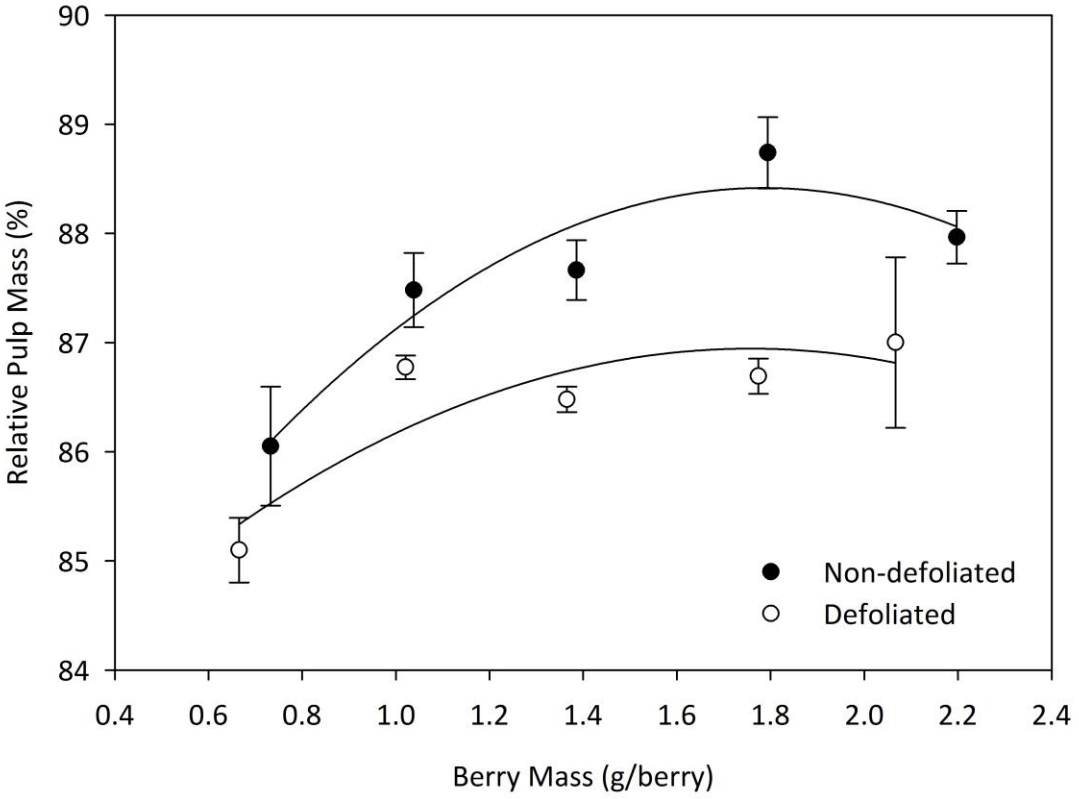

Figure B.9 Relative pulp mass per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

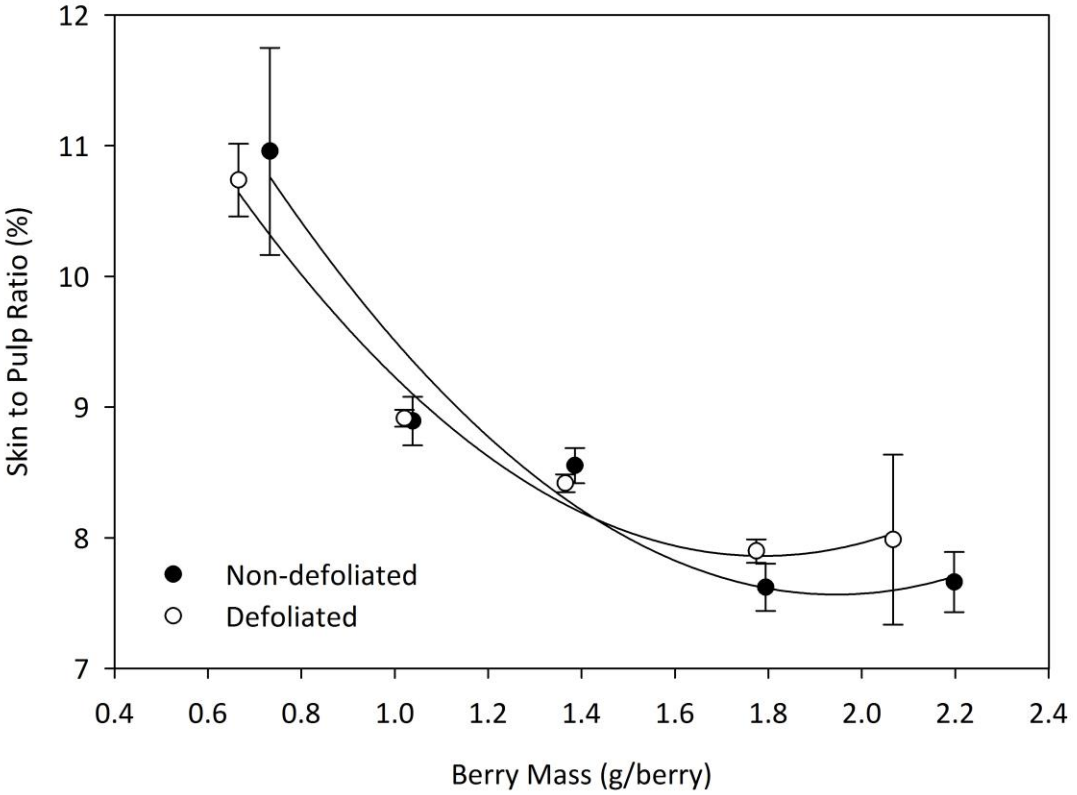

Figure B.10 Skin to pulp ratio per berry in five size categories for non-defoliated and defoliated vines. Plotted values represent means falling within each category. Sample size ranges of non-defoliated and defoliated vines were 4 to 60 and 3 to 222, respectively. Error bars represent standard error of the mean in y direction.

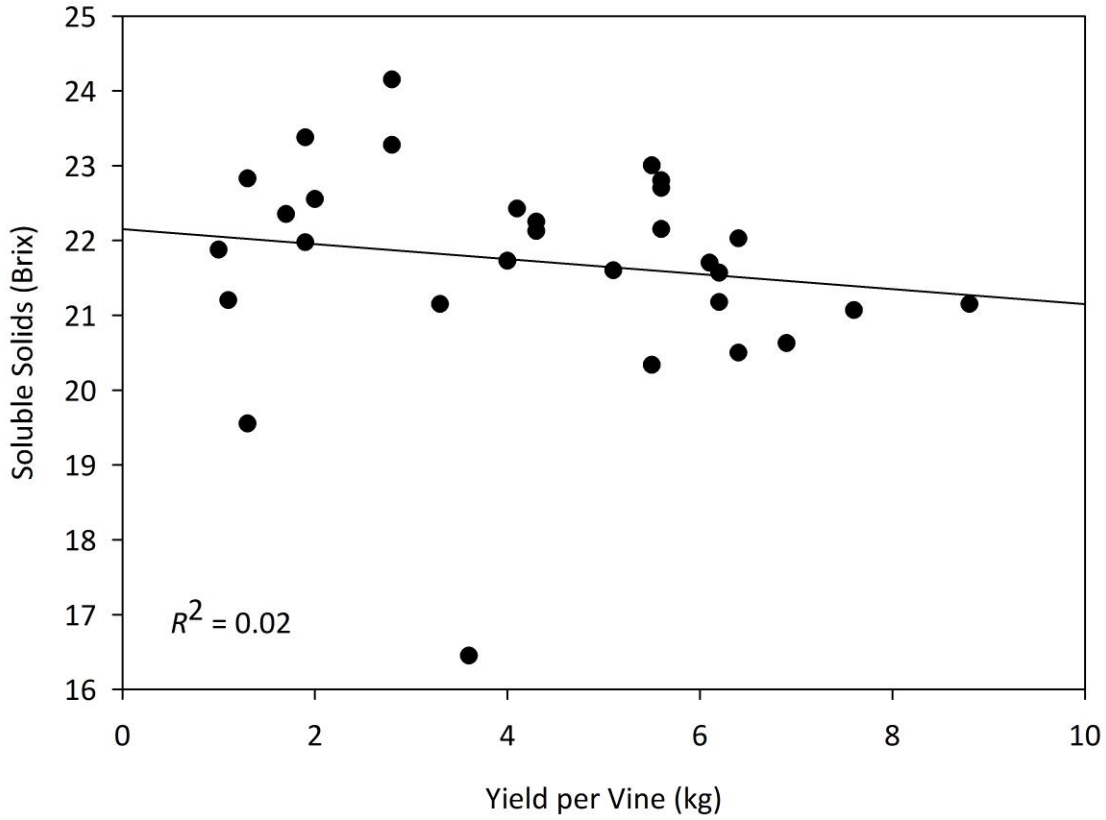

Figure B.11 Effect of vine yield in 2012 on soluble solids in grape juice (r = 0.15; *p* = 0.418).

APPENDIX C - FRUIT SET AND CLUSTER MORPHOLOGY AS A FUNCTION OF SOURCE AVAILABILITY AND SINK REQUIREMENT OF PINOT NOIR CLONE UCD29

Table C.1 Means of the yield components (± SE).

Treatment[z]	Yield per vine (kg)[y]	Pruning weight (kg)	Ravaz index
Leaf Removal (L)			
LNO	6.3 (0.8)	1.6 (0.2)	2.9 (1.1)
LYES	6.5 (0.8)	1.5 (0.2)	2.7 (1.1)
Sink Removal (S)			
SYES	5.1 (0.8)	1.7 (0.2)	1.8 (1.1)
SNO	7.7 (0.8)	1.3 (0.2)	3.8 (1.1)

[z] L = leaf removal; S = sinks removal; LNO = no leaves removed; LYES = leaves removed from 10 basal nodes; SYES = sinks removed; and SNO = sinks present.

[y] Means were based on seven replicates.

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