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Response of Pinot gris grapevines (*Vitis vinifera* L.) to
infestation by potato leafhoppers (*Empoasca fabae* Harris)

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Response of Pinot gris grapevines (*Vitis vinifera* L.) to infestation by potato leafhoppers (*Empoasca fabae* Harris)

By

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ABSTRACT

Response of Pinot gris grapevines (*Vitis vinifera* L.) to infestation by potato leafhoppers (*Empoasca fabae* Harris)

By

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Potato leafhoppers (*Empoasca fabae* Harris) can be pests in Michigan vineyards, but grapevine responses to infestations have not been well documented. In order to rectify this, experiments were conducted on potted grapevines at the whole-vine and single-leaf levels. Potted and fruitless Pinot gris (*Vitis vinifera* L.) grapevines grafted to rootstock 1103 Paulsen (*V. berlandieri* Planch. x *V. rupestris* Scheele) were infested with various levels of potato leafhopper (PLH) nymphs for seven days. Infestation severity was directly related to the leaf symptoms of cupping and discoloration and inversely related to both shoot and leaf growth. Leaf and root biomass tended to decline in response to infestation. Damage thresholds, defined as the number of insects necessary to cause damage to the plant (Pedigo *et al.* 1986), were determined for leaf growth, shoot growth and leaf symptoms. A threshold of 0.5 PLH was found for leaf cupping, leaf size, shoot length and internode length; a threshold of 1.0 PLH was found for leaf discoloration and leaves per vine; a threshold of 3.0 PLH was found for internodes per vine and 4.5 PLH for root fresh mass, root dry mass and total vine dry mass. Although PLH infestations caused decreases in shoot growth, leaf growth and biomass accumulation, the vines were able to tolerate low infestation levels without measurable decline in growth. In addition, the vines were able to recover for some growth reductions during the post-infestation period. In order to investigate the impact of PLH infestation on grapevine leaf photosynthesis, PLH nymphs were caged on 2.5 cm^2 portions of grapevine leaves and gas exchange was measured before and after the infestation period. PLH infestation level was inversely proportional to carbon assimilation (A), transpiration (E) and stomatal conductance (G_s) and directly proportional to internal CO_2 concentration (C_i). Decreases in A were correlated with decreased G_s and increased C_i and thus reductions in A were due to both stomatal and non-stomatal limitations. Damage thresholds existed for A, E, G_s and C_i at most leaf positions. Threshold levels for the four leaf positions from apex to base respectively were: 1, 2, 2 and 8 for A; 1, 1, 2 and none for E; 1, 1, 2 and 8 for G_s; and none, 4, 4 and 8 for C_i. These studies show that: 1) PLH infestations reduce shoot growth, leaf growth and biomass accumulation of potted Pinot gris grapevines; 2) PLH infestations reduce A, G_s and E and increase C_i of grapevine leaves; 3) damage thresholds exist for growth and photosynthetic performance of potted Pinot gris grapevines.

Dedication

This one goes out to all the seekers.

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List of Symbols, Units and Abbreviations

Symbol	Parameter	Units
PLH	potato leafhopper	
PLH-hrs	potato leafhopper hours	
A	net CO_2 assimilation	µmol CO_2 * m^{-2} * s^{-1}
A_{max}	maximum rate of CO_2 assimilation	µmol CO_2 * m^{-2} * s^{-1}
Ci	sub-stomatal CO_2 concentration	µl/l CO_2
Γ	CO_2 compensation point	µl/l CO_2
Φ	quantum efficiency	µmol CO_2 fixed/µmol photons
G_s	stomatal conductance	mmol H_2O* m^{-2} * s^{-1}
E	transpiration	mmol H_2O * m^{-2} * s^{-1}
k	carboxylation efficiency	µmol CO_2 fixed/ppm CO_2
PAR	photosynthetically active radiation	µmol photons * m^{-2} * s^{-1}
SE	standard error	
MRFE	most recently fully expanded leaf	
%SS	percent soluble solids	°BRIX

Literature Review

Introduction

Grapevines are one of the oldest known cultivated plants. Historical records indicate that cultivated *Vitis vinifera* L. originated in the region between the Black and Caspian seas (Winkler 1974, Mullins 1998) roughly 8,000 years ago (Mullins 1998). Grapevines are cultivated nearly everywhere in the world where human civilizations exist and where the climate is conducive to vine growth (Winkler 1974). Grapes are used for fresh fruit, raisins, juice, wine and as a seed oil extract.

According to the Michigan Grape and Wine Industry Council's website (2006), there are about 13,500 acres of vineyards in Michigan. Most of these are Concord and Niagara cultivars and are used for juice production. About 1,500 acres of this total are used for wine grapes making Michigan the eighth largest wine grape producer in the United States. About 58% of wine grapes in Michigan are *vinifera*; among these are Riesling, Chardonnay, Cabernet franc and Pinot gris. Since 1997 there has been a 24% increase in vineyard area, most of which is due to new *vinifera* plantings.

Michigan vineyards are subjected to a variety of stresses. This region is considered a cool climate viticultural area, which means the growing season is short and vines in Michigan can suffer from freeze damage and both early and late season frosts (Howell 2001). Although rainfall is generally not lacking, high humidity can lead to much disease pressure, especially from fungi (Agrios 1997). In addition to about two dozen pathogens that can infest vines here, there are also about two dozen insect species that can infest Michigan vineyards (Isaacs *et al.* 2003). Among these insects, the potato leafhopper (PLH) is one of the most important. PLH infestations of winegrape vineyards can lead to leaf discoloration and deformation, and the cultivar Pinot gris is one of the more susceptible cultivars to such infestations (Isaacs, personal communication 2001, 2006).

PLH infestations are commonly treated with pesticides at a cost of both time and money to growers (Wise *et al.* 2006). Although such resources are allocated to the control of PLH infestations, little is known about how the infestations actually affect grapevine productivity. Changes in leaf morphology and leaf color in response to infestations have been observed repeatedly (personal observation), but it is not clear how such symptoms affect vine growth. Knowing how PLH infestations affect vine growth would be valuable in making rational control decisions in the vineyard. Quantifying infestations in terms of their effects on vine growth and determination of damage thresholds could potentially reduce vineyard management costs.

A better understanding of how PLH affect grapevine productivity is necessary. To date, there are no scientific studies which document this relationship. However, there are many studies that show how PLH affect the growth of other host plant species such as alfalfa (*Medicago sativa* L.) (Womack 1984, Kabrick and Backus 1990, Ecale and Backus 1995b, Zhou and Backus 1999) and potato (*Solanum tuberosum* L.) (Walgenbach *et al.* 1985). These plant species generally exhibited stunted growth and reduced carbon assimilation when infestations were severe. If similar consequences occur in cases of PLH infestations of grapevines, grapevine crop balance might be altered, potentially leading to reduced vine productivity and vineyard profitability.

Grapevine Balance

The concept of grapevine balance dates back to the early 20th century. The French viticulturist M. L. Ravaz is credited with discovering the relationship between vegetative and reproductive growth of grapevines within a given season. The relationship became known as the Ravaz Index and it is the ratio of fruit yield and vegetative yield as measured by fresh pruning weights (Ravaz 1911). The Ravaz Index is a method of quantifying events that already occurred during the previous growing season.

In the 1920's and 1930's, the Michigan viticulturist Newton Partridge refined the Ravaz Index into a predictive model. He used the hindsight concept of the Ravaz Index and changed it to a model that could be used to estimate the potential yield of ripe fruit on a vine for the coming season. Partridge showed that one could estimate the yield potential of the vine for the upcoming season based upon the fresh mass of pruned canes from the previous growing season (Partridge 1925, 1926). Partridge observed that vines with heavier cane pruning weights had a greater capacity for larger yields the next growing season (Partridge 1925). Thus, pruning weights from year-1 could be used to estimate the fruiting potential of year-2 (Partridge 1926). He also observed that fruit quality as defined by °BRIX was influenced by pruning and thinning methods (Partridge 1931).

The observations made by Partridge are now known as the growth-yield relationship (Howell 2001). The growth-yield relationship is fundamentally about the accumulation, allocation and partitioning of vine resources between vegetative growth and reproductive yield. Today, the growth-yield relationship is often expressed as the amount of leaf area required to mature a given mass of fruit (Smart *et al.* 1990). The range of leaf area required to mature one gram of fruit can be wide (Smart 1985). According to a review by Howell, the range is 7-14 cm^2 per gram fruit (Howell 2001). This wide range is partly due to factors that can influence leaf photosynthesis (Kriedemann 1977). Such factors include the environment, both abiotic (Wareing *et al.* 1968, Flore and Lakso 1989, Petrie *et al.* 2000c), and biotic (Mercader and Isaacs 2003, Lehman 2005, Nail and Howell 2005), and grapevine canopy management (Smart 1985). These factors can influence vine growth and productivity through their influences on leaf photosynthesis (Petrie *et al.* 2000b, Nail and Howell 2005).

Photosynthesis

The most fundamental physiological process that affects grapevine productivity is leaf photosynthesis (Kriedemann 1977). Over 90% of plant dry matter is derived from atmospheric carbon fixed during photosynthesis (Flore and Lakso 1989). Grapevine photosynthesis occurs mainly in the chloroplasts of the leaf mesophyll cells (Kriedemann 1977), although other green tissues have been shown to be photosynthetically active (Kriedemann 1967, Pandey and Farmahan 1977). Sugar production *via* photosynthesis begins with the chloroplast. Triose phosphates are synthesized in the chloroplast then either stored as starch or exported from the chloroplast to the cytosol where they can be reduced into sucrose. These sugars can then be loaded into the phloem, translocated through the plant and unloaded in actively growing tissues called "sinks". Here, the sugars can be oxidized to produce energy compounds such as adenosine triphosphate

(ATP), or reduced further to make starch, cellulose, tissues and organs (Taiz and Zeiger 1998).

Photosynthetic rates can be measured directly as the amount of O_2 evolved by the plant (Ladd Jr. 1964) or the amount of CO_2 assimilated by the plant (Wareing *et al.* 1968 Womack 1984, Baysdorfer and Bassham 1985, Smart *et al.* 1988, Flinn *et al.* 1990, Layne and Flore 1995, Edson *et al.* 1995a, Petrie *et al.* 2000c). Such gas exchange measurements can be conducted on whole plants (Layne and Flore 1995, Edson *et al.* 1995a) or individual leaves and leaf regions (Layne and Flore 1992, Edson *et al.* 1995a, Petrie *et al.* 2000c). Photosynthetic carbon assimilation can also be measured indirectly as biomass accumulation by the plant. Biomass accumulation can be measured as the size or mass of plants or portions of plants (Edson *et al.* 1995b, Miller *et al.* 1996b, Petrie *et al.* 2000b). Such techniques have lead to a greater understanding of the factors that can influence photosynthesis of grapevines and other plants. Several such factors that can influence photosynthesis include those of the abiotic environment.

Abiotic Environment

Several components of the abiotic environment are known to influence the rate of net photosynthesis (Pn) of plants. These include light (Flore and Lakso 1989), temperature (Flore and Lakso 1989), ambient CO_2 concentration (Wareing *et al.* 1968, Stoev and Slavcheva 1979), water content of the soil and atmosphere (Flore and Lakso 1989), wind (Jackson 2000) and nutrient availability (Jackson 2000). Additionally, there are diurnal effects on photosynthesis and leaves will typically show a decline in Pn in the afternoon (Kriedemann and Smart 1971). The general photosynthetic reaction is CO_2 +

H_2O + light = $C_6H_{12}O_6 + O_2$. From this equation it should be noted that the main substrates for plant photosynthesis are atmospheric CO_2 and water. In the presence of adequate light, and within a range of temperature optima, these substrates can be synthesized into carbohydrates with oxygen given off as a byproduct.

Light provides the energy that drives photosynthesis. At low light levels, CO_2 losses due to respiration can exceed CO_2 gains due to photosynthesis (Kriedemann 1968, Stoev and Slavcheva 1979). As light levels increase, the light compensation point will be reached, defined as no net gain or loss in CO_2 exchange by the leaf (Layne and Flore 1995). Beyond the light compensation point, the rate of grapevine leaf photosynthesis increased with increased light intensity striking the leaf (Kriedemann 1968, Wareing *et al.* 1968, Kriedemann 1977, Smart *et al.* 1988). However, there is a point where photosynthetic rates no longer increase in response to increasing light (Kriedemann 1968), and this is referred to as the light saturation point. Grapevine leaves will typically reach the light saturation point around one third to one half full sunlight (Kriedemann 1977). In Michigan, this equates to 800-1000 $\mu mol*m^{-2}*s^{-1}$ PAR (personal observation).

Temperature is another abiotic influence on leaf photosynthesis. Temperature influences the rate of Pn over a range of light levels and CO_2 concentrations (Kriedemann 1968, Stoev and Slavcheva 1979), increasing up to a temperature optimum of 25°C for greenhouse grown vines and 30°C for field grown vines (Kriedemann 1968). Leaf Pn declined at temperatures exceeding about 35-40°C (Kriedemann 1968, Stoev and Slavcheva 1979). This decline in Pn due to excessive heat is likely due to enzyme destabilization, stomatal closure and tissue desiccation (Kriedemann 1977).

CO_2 is one of two main substrates for sugar synthesis. Ambient CO_2 levels influence the rate of carbon assimilation (Wareing *et al.* 1968, Stoev and Slavcheva 1979). Increasing ambient CO_2 concentration resulted in elevated rates of Pn over a range of light levels (Wareing *et al.* 1968). However, as with light, there is an optimum level of CO_2 above which increasing CO_2 concentrations will not result in significant increases in Pn (Kriedemann 1977, Layne and Flore 1992, 1995).

Water is the other main substrate for the reactions of photosynthesis. Water content of the soil and its influence on leaf water status can influence Pn (Flore and Lakso 1989). Soil water content can inhibit plant photosynthesis when there is either drought (Flore and Lakso 1989) or flooding (Davies and Flore 1986, Larson *et al.* 1991, Beckman *et al.* 1992, Blanke and Cooke 2004). In both cases, stomatal closure is involved in the inhibition of Pn (Bradford and Hsiao 1982, Davies and Flore 1986, Crane and Davies 1988, Flore and Lakso 1989, Larson *et al.* 1991, Blanke and Cooke 2004). However, C_i is generally either not affected or it will increase in response to flooding (Larson *et al.* 1991, Beckman *et al.* 1992, Blanke and Cooke 2004) or drought (Flore and Lakso 1989).

Photosynthesis is indirectly affected by airflow through the canopy. Wind can shift leaves in the canopy, allowing greater sunlight exposure of shaded leaves. As previously noted, low light levels can inhibit leaf photosynthesis (Kriedemann 1968). Wind can also reduce canopy moisture content by reducing humidity and drying wet leaves (English *et al.* 1990, Jackson 2000). High humidity in the canopy can facilitate fungal infections of the leaves (Agrios 1997) thus potentially reducing leaf photosynthesis (Lehman 2005, Nail and Howell 2005).

These aforementioned components of the abiotic environment can influence plant photosynthesis. Thus, they can impact the carbohydrate status of the vine and vine productivity as well. Although humans can alter any of these environmental factors artificially, doing so is largely impractical for commercial purposes, perhaps with the exception of irrigation. However, modification of the vine *via* canopy management techniques can be used by the viticulturist in order to influence how the abiotic environment interacts with the vine.

Canopy Architecture

The canopy is defined as the shoot and leaf system (Shaulis and Smart 1974). It is all of the above ground portions of the grapevine. Canopy architecture refers to the relative amounts of each organ (shoots, leaves, fruit and wood) and also refers to how these tissues are organized in space. The architecture of the canopy can alter the microenvironment of the vine and thus influence how the abiotic environment interacts with the vine (Smart 1985). Thus, one can change the architecture of the canopy in order to optimize the influence of the abiotic environment on the vine and hence influence vine Pn.

One of the most important aspects of canopy architecture is its impact on light interception. Light interception varies over different portions of the canopy indicating that vine architecture will influence light interception by the canopy (Smart 1973, Smart *et al.* 1990). Grapevine leaves absorb about 90% of the light that strikes them, with only about 5% reflected and 5% transmitted (Smart *et al.* 1988). Thus, inter-vine shading of the leaves can reduce carbon assimilation by the canopy. Leaves growing in the shade have been shown to have less photosynthetic potential than leaves growing in the sun (Smart *et al.* 1988, Intrieri *et al.* 1995), and can be parasitic on vine carbohydrates below the light compensation point. In addition to the leaves, grapes have also been shown to be photosynthetically active (Kriedemann 1967, Pandey and Farmahan 1977), thus fruit exposure to the light can also influence carbon assimilation of the vine. During the ripening period, shade reduced berry color change, mean berry weight, %SS and increased TA (Smart *et al.* 1988), a consequence that could have been due to light, temperature or a combination of the two.

Source/sink relations of grapevines

In addition to the canopy architecture influence on light interception by leaves, it also affects photoassimilate allocation and partitioning within the vine as it influences source/sink relations of the vine. The overall carbon budget of the vine can be described in terms of the ratio of carbon sources to carbon sinks. Sink strength has been defined as assimilate demand (Marcelis *et al.* 2004), and as the product of sink size and sink activity (Flore and Lakso 1989, Taiz and Zeiger 1998). Source strength is defined as assimilate supply (Marcelis *et al.* 2004). It should be noted that the relative strength of any given sink or source changes over the course of the season (Mullins *et al.* 1992). The main source of carbon used for growth and metabolism comes from leaf photosynthesis (Wardlaw 1990). However, young leaves are net carbohydrate sinks and do not become net sources to the vine until they are one third to one half of their final size (Hale and Weaver 1962).

Many studies have been conducted showing how altering vine architecture can influence source/sink relations and thus carbohydrate accumulation, allocation and partitioning. The fruit is a major carbohydrate sink and by veraison will become the dominant sink. Altering crop level can affect carbohydrate translocation patterns of the vine (Hale and Weaver 1962) and also cause changes in leaf photosynthetic rates (Gucci *et al.* 1991, Edson *et al.* 1995a, Iacono *et al.* 1995, Petrie *et al.* 2000c). Reducing crop level caused decreased total fruit yields (Partridge 1931, Kaps and Cahoon 1989, Edson and Howell 1993, Edson *et al.* 1995b) but lead to increases in average berry size (Kaps and Cahoon 1989), cluster weight (Partridge 1931, Edson and Howell 1993, Edson *et al.* 1995b), berries/cluster (Edson and Howell 1993, Edson *et al.* 1995b), %SS of the fruit (Partridge 1931, Kaps and Cahoon 1989) and juice sugar concentration (Iacono *et al.* 1995). Increasing clusters per vine caused reductions in leaf area per vine, leaf area per gram fruit and leaf area per gram total vine dry mass (Edson and Howell 1993).

In addition to crop level influences on reproductive growth, it also influences vegetative growth of the vine. Increasing crop level caused decreases in shoot length, internode length, leaf size and leaf area per vine (Edson *et al.* 1993, Edson and Howell 1993, Edson *et al.* 1995b), decreased leaf area per gram fruit (Edson and Howell 1993) and decreased leaf area per gram total vine dry mass (Edson and Howell 1993). These results were reflected in biomass partitioning which shows that increasing clusters per vine resulted in increased dry mass for fruit but reduced dry mass for leaves, shoots and roots, but with no apparent effect on total vine biomass (Edson and Howell 1993).

Similar to reproductive sinks, the number and activity of vegetative sinks can also influence vine biomass partitioning. Shoot numbers are commonly varied by pruning and by altering the number of nodes retained while pruning. As node number retained per vine increases, there is typically an increase in the number of shoots per vine (Smithyman *et al.* 1997, Smithyman *et al.* 1998, Miller and Howell 1998). This results in higher yields (Byrne and Howell 1978, Miller *et al.* 1993, Smithyman *et al.* 1997, Miller and Howell 1998), but reduced vegetative growth (Miller *et al.* 1993, Smithyman *et al.* 1997, Smithyman *et al.* 1998, Miller and Howell 1998). In some cases, higher yields are correlated with reduced fruit maturity as measured by fruit composition values (Byrne and Howell 1978, Miller *et al.* 1993)

A study varying shoot numbers within a population of fruitless potted Chambourcin (Joannes Seyve 26-205) grapevines showed that increasing the number of vegetative sinks also influenced biomass partitioning (Miller *et al.* 1996a, Miller *et al.* 1996c). As the number of shoots increased, individual shoots were shorter, had fewer and smaller leaves and shorter internodes (Miller *et al.* 1996a, Miller *et al.* 1996c). This indicates within-vine competition; as the number of vegetative sinks increased, there were fewer vine resources allocated to each sink. However, the greater number of sinks did lead to higher vegetative growth on a per vine basis in terms of leaves per vine and total leaf area per vine (Miller *et al.* 1996a, Miller *et al.* 1996c). Finally, vines with higher shoot numbers had greater total dry mass by harvest (Miller *et al.* 1996b), indicating that vines with relatively few sinks were sink-limited.

Defoliation is another means by which vine growth can be altered. Reducing source strength by defoliation can decrease the size of the leaf sink, at least temporarily, thus causing pronounced effects on vine growth. Mechanical defoliation of potted grapevines resulted in shorter internodes, smaller leaves and thinner shoots (Petrie *et al.*

2000b). Defoliation caused reductions in yield per vine (Koblet *et al.* 1994), cluster weight (Koblet *et al.* 1994, Petrie *et al.* 2000a), berry weight (Koblet *et al.* 1994, Petrie *et al.* 2000a), %SS (Mansfield and Howell 1981, Koblet *et al.* 1994, Petrie *et al.* 2000a), fruit sugar content (Iacono *et al.* 1995, Petrie *et al.* 2000a), increased titratable acidity (Koblet *et al.* 1994) and delayed berry maturation (Mansfield and Howell 1981). Defoliation also reduced total vine biomass accumulation (Petrie *et al.* 2000b). These studies on defoliation show that removal of the principal source of vine sugars can result in fewer resources available for growth. However, the vine seems able to compensate to some degree for shifts in source/sink balance.

Compensatory mechanisms

The studies cited above show that varying the source/sink balance of grapevines leads to morphological changes in vine growth. When the balance is shifted, the relative allocation and partitioning of assimilate to vegetative and reproductive tissue is affected. However, in addition to changes in biomass, there are also physiological changes occurring in grapevines in response to shifts in source/sink balance. Leaf Pn can be stimulated by reducing source strength or by increasing sink strength, a phenomenon known as photosynthetic compensation (Flore and Lakso 1989, Wardlaw 1990).

Photosynthetic compensation has been demonstrated in several plant species. Reducing source strength by defoliation caused increased Pn for grapevines (Iacono *et al.* 1995, Intrieri *et al.* 1997, Petrie et al. 2000c), alfalfa (Baysdorfer and Bassham 1985), cherry (*Prunus cerasus* L.) (Layne and Flore 1992, 1995) and both bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) (Wareing *et al.* 1968). Reducing leaf area per vine by varying the number of shoots retained resulted in no differences in vine dry mass throughout most of the season (Miller *et al.* 1996b), indicating that vines with low leaf area were operating at higher photosynthetic rates. Thus, it appears that leaves of grapevines and other plants can increase Pn in situations where source strength is insufficient to meet the energy demands of the sinks.

Similarly, increasing the sink strength of grapevines can also stimulate leaf Pn. Situations where sink strength was increased by increasing crop level resulted in reductions in total leaf area but increases in leaf assimilation rates (Edson *et al.* 1995b, Petrie *et al.* 2000b) ultimately resulting in similar total vine biomass accumulation among vines that differed in crop level (Edson *et al.* 1995b, Petrie *et al.* 2000b). A study in which cluster numbers were varied on potted vines resulted in similar total leaf area per vine yet vines with higher crop level were able to ripen more fruit to similar maturity levels (Miller *et al.* 1996c). Since fruit maturity, as measured by %SS, pH and titratable acidity, was similar among vines that differed in leaf area per gram fruit, vines with less leaf area must have been operating at higher rates of leaf Pn (Miller *et al.* 1996c). Similar situations occurred where 12% of the main leaves were removed from grapevines but there were no differences in yields or fruit composition by the end of the study (Koblet *et al.* 1994), and where half of the leaves were removed from shoots of Concord vines with no apparent affect on %SS, bud fruitfulness or subsequent vine size (Mansfield and Howell 1981).

The biotic environment and damage thresholds

As suggested above, the abiotic environment directly and indirectly influences vine Pn. Additionally, Pn can also be influenced by the biotic environment. In Michigan, there are over two dozen pathogens that can cause disease to grapevines and there are also over two dozen insects that can infest grapevines (Isaacs *et al.* 2003). Every portion of the grapevine can be subjected to disease by pathogens or infestation by insects (Isaacs *et al.* 2003). Among the host of pathogens and insects known to affect grapevines in Michigan, some have been shown to infest grapevine leaves and reduce leaf Pn. These include powdery mildew (*Uncinula necator* Burr.) (Shtienberg 1992, Nail and Howell 2005), downy mildew (*Plasmopara viticola* Berk. and Curt. ex de Bary) (Lehman 2005), rose chafers (*Macrodactylus subspinosus* F.) (Mercader and Isaacs 2003) and Japanese beetles (*Popillia japonica* Newman) (Mercader and Isaacs 2003).

Another foliar-feeding organism of grapevines in Michigan is the leafhopper. The two most important species in Michigan are the Eastern grape leafhopper (*Erythroneura comes* Say) and the potato leafhopper (*Empoasca fabae* Harris) (Isaacs *et al.* 2003). Both feed on the underside of grapevine leaves and can cause damage as they feed. Both are generalists and known to feed on several species of plants (DeLong 1931, Lamp *et al.* 1994, Martinson *et al.* 1994). In general, *vinifera* cultivars tend to be less tolerant than *V. labrusca* L. cultivars (Isaacs *et al.* 2003). Although very little is known about how PLH infestations affect grapevines, the Eastern grape leafhopper (*Erythroneura comes* Say) reduced yields (Martinson *et al.* 1997), berry weights (Martinson *et al.* 1997) and juice sugars (%SS) (Van Dine 1923) of Concord (*Vitis labrusca* L.) grapevines.

These various diseases and insects in Michigan vineyards are known to infest vine canopies, and some are known to impact leaf photosynthesis. The concept of photosynthetic compensation discussed above is one example of the ability of grapevines to tolerate a degree of imbalance in source/sink relations, and this has been demonstrated by plant responses to abiotic defoliation discussed above (Layne and Flore 1992, Layne and Flore 1995, Petrie *et al.* 2000a, 2000b, 2000c). A second example is the concept of feedback inhibition, a situation where end-products of photosynthesis can inhibit further production of assimilate which is especially pronounced in response to low sink demand (Layne and Flore 1995). Although there are no examples in the literature of similar grapevine responses to biotic reductions in leaf area, the mechanism does seem to exist as evidenced by the affects of defoliation on Pn.

The ability of plants to tolerate some level of stress is integral in the concept of thresholds. There have been several different thresholds described in the literature in regards to biotic stress (Pedigo *et al.* 1986, Pedigo and Higley 1992, Hunt *et al.* 2000). These thresholds focus on the ultimate goal of determining the economically acceptable levels of damage done to crops by pests, with damage being defined as "measurable loss in host utility" (Pedigo *et al.* 1986). Before these economic thresholds can be defined, it is necessary to quantify plant growth in response to infestations by a specific pest; this has not been done with PLH infestations of grapevines to date. The damage threshold, also known as the damage boundary, is defined as the number of insects necessary to cause damage to the plant (Pedigo *et al.* 1986). Determination of damage thresholds is one of the objectives of the research conducted for this dissertation.

Empoasca fabae Harris, the potato leafhopper

The potato leafhopper (PLH) is a member of the family *Cicadellidae* which contains about 3,000 North American species (Bland and Jaques 1978). PLH are distributed throughout nearly all of the Eastern United States, and have been found on plants from 220 species, 100 genera and 26 families (DeLong 1931, Lamp *et al.* 1989, Lamp *et al.* 1994). PLH have seven developmental stages beginning with the egg and ending with the adult. The eggs are about 1 mm long, elongate and whitish in color. In about 10 days the eggs hatch into nymphs. The nymphs progress through five stages of development called instars and during each instar the nymphs lack functional wings. The developing wings, called wingpads, gradually grow larger as the nymphs proceed through the instars eventually becoming fully functional wings by adulthood. The adults grow to be about 3 mm long, are an iridescent light-green color and have a life-span of about 28 days. PLH are not native to MI and do not overwinter here (DeLong 1931, Medler 1957, Shields and Testa 1999). They overwinter in the southern states along the coast of the Gulf of Mexico (Medler 1957, Shields and Testa 1999). As the weather warms during the early spring, they migrate north eventually arriving in Michigan in late May to early June (Carlson *et al.* 1992). As Fall temperatures cool, they complete their annual migration cycle by returning to the South (Shields and Testa 1999) or they die.

Leafhopper infestations and plants

PLH feed by using their needle-like mouthparts, called stylets, to probe plant tissues, lacerate cells and then ingest the fluids that are flushed from these cells. This feeding behavior can cause plants to develop 'hopperburn', a general yellowing and wilting of plant tissues. Hopperburn is caused by mechanical damage from probing and laceration, and chemical damage from the insect's saliva (Medler 1941, Backus and Hunter 1989, Hunter and Backus 1989, Kabrick and Backus 1990, Ecale and Backus 1995a, Ecale and Backus 1995b). PLH feeding behavior has been termed 'lacerate-and-flush' in reference to their tendencies to rupture cells and tissues with their stylets and then ingest the fluids that are flushed out in the process (Backus and Hunter 1989, Hunter and Backus 1989, Kabrick and Backus 1990).

Stylet probing of alfalfa (*Medicago sativa* L.) can cause abnormally enlarged and abnormally divided cells of the phloem (Medler 1941, Kabrick and Backus 1990, Zhou and Backus 1999) and vascular cambium (Kabrick and Backus 1990), disorganized phloem cells (Kabrick and Backus 1990, Zhou and Backus 1999), collapsed and constricted phloem (Kabrick and Backus 1990, Ecale and Backus 1995b, Zhou and Backus 1999), damaged mesophyll cells (Medler 1941, Hunter and Backus 1989) and damaged xylem (Ecale and Backus 1995b). This range of tissue damage is likely responsible for observations of the numerous altered physiological processes in alfalfa in response to PLH infestations. Assimilation and transpiration of alfalfa were both found to be inversely proportional to the number and duration of PLH exposed to alfalfa stems (Womack 1984). Stomatal conductance, assimilation and transpiration were all inversely proportional to the number of adult female PLH that infested alfalfa (Flinn and Hower 1984). Sugar translocation beyond fed-upon phloem tissues was reduced and sugar concentration upstream from damaged tissue increased for alfalfa in response to PLH feeding (Flinn *et al.* 1990, Nielsen *et al.* 1990, Nielsen *et al.* 1999, Lamp *et al.* 2001).

These studies indicate that PLH-damaged phloem tissue can act as a biological dam, inhibiting the flow of photoassimilates beyond the vicinity of stylet probing.

Reductions in the rates of CO_2 assimilation and carbohydrate translocation mean that PLH feeding can reduce source strength of the host plants, at least temporarily and in the locality of feeding damage. When carbohydrate source strength is reduced, there will be fewer resources for plant growth and development and this can manifest as a general reduction in quantity and quality of yields. Infestations at the level of individual plants and plant populations of alfalfa showed height reduction (Poos and Johnson 1936, Kindler *et al.* 1973, Faris *et al.* 1981, Flinn and Hower 1984, Hower and Flinn 1986), leaf and stem wilting (Harman *et al.* 1995), reduced leaf number and reduction in both fresh and dry weights (Poos and Johnson 1936, Kindler *et al.* 1973, Flinn and Hower 1984, Hower and Flinn 1986, Flinn *et al.* 1990, Hutchins and Pedigo 1990). Changes in the nutritional value of alfalfa have been reported as reduced protein content (Poos and Johnson 1936, Kindler *et al.* 1973, Faris *et al.* 1981, Hower and Flinn 1986, Flinn *et al.* 1990, Hutchins and Pedigo 1990), fat content (Poos and Johnson 1936) and fiber content (Poos and Johnson 1936, Hutchins and Pedigo 1990). Infestations also caused delayed alfalfa growth within a growth cycle and regrowth failure in a cycle that followed severe infestations (Flinn *et al.* 1990, Hutchins and Pedigo 1990).

Although damage by PLH can be severe, some evidence exists that plants can compensate for feeding damage. Walgenbach *et al.* (1985) observed partial recovery of assimilation rates for potato (*Solanum tuberosum* L.) following PLH infestations. Infestation of alfalfa showed eventual phloem regrowth whereby new sieve tubes circumvented damaged areas of phloem (Zhou and Backus 1999). Another study on alfalfa showed phloem damage due to PLH probing, but the phloem appeared normal again eight days after the infestation period (Ecale and Backus 1995b). These studies indicated a degree of tolerance and compensation of some host plants to PLH infestations. Compensation occurred as enhanced rates of photosynthesis and altered tissue growth.

In conclusion, the responses of alfalfa to PLH infestations can be seen as set of symptoms occurring from the cellular level to the population level (Flinn and Hower 1984, Hower and Flinn 1986, Kabrick and Backus 1990, Ecale and Backus 1995b, Zhou and Backus 1999). Stylet probing directly damages alfalfa at the level of cells and tissues, but the indirect affects can extend throughout an individual plant and even to entire populations of plants (Flinn *et al.* 1990, Hutchins and Pedigo 1990). Carbon assimilation and sugar translocation can both be inhibited, leading to reduced vegetative growth and in some cases death (Womack 1984, Flinn *et al.* 1990, Hutchins and Pedigo 1990, Nielsen *et al.* 1990, Lamp *et al.* 2001). In general, probing damage can be thought of as reducing source strength of the host plant, which in turn can reduce both the quantity and quality of yields. Although studies tend to focus on alfalfa, possibly one might find similar responses to PLH by other host species, including grapevines.

Quantifying potato leafhopper infestations of grapevines

PLH infestations in Michigan vineyards are typically controlled by the use of one or more insecticide sprays (Wise *et al.* 2006). Pesticide application is a cost to growers and requires the use of fossil fuels and other non-renewable energy resources. Additionally, there is increasing public concern about the impact these chemicals will have on human health and the environment. If pesticide use can be reduced while maintaining long-term productivity of the vineyard, growers could reduce their costs, resources would be conserved and any potential environmental impact of pesticide use could be limited. A better understanding of grapevine tolerance thresholds to PLH infestations is necessary in order to more efficiently use pesticides. Knowing the severity of PLH infestations required to reduce grapevine productivity and/or fruit quality would be valuable in order to make rational control decisions in the vineyard.

Dissertation Goals and Hypotheses

The goals of the research for this dissertation were to: 1) quantify PLH damage to grapevines in terms of vine vegetative growth, damage thresholds and recovery mechanisms; 2) characterize the impact of PLH infestations on leaf photosynthesis in terms of gas exchange rates, thresholds and recovery mechanisms. The specific hypotheses are: 1) PLH infestations will reduce grapevine leaf and shoot growth; 2) PLH infestations will reduce vine biomass accumulation; 3) there will be damage thresholds of PLH infestation below which no measurable reductions in growth or biomass accumulation will occur; 4) the vine will recover for growth reductions during the post-infestation period; 5) grapevine leaf photosynthesis will decrease in response to PLH infestation; 6) there will be damage thresholds for leaf gas exchange; 7) photosynthetic compensation to PLH infestations will occur for grapevine leaves.

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Chapter 1

Vegetative growth responses of Pinot gris (*Vitis vinifera* L.) grapevines to infestation by potato leafhoppers (*Empoasca fabae* Harris).

Abstract

Potato leafhoppers (*Empoasca fabae* Harris) can be pests in Michigan vineyards, but grapevine responses to infestations by this insect have not been well documented. To rectify this, two experiments were conducted on potted and fruitless Pinot gris (*Vitis vinifera* L.) grapevines grafted to rootstock 1103 Paulsen (*V. berlandieri* Planch. x *V. rupestris* Scheele) and infested for seven days with a range of potato leafhopper (PLH) nymphs from 0.0-4.5 per leaf. Shoot growth, leaf growth and infestation symptoms of the leaves were quantified before and after the infestation period and biomass of vegetative vine structure was measured at the end of each experiment. Infestation severity was directly related to the leaf symptoms of cupping and discoloration and inversely related to both shoot and leaf growth. Both leaf and root mass declined in response to infestation level. Damage thresholds were observed for leaf symptoms, leaf growth and shoot growth for both experiments. Vine recovery, as measured by the influence on growth reductions by infestation, was observed in a second experiment (Experiment II). PLH infestations decreased shoot and leaf growth and vine biomass, but the vines were able to recover from low infestation levels in terms of leaf and shoot growth during the post-infestation period.

Introduction

Potato leafhoppers, *Empoasca fabae* Harris (PLH) can be grapevine pests in Michigan and the Great Lakes region, but grapevine responses to infestations have not been well documented. Knowing how PLH populations affect vineyard productivity would be a useful tool for vineyard management. If there is a relationship between the severity of a PLH infestation and changes in the quantity or quality of grapevine growth, then damage thresholds could be defined. Damage thresholds have been defined as the number of insects necessary to cause damage to the plant (Pedigo *et al.* 1986). Such thresholds could be of value in making control decisions based upon estimated losses in vineyard productivity rather than the mere presence, absence or quantity of this insect. Utilization of such thresholds could decrease the use of insecticides, and thus reduce vineyard management costs and prevent any potential environmental pollution from insecticide use.

An area of major importance in viticulture is carbohydrate source/sink relations. Shifts in source/sink relations can result in changes in vine growth. Reducing carbohydrate source strength of potted Pinot noir (*Vitis vinifera* L.) by defoliation resulted in decreased rates of berry maturation (Petrie *et al.* 2000a) and reduced biomass accumulation (Petrie *et al.* 2000b). Mechanical defoliation of Niagara (*Vitis labrusca* L.) grapevines at bloom resulted in stunted shoots and lower dry mass of roots (Mercader and Isaacs 2003, Mercader and Isaacs 2004). Increasing vegetative sink number and competition by increasing shoot numbers of potted Chambourcin (Joannes Seyve 26-205) grapevines resulted in shoots that were shorter, had fewer and smaller leaves and shorter internodes (Miller *et al.* 1996a). Similarly, increasing reproductive sink strength by retaining higher numbers of clusters on potted Seyval (Seyve-Villard 5-276) grapevines caused decreases in shoot length, leaf area and cluster weight (Edson *et al.* 1993).

These studies show that altering carbohydrate source/sink relations can have profound affects on vine morphology. Decreasing the ratio of carbohydrate source strength to carbohydrate sink strength can lead to deficits in the vine resources available for plant growth and metabolism. Since the leaves are the main source of carbohydrate production for grapevines, disruption of leaf function by foliar-feeding insects could reduce source strength and limit vine growth, productivity and fruit quality. PLH are foliar feeders of grapevines and thus infestations could lead to impaired leaf function. Although grapevine responses to PLH infestations have not been well established, the impact of infestations on other PLH host species has been studied extensively.

The photosynthetic activity of alfalfa (*Medicago sativa* L.) and potato (*Solanum tuberosum* L.) leaves showed reduced rates of CO_2 assimilation, transpiration and stomatal conductance and disrupted translocation of sugars in response to PLH exposure (Hibbs *et al.* 1964, Ladd Jr. and Rawlins 1964, Womack 1984, Walgenbach and Wyman 1985, Flinn and Hower 1990, Nielsen *et al.* 1990, Nielsen *et al.* 1999, Lamp *et al.* 2001). Infestations of individual alfalfa plants resulted in height reduction (Poos and Johnson 1936, Flinn and Hower 1984, Hower and Flinn 1986), leaf and stem wilting (Harman *et al.* 1995), reduced leaf number and decreases in both fresh and dry masses (Poos and Johnson 1936, Flinn and Hower 1984, Hower and Flinn 1986, Flinn *et al.* 1990, Hutchins and Pedigo 1990).

The studies described above show that PLH infestations can disrupt leaf function of both alfalfa and potato, thereby reducing the strength of the main source of carbohydrate synthesis. Such source strength reductions can result in stunted growth of the infested plants. It seems likely that other host plants, including grapevines, will show similar responses to reductions in source strength due to PLH infestations.

The goals of this research were: 1) to determine whether PLH infestations on grapevines will reduce the quantity of vegetative growth; 2) to determine whether there is a relationship between the symptoms of infestation and the quantity of growth; 3) to define damage thresholds for any changes in vegetative growth that might occur; and 4) determine whether vines can recover from PLH induced growth reductions.

Materials and Methods

Two experiments were conducted to test the effects of PLH infestations on the growth and biomass accumulation of grapevines. This section will describe the materials and methods common to both experiments; this section will be followed by the unique details of each experiment.

Plant material: The vines used for both studies were Pinot gris (*Vitis vinifera* L.) grafted to rootstock 1103 Paulsen (*V. berlandieri* Planch. x *V. rupestris* Scheele); the vines for Experiment I were three years old, those from Experiment II were two years old. For both experiments, the vines were grown in black plastic pots with ten liters of steam sterilized soil composed of 50% sandy loam, 30% sphagnum peat and 20% washed sand. The vines were grown under natural light and watered as needed, usually three times per week. All flower clusters were removed; lateral shoots and non-count shoots were removed as they appeared. The vines were fertilized monthly with 600 ml of a solution made by mixing five tablespoons (about 60 g) of 15-30-15 fertilizer with 19 liters of water. This solution contained approximately 475 mg/l of both nitrogen and potassium, 950 mg/l of phosphorus and trace amounts of boron, copper, iron, manganese, molybdenum and zinc.

Insect Source: Second and third instar PLH nymphs were used to infest the vines for this study. Adult PLH were collected from a field mixed with alfalfa (*Medicago sativa* L.) and clover (*Trifolium pratense* L.) using a canvas sweep net. These insects were placed into collapsible cages that measured 61 cm in length, width and height (BioQuip Products, Inc., Gardena CA, model 1450D) and reared indoors on bean plants

(*Vicia faba* L.). The second and third instar nymphal offspring of these adults were used for the experiments. To collect nymphs for applying treatments, a piece of white valais sheers fabric was placed inside the body of an aspirator, then enough nymphs for one vine were aspirated and rendered temporarily immobile using CO_2 gas. While still immobile, the aspirator was dismantled and the fabric containing the nymphs was removed and placed on the soil at the base of the trunk. The vine was then enclosed in a bag made from this same fabric until insect removal occurred. This process was repeated until all the vines received the appropriate number of PLH nymphs. The fabric bags reduced the amount of photosynthetically active radiation by about 1/3 and reduced air flow only slightly (data not shown).

Measurements: Every leaf was assessed visually for cupping. For Experiment I, leaves were scored for cupping as a 1, 2 or 3. Looking down on the leaf, a score of 1 was given if the leaf was the normal flat to slightly concave shape. A score of 2 was given to a leaf that was slightly to moderately convex with margins curling inward towards the abaxial surface, but not overlapping the leaf. A score of 3 was given to a leaf that was severely convex with margins curling inward towards the abaxial surface and overlapping the central portions of the leaf. The same protocol was used for Experiment II except that leaves were scored as either a 1 (normal), 2 (slight cupping), 3 (moderate cupping) or 4 (severe cupping). For both experiments, the percentage of leaves cupped was quantified as the number of leaves showing any degree of cupping as a proportion of the total number of leaves.

Discoloration was assessed visually and expressed as the percentage of tissue per leaf that was yellow to light green and given a score ranging from zero to 100% in increments of ten percent. The percentage of leaves discolored was recorded as the number of leaves showing any amount of discoloration as a proportion of the total number of leaves. The variable '% non-zero leaf' was the average percent discoloration per leaf of only those leaves that were discolored.

Internode lengths and midrib lengths were measured with a ruler to the nearest millimeter. The length of an internode was the linear distance between two nodes and the length of a midrib was the linear distance between the basal and apical ends of the primary vein that bisects the leaf blade. Shoot length was the sum of the lengths of all internodes for a given shoot. At the end of the experiment, biomass accumulation was measured as the fresh and dry mass of the leaves, shoots, wood and roots of each vine. The leaves included both the lamina and petiole, shoot mass included all of the current season's shoot growth except the leaves, roots included all growth from the main trunk axis below the graft union excluding the main axis, and all remaining tissue was categorized as the wood. Total vine biomass was the sum of these four tissue types. Dry mass was attained from plant material that was dried in an oven at 49°C until no further reduction in mass occurred.

Experiment I

Plant Material and Vine Training: On 23 June 2004, dormant vines were moved from a coldroom at a temperature of 4°C into a greenhouse. Each vine was trained to three shoots and no pesticides were used. Three 1.5 m long bamboo stakes were inserted into the soil near the outer rim of the pots as trellises for the shoots. Prior to PLH infestation, the vines appeared healthy and normal showing no symptoms of nutritional imbalances or pest damage with the exception of very mild amounts of defoliation from Japanese beetles (*Popillia japonica* Newman) totaling well under 1% leaf area removed (data not shown).

Measurements and Treatment Application: All data were collected in 2004. The first set was collected prior to treatment application on July 14th and 15th and included midrib lengths, internode lengths, leaf cupping and leaf discoloration. The treatment factor was the number of PLH nymphs per vine and there were six levels: 0, 25, 50, 75, 100 and 150 nymphs per vine. The PLH treatments were added to the vines on July 19th and removed from the vines and counted on July 26th for a total stress period of seven days. The second data set was collected on July 27th and 28th and included the same measurements as the first set. Destructive harvest for fresh masses occurred on August 26th and dry masses were obtained on October 15th.

Leaf Area Analysis: Leaf area was estimated by midrib length. During a previous study, midrib length and leaf area were measured on 69 leaves from the potted Pinot gris vines. For each leaf, midrib length was measured with a ruler and leaf area was measured with a leaf area meter (Li3000, LiCor Inc., Lincoln, NE). A linear regression between these variables was significant ($p<0.0001$, $R^2=0.80$), the relationship was $y = 0.87x - 12.86$, where y is leaf area and x is midrib length, and this equation was used to estimate leaf area for this study.

Experimental Design and Statistical Analysis: The vines were arranged as a randomized complete block design with six treatments and seven blocks using initial leaf number per vine as the blocking factor. Midrib length, internode length, leaf cupping and leaf discoloration were analyzed by ANCOVA using the pre-treatment measurements for each respective variable as covariates. Biomass accumulation was measured at the end of the experiment and analyzed using ANOVA. All statistical analyses were performed using SAS version 8 (SAS Institute, Cary NC). All figures were created using SigmaPlot 8.0 including best-fit equations and R^2 values (Systat Software, Richmond CA).

Herbicide Damage: Shortly after treatment removal, some vines were showing symptoms of herbicide damage including unusually short and thin internodes and leaves that were very small and fan-shaped. All vines showing these symptoms at the time of the second set of measurements (July 27th and 28th) were eliminated from the analysis of midribs, internodes, leaf cupping and leaf discoloration. By August 12th, all but four vines were showing these symptoms and the experiment was ended prematurely as a result of the prevalence of these symptoms. The symptoms of damage resembled those caused by 2,4-Dichlorophenoxyacetic acid, and we suspect that this herbicide caused the damage to the vines. Fresh and dry weight data were included in the results section, but these data should be viewed with caution due to this problem.

Experiment II

Plant Material and Vine Training: The vines were purchased from a nursery and each was weighed and labeled prior to potting. All data were collected in 2005. The vines were potted on June 29th (day-1) and prior to infestation, were normal in appearance with no symptoms of pest damage or nutritional imbalances. A 1.5 meter length of bamboo was then inserted into the soil at the edge of each pot for shoot training. Each vine was trained to one shoot.

Measurements and Treatment Application: This experiment lasted for 114 days. At three times throughout the course of this experiment, the lengths of all internodes and leaf midribs were measured and every leaf was scored for degree of cupping and amount of discoloration. On day 1 (June 29th), the vines were potted. The data for Time-1 were collected on days 27-29; included in this data set was the leaf area regression (see section for Leaf Area Analysis). On day 30, the leaf area of the experimental vines was measured and the vines were arranged into blocks based on leaf area per vine. On day 31, the treatments were randomly applied to each vine within a block, thus initiating the infestation period. There were six treatment levels: 0.0, 0.5, 1.0, 1.5, 3.0 and 4.5 PLH nymphs per leaf. The infestation period lasted for seven days, ending on day 38 when the PLH were aspirated off of each vine and counted. The data for Time-2 were collected on days 41-43, three days after the infestation was terminated. The data for Time-3 were collected on days 111-113, 73 days after the infestation was terminated. On day 114, the vines were destructively analyzed for biomass accumulation.

Leaf Area Analysis: Leaf area was estimated by midrib length. During Time-1, midrib length and leaf area were measured on 469 leaves from the potted Pinot gris vines. For each leaf, midrib length was measured with a ruler and leaf area was measured with a leaf area meter (Li3000, LiCor Inc., Lincoln, NE). A nonlinear regression between these variables was significant ($p<0.0001$, $R^2=0.95$), the relationship was $y = 0.0125x^{1.9955}$, where y is leaf area and x is midrib length. This equation was used to estimate leaf area for this study.

Experimental Design and Statistical Analysis: This experiment was a randomized complete block design with repeated measures. The blocking factor was leaf area and the treatment factor was the number of PLH nymphs per leaf. Multiple comparison tests at the 5% level were used to examine pairwise differences among all means for all of the analyses using LSD.

All midrib and internode length data were analyzed using a linear mixed model with treatment level and time as fixed effects and block as a random effect. The Shapiro-Wilk test was used to determine whether the residuals were normal; in cases where the residuals were not normal, the data were transformed. Log transformations were performed on shoot length and leaf area in order to normalize the residuals. Leaf cupping and discoloration were analyzed using a linear mixed model with treatment level as a fixed effect and block as a random effect. Biomass data were analyzed by ANCOVA with initial pre-planting vine mass used as the covariate, treatment level was used as a fixed effect and block as a random effect. A log transformation was performed on the root fresh mass in order to normalize the residuals. With the exception of the figures, all statistical analysis was performed using SAS version 8 (SAS Institute, Cary NC). All figures were created using SigmaPlot 8.0 including best-fit equations and R^2 values (Systat Software, Richmond CA).

Pest Problems and Spray Applications: A minor outbreak of powdery mildew (*Uncinula necator* Schw.) occurred and the vines were sprayed on days 56 and 62 with Compass and Terraguard respectively. Later in the season, the vines were showing mild symptoms of two-spotted spider mite (*Tetranychus urticae* Koch) damage and were sprayed with Floramite SC on day 90.

Results

Experiment I

Table 1 shows the treatment levels used in this study and their equivalents in terms of PLH per leaf, and the stress severity in terms of PLH-hours (PLH-hrs). PLH-hrs are the product of the number of PLH in the infestation and the number of hours of the infestation. PLH-hrs are expressed on a per vine and per leaf basis. In order to calculate leaf size and leaf area/vine, a regression was performed between midrib length and leaf area (Figure 1). There was a significant linear relationship between midrib length and leaf area at the 0.1% level with an adjusted R^2 value of 0.80 (Figure 1).

Leaf Symptoms: The typical PLH feeding symptoms of downward leaf cupping and yellow leaf discoloration both increased with infestation severity. Leaf cupping data are shown in Table 2. The average number of leaves that were cupped after the infestation period ranged from 5.1 for the Control to 27.4 for the highest infestation level, or 9.3% to 56.8% of the total leaves per vine that were cupped respectively. The average score of the cupped leaves ranged from 2.01 (slight cupping) for the Control to 2.42 (moderate cupping) for the 150 PLH/vine level. Linear regressions between the number of PLH per vine and leaf cupping were all positive and significant at the 0.1% level. Table 3 shows leaf discoloration data. The number of leaves discolored after the infestation period ranged from 3.8 for the Control to 19.6 for the 150 PLH/vine level; or 6.9% to 40.5% of the total leaves per vine that were discolored respectively. The average percent discoloration of the discolored leaves ranged from 12.0% for the Control to 29.2% for the 150 PLH/vine level. Linear regressions between the number of PLH per vine and leaf discoloration were all significant at the 0.1% level.

A significant non-linear relationship existed between the number of PLH per vine and the percentage of leaves that were cupped at the 0.1% level with an adjusted R^2 value of 0.68 (Figure 2). There was also a significant non-linear relationship between the number of PLH per vine and the percentage of leaves that were discolored at the 0.1% level with an adjusted R^2 value of 0.54 (Figure 3).

Shoot and Leaf Growth: Table 4 and Table 5 show how leaf and shoot growth were affected by PLH infestations. Infestation severity was inversely related to leaf number/vine and leaf area/vine (Table 4). Likewise, shoot length and internodes/vine were both inversely proportional to infestation severity (Table 5). All four of these variables showed significant linear and quadratic regressions at the 0.1% level when plotted against treatment levels. Internode length and leaf size were not significantly different among treatments and showed no clear trends in relation to infestation severity.

Biomass Accumulation: Vine fresh and dry mass data showed few clear trends (Table 6). Based upon total fresh and total dry masses, vines that received 0, 25 or 50 PLH per vine tended to be larger than vines receiving 75, 100 or 150 PLH per vine, although statistically there were no significant differences between any level and the Control. A linear regression between leaf fresh mass and PLH per vine was significant at the 5% level with an R^2 of 0.67, indicating that infestation severity resulted in a reduction in leaf fresh mass.

Damage Thresholds: Table 7 shows damage thresholds for Experiment I. Leaves/vine, shoot length and internodes/vine did not differ from the Control until an infestation level of 25 PLH/vine. Leaf cupping, leaf discoloration and leaf area per vine did not differ from the Control until infestation severities reached 50 PLH/vine. Although biomass data showed no clear trends, leaf fresh mass was reduced at infestation severities of 75 and 150 PLH/vine, but not at 100 PLH/vine. Thus, the damage threshold for leaf fresh mass might have occurred between 75-150 PLH/vine.

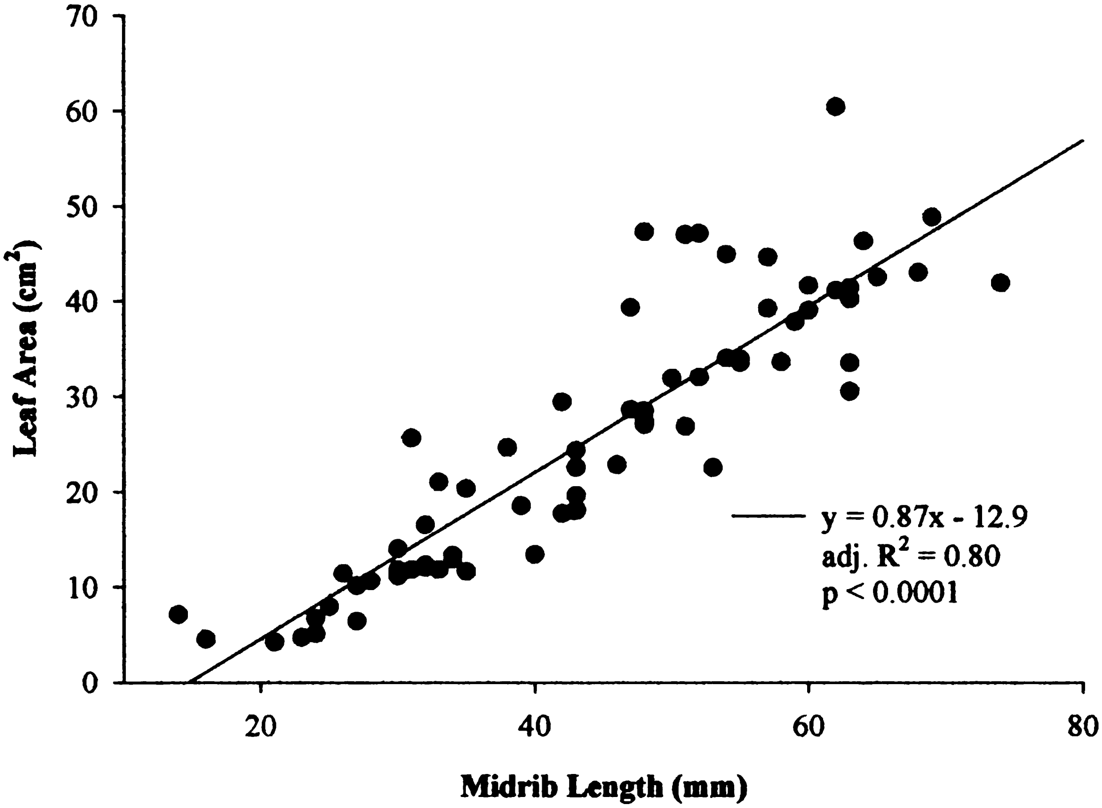

Figure 1. Relationship between midrib length and leaf area of Pinot gris leaves. The midrib lengths of 69 leaves were measured then each leaf was scanned with a leaf area meter. A linear correlation analysis was performed and the relationship was used to estimate the leaf area of experimental vines.

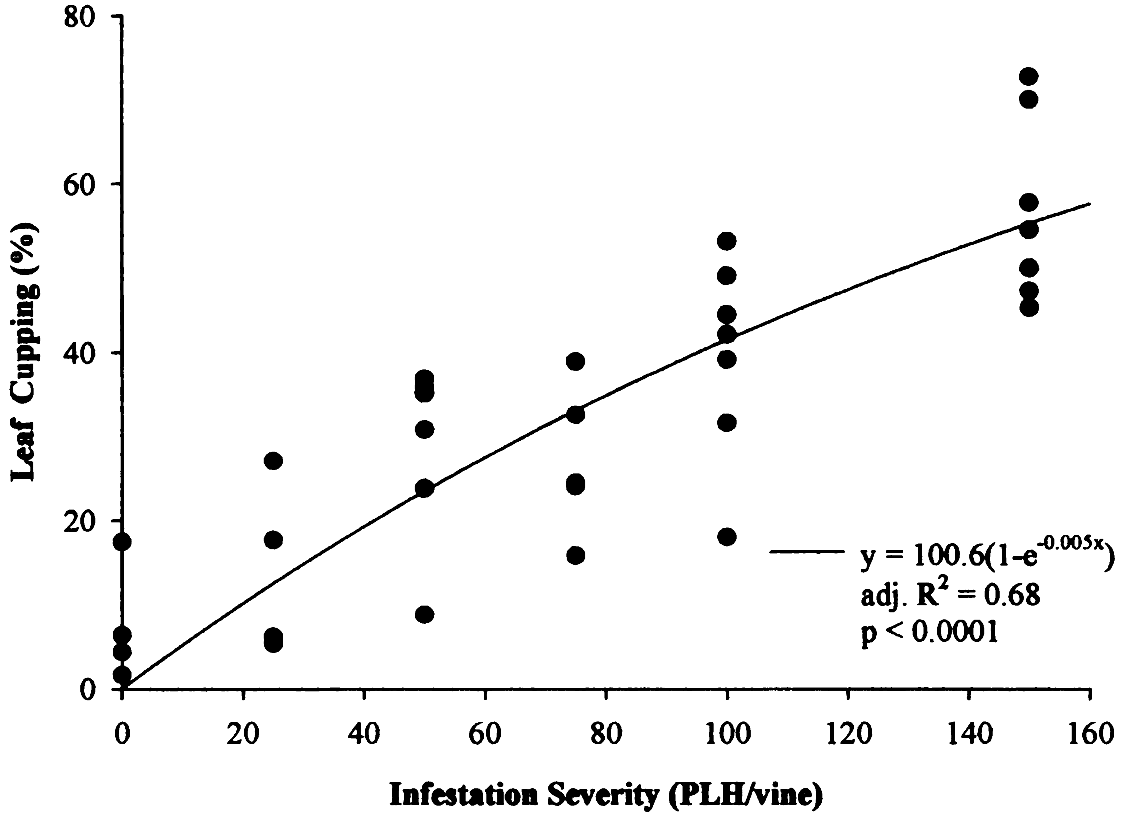

Figure 2. The relationship between infestation severity and leaf cupping of Pinot gris grapevines. Leaf cupping is expressed as the percentage of the total leaf number that exhibited downward cupping in response to PLH infestation. Infestation severity is expressed as the number of individuals in the infestation per vine.

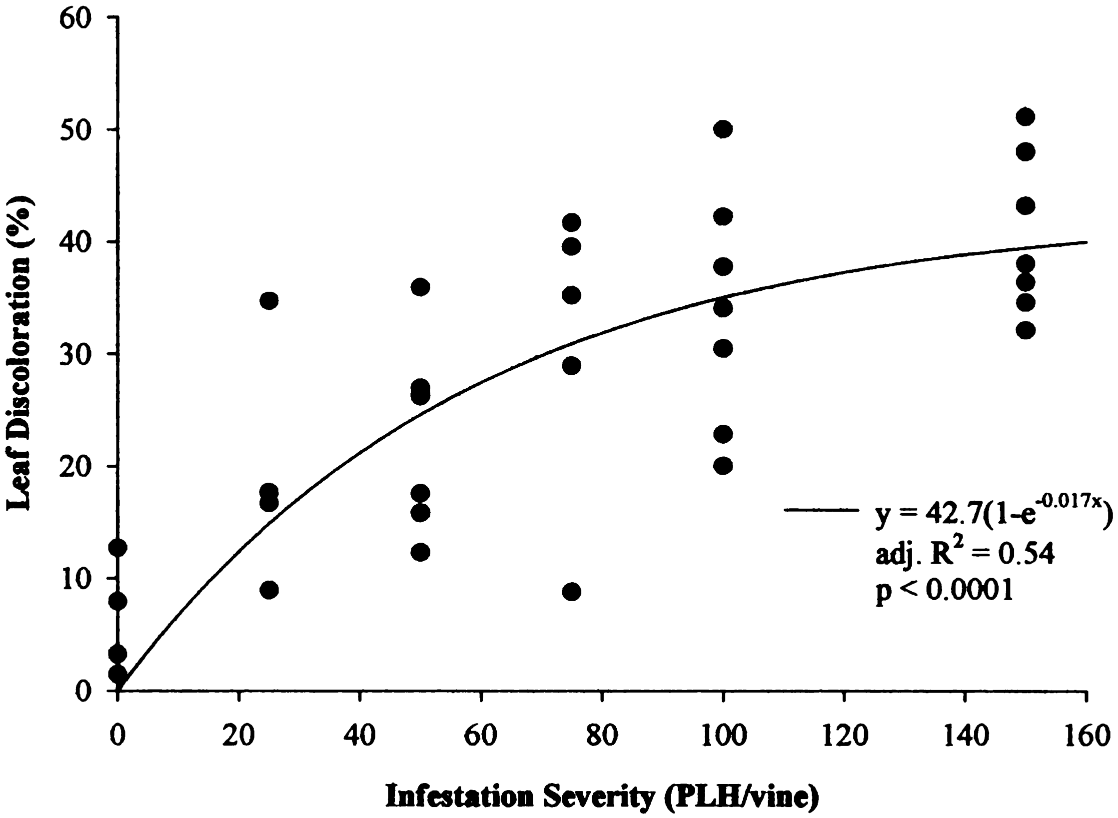

Figure 3. The relationship between infestation severity and leaf discoloration of Pinot gris leaves. Leaf discoloration is expressed as the percentage of the total leaf number that exhibited discoloration in response to PLH infestation. Infestation severity is expressed as the number of individuals in the infestation per vine.

Table 1. Treatment levels and their equivalents in terms of potato leafhoppers (PLH) per leaf and potato leafhopper hours (PLH-hrs.) per vine and per leaf (Experiment I).

Treatment (PLH/vine)	**PLH/leaf**	**Stress severity (PLH-hrs)**	
		per vine	**per leaf**
0	0.0	0	0.0
25	0.6	4,234	101.5
50	1.2	8,466	201.1
75	1.8	12,699	294.6
100	2.5	16,939	407.2
150	3.7	25,413	609.4

Table 2. Leaf cupping of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days (Experiment I).

	leaves per vine			
PLH/vine	**total**	**cupped**	**% cupped**	**score**[c]
0	62.1 a	5.1 d	9.3 d	2.01 c
25	55.2 b	6.3 d	10.0 d	2.00 c
50	50.5 c	14.7 bc	28.8 c	2.18 b
75	45.2 d	12.1 c	26.8 c	2.28 ab
100	48.2 cd	18.9 b	39.6 b	2.30 ab
150	48.7 cd	27.4 a	56.8 a	2.42 a
F-values	***	***	***	***
linear R^2	0.70 ***	0.79 ***	0.83 ***	0.65 ***
quadratic R^2	0.87 ***	ns	ns	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

[c] Score is the average cupping score of cupped leaves only; 1=normal, 2=slight/moderate, 3=severe

Table 3. Leaf discoloration of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days (Experiment I).

	leaves per vine			
PLH/vine	**total**	**discolored**	**% discolored**	**non-zero leaf (%)**[c]
0	62.1 a	3.8 d	6.9 d	12.0 c
25	55.2 b	8.9 cd	15.4 cd	14.2 bc
50	50.5 c	11.3 c	23.0 bc	19.9 bc
75	45.2 d	14.1 bc	31.5 ab	20.6 bc
100	48.2 cd	15.9 ab	33.9 a	22.6 ab
150	48.7 cd	19.6 a	40.5 a	29.2 a
F-values	***	***	***	***
linear R^2	0.70 ***	0.67 ***	0.70 ***	0.57 ***
quadratic R^2	0.87 ***	ns	ns	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

[c] Average of discolored leaves only.

Table 4. Leaf production of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days (Experiment I).

PLH/vine	Leaves/vine	Leaf area/ vine (cm^2)	Leaf size (cm^2)
0	62.1 a	2,172 a	34.6 b
25	55.2 b	2,085 a	38.5 a
50	50.5 c	1,860 b	36.3 ab
75	45.2 d	1,811 b	37.3 ab
100	48.2 cd	1,813 b	37.1 ab
150	48.7 cd	1,823 b	37.1 ab
F-values	***	***	*
linear R^2	0.70 ***	0.92 ***	ns
quadratic R^2	0.87 ***	0.95 ***	ns

[a]Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b]Significant F-values and R^2-values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 5. Shoot production of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days (Experiment I).

PLH/vine	Shoot length (mm)	Internodes/ vine	Internode length (mm)
0	832 a	69.7 a	35
25	693 b	62.5 b	34
50	632 c	55.3 c	34
75	583 d	51.6 d	33
100	603 cd	53.6 cd	33
150	612 cd	53.2 cd	34
F-values	***	***	ns
linear R^2	0.90 ***	0.75 ***	ns
quadratic R^2	0.97 ***	0.91 ***	ns

[a]Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b]Significant F-values and R^2-values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 6. Biomass production of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days: I. fresh biomass, II. dry biomass (Experiment I).

I.

	Fresh biomass (g)				
PLH/vine	**Leaves**	**Shoots**	**Wood**	**Roots**	**Total**
0	58.4 a	35.5	65.4 b	43.7 ab	203.0 ab
25	54.6 ab	35.5	83.9 a	55.1 a	229.2 a
50	52.4 ab	34.5	62.7 b	47.3 ab	197.0 ab
75	45.2 b	27.9	61.6 b	42.6 ab	177.2 b
100	47.5 ab	32.7	64.4 b	35.2 b	179.9 b
150	47.4 b	30.8	69.7 b	40.5 ab	188.3 b
F-values	*	ns	**	*	*
linear R^2	0.67 *	ns	ns	ns	ns
quadratic R^2	ns	ns	ns	ns	ns

II.

	Dry biomass (g)				
PLH/vine	**Leaves**	**Shoots**	**Wood**	**Roots**	**Total**
0	14.7	14.0	38.3 b	23.6 ab	90.5 ab
25	14.0	13.4	48.6 a	33.2 a	109.3 a
50	13.6	13.0	36.0 b	27.5 ab	90.2 ab
75	12.2	10.9	36.0 b	24.5 ab	83.6 b
100	12.8	12.4	37.4 b	19.3 b	82.0 b
150	13.3	10.7	40.3 b	24.1 ab	88.4 ab
F-values	ns	ns	**	*	*
linear R^2	ns	ns	ns	ns	ns
quadratic R^2	ns	ns	ns	ns	ns

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[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2-values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 7. Damage thresholds for Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days in terms of PLH per leaf, PLH per vine and potato leafhopper hours (PLH-hrs.) per leaf and PLH-hrs. per vine (Experiment I).

Variable	PLH/leaf	PLH/vine	PLH-hrs. per leaf	PLH-hrs. per vine
symptoms				
leaves cupped/vine	1.2	50	201.1	8,466
leaves discolored/vine	1.2	50	201.1	8,466
leaf and shoot growth				
leaves/vine	0.6	25	101.5	4,234
leaf area/vine	1.2	50	201.1	8,466
leaf size	ns	ns	ns	ns
shoot length	0.6	25	101.5	4,234
internodes/vine	0.6	25	101.5	4,234
internode length	ns	ns	ns	ns
biomass				
leaves fresh mass	1.8 (?)	75 (?)	294.6 (?)	12,699 (?)
leaves dry mass	ns	ns	ns	ns
shoots fresh mass	ns	ns	ns	ns
shoots dry mass	ns	ns	ns	ns
wood fresh mass	ns	ns	ns	ns
wood dry mass	ns	ns	ns	ns
roots fresh	ns	ns	ns	ns
roots dry	ns	ns	ns	ns
total vine fresh	ns	ns	ns	ns
total vine dry	ns	ns	ns	ns

[a] Numbers represent the lowest infestation level that differed significantly from the Control; (?) means no clear trend existed; 'ns' means no level differed significantly from the Control.

Experiment II

Table 8 shows the treatment levels and their equivalents in terms of PLH/vine, PLH-hrs/leaf and PLH-hrs/vine. In order to calculate leaf size and leaf area/vine, a regression was performed between midrib length and leaf area (Figure 4). This relationship was significant at the 0.1% level with an adjusted $R^2 = 0.84$.

Leaf Symptoms: In cases where PLH infestations on grapevines were severe, leaves appeared discolored and cupped. Discoloration typically appeared as fluorescent yellow or light green wedges with the wide end at the leaf margins and the cupping was downward with the abaxial surface on the inside of the cup. At Time-1, these symptoms were absent, and all leaves were normal in appearance (data not shown).

By Time-2, symptoms of PLH infestation were apparent and were directly related to infestation level. As infestation severity increased, so too did the amount of cupped leaves (Table 11). As few as 0.5 PLH/leaf resulted in significantly more cupped leaves than the Control. The range in the number of cupped leaves per vine was from 1.5 for the Control to 12.6 for the 4.5 PLH/leaf level; in terms of the percentage of cupped leaves per vine, the range was 5.3% to 58.7% respectively. A non-linear regression between PLH/leaf and the percentage of leaves cupped was significant at the 0.1% level with an adjusted $R^2 = 0.82$ (Figure 5). The average score per cupped leaves was 2.0 (slight) for the Control and increased up to 3.1 (moderate) for the 4.5 PLH/leaf level. Linear regressions between PLH/leaf and all leaf cupping variables were significant at the 0.1% level.

Leaf discoloration was directly related to infestation severity (Table 12). The number of leaves that were discolored per vine ranged from 0.0 for the Control to 6.6 for the 4.5 PLH/leaf level, or 0.0% to 30.7% of the total leaf number was discolored. A significant non-linear regression existed between PLH/leaf and the % leaves discolored at the 0.1% level with an adjusted $R^2 = 0.82$ (Figure 6). The average % discoloration of discolored leaves ranged from 0.0% for the Control to 33.1% for the 4.5 PLH/leaf level.

Shoot and Leaf Growth: Leaf and shoot growth status prior to the infestation period (Time-1) are shown in Table 9 and Table 10 respectively. Very few significant differences existed among treatments at Time-1. At this time, there were no significant differences among treatments in terms of internodes/vine, leaves/vine, leaf size and leaf area/vine. Although there were slight differences among treatments in terms of internode length, no level differed significantly from the Control. Only the 0.5 PLH/vine level differed from the Control for shoot length.

At Time-2, three days after the end of the infestation period, every variable for leaf and shoot growth showed significant differences among treatments at the 0.1% level. In addition, each variable showed an inverse linear relationship to PLH/leaf that was significant at the 1.0% level or higher. Leaf growth was reduced by PLH infestation (Table 13). Infestation resulted in fewer leaves/vine, less leaf area/vine and smaller leaves. Shoot growth was also reduced by infestation (Table 14). Infestations reduced shoot length and internodes/vine and average internode length.

At Time-3, ten weeks after the end of the infestation period, the data show a different trend than at Time-2. Leaf growth (Table 15) and shoot growth (Table 16) still showed significant differences among treatments. Although significant differences among treatments still existed for internodes/vine, leaves/vine, leaf size and leaf area/vine, there were no longer differences among treatments in terms of internode length and shoot length. Where significant differences did exist, it was typically one of the middle treatment levels that showed the growth stimulation and in all cases, the Control vines did not differ from the 4.5 PLH/leaf vines. For all variables where significant differences did exist, the 1.0 PLH/leaf level was always among the highest values.

Biomass Accumulation: Relative to the Control, very few statistically significant differences existed in biomass accumulation data (Table 17). Vines that received 1.0 PLH/leaf had the highest fresh and dry masses of leaves and highest fresh mass of shoots, and these data were significantly greater than the Control vines. These data are a reflection of the shoot and leaf growth data at Time-3, where this group also had the greatest amount of leaf area and was among the vines with the longest shoots, largest leaves and most leaves per vine. In terms of root fresh mass, root dry mass and total vine dry mass the vines that received 4.5 PLH/leaf were the smallest among treatments and were significantly lower than the Control.

Damage Thresholds: Table 18 shows damage thresholds for Experiment II. Leaf cupping was significantly higher than the Control at the lowest infestation severity of 0.5 PLH/leaf; leaf discoloration did not increase relative to the Control until 1.0 PLH/leaf. Leaf size, shoot length and internode length were significantly lower than the Control at 0.5 PLH/leaf. Leaves/vine began to decrease at 1.0 PLH/leaf. Leaf area/vine and internodes/vine did not differ from the Control until an infestation severity of 3.0 PLH/leaf; however, the trend for leaf area/vine was not very clear. Relative to the Control, root mass and total vine dry mass were not reduced until infestations reached 4.5 PLH/leaf.

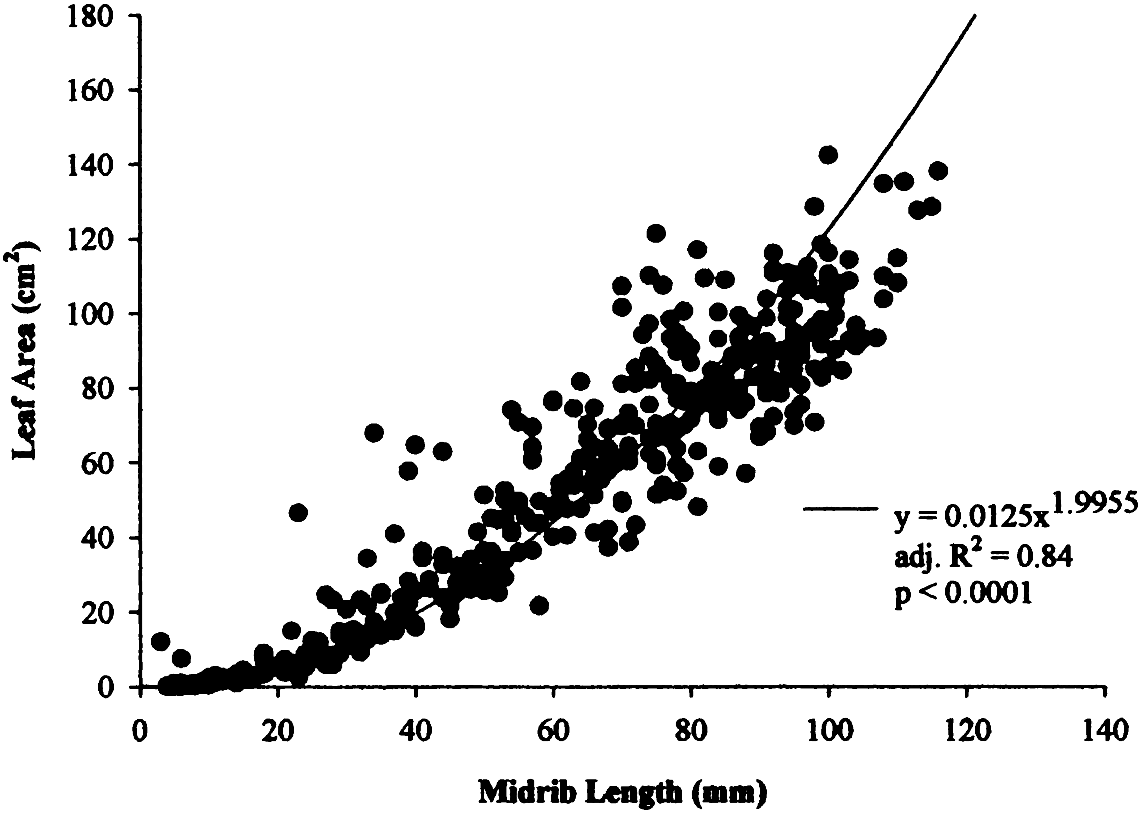

Figure 4. Relationship between midrib length and leaf area of Pinot gris leaves. The midrib lengths of 469 leaves were measured then each leaf was scanned with a leaf area meter. A non-linear correlation was performed and the relationship was used to estimate the leaf area of experimental vines.

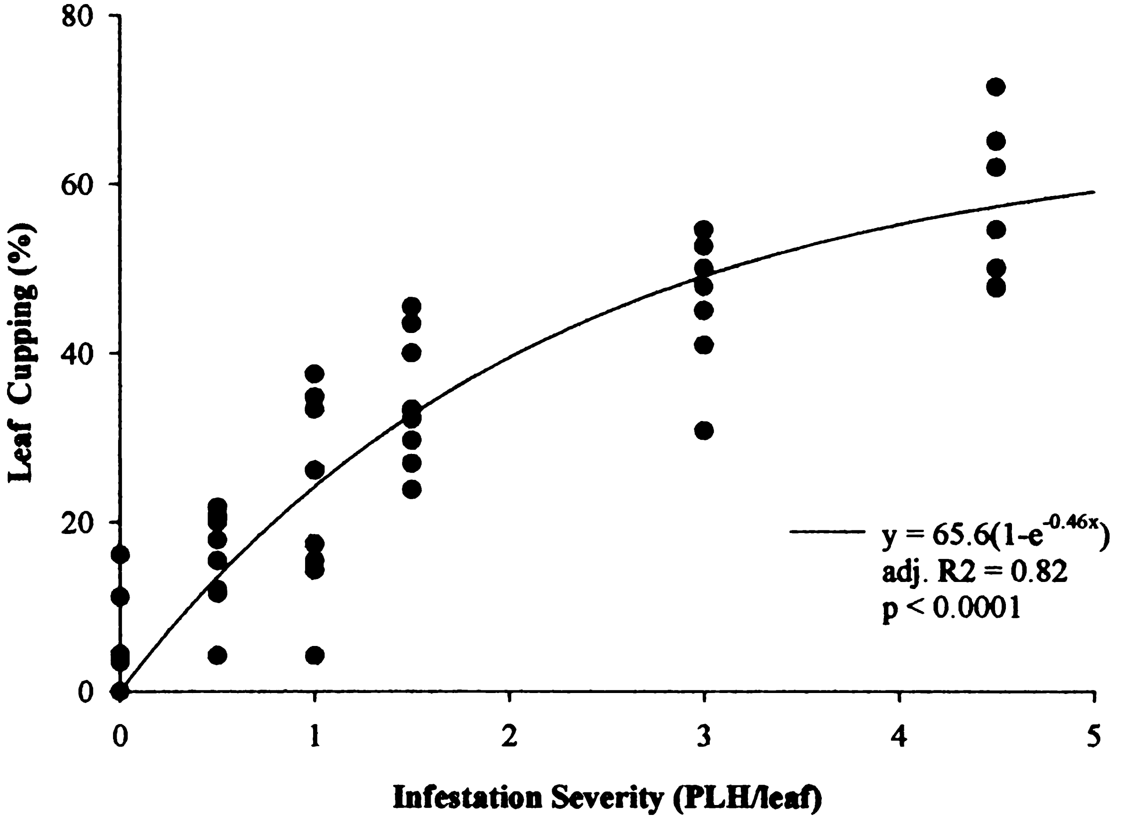

Figure 5. The relationship between infestation severity and leaf cupping for Pinot gris grapevines. Leaf cupping is expressed as the percentage of the total leaf number that exhibited downward cupping in response to PLH infestation. Infestation severity is expressed as the number of individuals in the infestation per leaf.

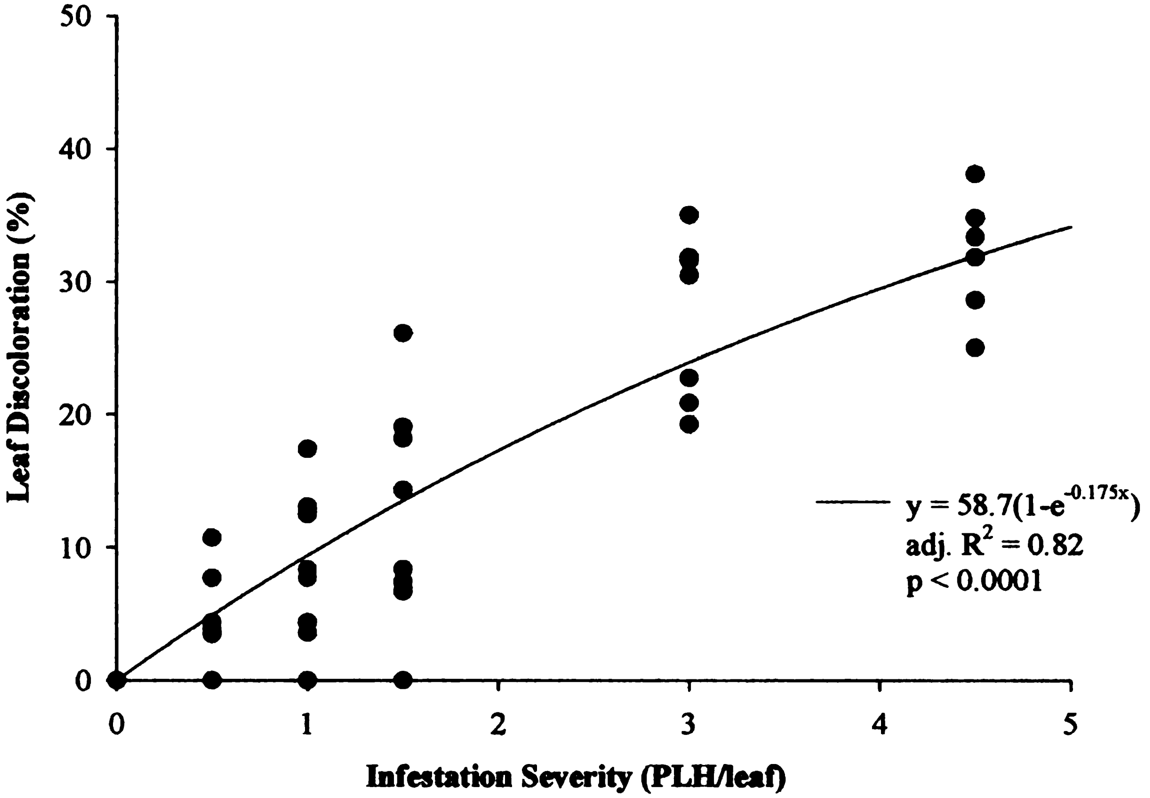

Figure 6. The relationship between infestation severity and leaf discoloration of Pinot gris leaves. Leaf discoloration is expressed as the percentage of the total leaf number that exhibited discoloration in response to PLH infestation. Infestation severity is expressed as the number of individuals in the infestation per leaf.

Table 8. Treatment levels and their equivalents in terms of potato leafhoppers (PLH) per vine and potato leafhopper hours (PLH-hrs.) per vine and per leaf (Experiment II).

Treatment (PLH/leaf)	**PLH/vine**	**Stress severity (PLH-hrs)**	
		per leaf	**per vine**
0.0	0.0	0	0
0.5	8.4	79	1,394
1.0	16.4	164	2,726
1.5	25.0	245	4,162
3.0	52.3	494	8,702
4.5	74.8	733	12,456

Table 9. Leaf production of Pinot gris grapevines prior to the potato leafhopper (PLH) infestation period (Time-1, Experiment II).

PLH/leaf	**Leaves/vine**	**Leaf area/ vine (cm^2)**	**Leaf size (cm^2)**
0.0	17.0	731	31.8
0.5	18.0	680	29.8
1.0	17.0	728	33.3
1.5	17.0	729	32.9
3.0	18.0	700	29.9
4.5	17.0	728	31.7
F-values	ns	ns	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 10. Shoot production of Pinot gris grapevines prior to the potato leafhopper (PLH) infestation period (Time-1, Experiment II).

PLH/leaf	**Shoot length (mm)**	**Internodes/ vine**	**Internode length (mm)**
0.0	854 a	21	42 ab
0.5	769 b	20	39 ab
1.0	801 ab	20	41 ab
1.5	805 ab	20	40 ab
3.0	795 ab	21	37 b
4.5	816 ab	20	42 a
F-values	*	ns	*

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 11. Leaf cupping of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days (Time-2, Experiment II).

	leaves per vine			
PLH/leaf	**total**	**cupped**	**% cupped**	**score**[c]
0.0	27.0 a	1.5 e	5.3 e	2.0 c
0.5	25.8 ab	4.0 d	15.4 d	2.2 bc
1.0	24.4 b	5.5 d	22.9 d	2.4 b
1.5	25.1 ab	8.6 c	34.3 c	2.4 b
3.0	22.3 c	10.3 b	46.5 b	2.9 a
4.5	21.6 c	12.6 a	58.7 a	3.1 a
F-values	***	***	***	***
linear R^2	0.78***	0.83***	0.86***	0.70***
quadratic R^2	ns	0.88***	0.89**	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

[c] Score is the average cupping score of cupped leaves only; 1=normal, 2=slight, 3=moderate, 4=severe

Table 12. Leaf discoloration of Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days (Time-2, Experiment II).

	leaves per vine			
PLH/leaf	**total**	**discolored**	**% discolored**	**non-zero leaf (%)**[c]
0.0	27.0 a	0.0 d	0.0 d	0.0 c
0.5	25.8 ab	1.0 cd	3.8 cd	12.9 b
1.0	24.4 b	2.0 bc	8.4 bc	14.3 b
1.5	25.1 ab	3.0 b	12.5 b	11.3 b
3.0	22.3 c	5.9 a	26.8 a	31.1 a
4.5	21.6 c	6.6 a	30.7 a	33.1 a
F-values	***	***	***	***
linear R^2	0.78***	0.85***	0.86***	0.68*
quadratic R^2	ns	0.87**	0.88*	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

[c] Average of discolored leaves only.

Table 13. Leaf production of Pinot gris grapevines three days after the potato leafhopper (PLH) infestation period (Time-2, Experiment II).

PLH/leaf	Leaves/vine	Leaf area/ vine (cm^2)	Leaf size (cm^2)
0.0	27 a	1,576 a	47.4 a
0.5	26 ab	1,329 b	41.9 bc
1.0	24 b	1,415 b	45.5 ab
1.5	25 ab	1,362 b	43.1 abc
3.0	22 c	1,124 c	39.8 c
4.5	22 c	1,182 c	41.7 bc
F-values	***	***	**
linear R^2	0.78***	0.79***	0.63**
quadratic R^2	ns	0.83**	0.67*

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 14. Shoot production of Pinot gris grapevines three days after the potato leafhopper (PLH) infestation period (Time-2, Experiment II).

PLH/leaf	Shoot length (mm)	Internodes/ vine	Internode length (mm)
0.0	1,328 a	30 a	45 a
0.5	1,108 b	28 ab	40 b
1.0	1,089 b	27 ab	40 b
1.5	1,075 bc	28 ab	39 b
3.0	942 d	26 b	37 b
4.5	971 cd	24 c	41 b
F-values	***	***	***
linear R^2	0.84***	0.81***	0.81**
quadratic R^2	0.90***	ns	0.86**

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 15. Leaf production of Pinot gris grapevines ten weeks after the potato leafhopper (PLH) infestation period (Time-3, Experiment II).

PLH/leaf	Leaves/vine	Leaf area/ vine (cm^2)	Leaf size (cm^2)
0.0	34 c	2,599 b	74.9 ab
0.5	36 ab	2,645 b	71.3 bc
1.0	38 a	3,069 a	78.1 a
1.5	36 ab	2,725 b	73.4 ab
3.0	37 a	2,639 b	68.0 c
4.5	35 bc	2,547 b	70.4 bc
F-values	***	***	***
linear R^2	ns	ns	ns
quadratic R^2	0.48**	0.33*	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 16. Shoot production of Pinot gris grapevines ten weeks after the potato leafhopper (PLH) infestation period (Time-3, Experiment II).

PLH/leaf	Shoot length (mm)	Internodes/ vine	Internode length (mm)
0.0	1,738	37 d	48
0.5	1,707	38 bcd	44
1.0	1,850	41 a	46
1.5	1,762	39 abc	45
3.0	1,787	40 ab	44
4.5	1,736	37 cd	47
F-values	ns	***	ns
linear R^2	ns	ns	ns
quadratic R^2	ns	0.55**	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 17. Biomass production of Pinot gris grapevines ten weeks after the potato leafhopper (PLH) infestation period: I. fresh biomass, II. dry biomass (Time-3, Experiment II).

I.

PLH/leaf	Fresh Mass (g) Initial	Leaves	Shoots	Wood	Roots	Total
0.0	72.4 a	51.5 b	32.2 b	64.8 ab	81.7 ab	230.2 ab
0.5	56.5 b	55.1 b	33.8 ab	69.0 a	85.3 ab	243.1 a
1.0	68.2 ab	61.8 a	37.0 a	59.4 b	89.7 a	247.9 a
1.5	66.9 ab	54.2 b	33.9 ab	60.2 b	71.7 bc	220.0 b
3.0	56.1 b	55.9 b	34.1 ab	61.0 b	75.5 abc	226.4 ab
4.5	66.9 ab	53.6 b	35.3 ab	60.2 b	61.1 c	210.2 b
F-values	*	***	*	*	***	**
linear R^2	ns	ns	ns	ns	0.29**	0.18*
quadratic R^2	ns	ns	ns	ns	ns	ns

II.

PLH/leaf	Dry Mass (g) Leaves	Shoots	Wood	Roots	Total
0.0	17.0 b	17.6	37.2 ab	44.4 ab	116.1 ab
0.5	17.9 b	18.3	38.9 a	41.4 ab	116.5 ab
1.0	20.1 a	19.9	33.7 b	48.3 a	122.1 a
1.5	17.4 b	18.4	34.6 ab	38.3 bc	108.6 bc
3.0	17.9 b	18.3	34.7 ab	41.2 ab	112.1 abc
4.5	17.3 b	18.9	34.0 b	33.6 c	103.8 c
F-values	**	ns	*	**	***
linear R^2	ns	ns	ns	0.26**	0.19*
quadratic R^2	ns	ns	ns	ns	ns

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2-values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/vine.

Table 18. Damage thresholds for Pinot gris grapevines in response to infestation by potato leafhopper (PLH) nymphs for seven days in terms of PLH per leaf, PLH per vine and potato leafhopper hours (PLH-hrs.) per leaf and PLH-hrs. per vine (Time-3, Experiment II).

Variable	**PLH/leaf**	**PLH/vine**	**PLH-hrs. per leaf**	**PLH-hrs. per vine**
symptoms				
leaves cupped/vine	0.5	8.4	79	1,394
leaves discolored/vine	1.0	16.4	164	2,726
leaf and shoot growth				
leaves/vine	1.0	16.4	164	2,726
leaf area/vine	ns	ns	ns	ns
leaf size	0.5	8.4	79	1,394
shoot length	0.5	8.4	79	1,394
internodes/vine	3.0	52.3	494	8,702
internode length	0.5	8.4	79	1,394
biomass				
leaves fresh mass	ns	ns	ns	ns
leaves dry mass	ns	ns	ns	ns
shoots fresh mass	1.0 (?)	16.4 (?)	164 (?)	2,726 (?)
shoots dry mass	ns	ns	ns	ns
wood fresh mass	ns	ns	ns	ns
wood dry mass	ns	ns	ns	ns
roots fresh	4.5	74.8	733	12,456
roots dry	4.5	74.8	733	12,456
total vine fresh	ns	ns	ns	ns
total vine dry	4.5	74.8	733	12,456

[a] Numbers represent the lowest infestation level that differed significantly from the Control; (?) means no clear trend existed; 'ns' means no level differed significantly from the Control.

Discussion

The results of these experiments show that PLH infestations caused transient vegetative growth reduction of potted Pinot gris grapevines. The number of PLH per vine was well correlated with both a reduction in the quantity of vegetative growth and with an increase in the PLH feeding symptoms of leaf cupping and leaf discoloration. More specifically, as the number of PLH per vine increased, there was a decrease in shoot length, internodes/vine, leaves/vine and leaf area/vine for Experiment I. For Experiment II, infestation caused transient decreases in internodes/vine, average internode length, shoot length, leaves/vine, leaf size and leaf area/vine. Biomass data for Experiment I showed few clear trends, with the exception of a decrease in leaf fresh mass. Biomass data for Experiment II showed that leaf and shoot mass of 1.0 PLH/leaf vines was largest and root mass of the 4.5 PLH/leaf level was the lowest.

For Experiment I, reduced shoot length appears to have been caused by fewer internodes per shoot as opposed to a reduction in internode length. There were no significant differences in internode length among treatments, but the number of internodes per vine was inversely related to infestation severity (Table 5). Internodes/vine was inversely related to the treatment levels; a linear regression was significant at the 0.1% level with an R^2 of 0.75. For Experiment II, decreased shoot length was due to both reduced internode lengths and fewer internodes per vine at Time-2. By Time-3, after ten weeks of growth in the absence of infestations, shoot length was no longer significantly different among treatments. At Time-3, average internode length showed no significant differences among treatments, although there were some differences among treatments in terms of internodes/vine. Vines receiving 1.0 PLH/leaf had the most internodes, which might be indicative of compensatory or stimulatory growth in response to low levels of infestation.

Leaf area per vine for Experiment I was reduced due to fewer leaves per vine rather than to smaller leaves. Only the 25 PLH/vine level differed from the Control and neither linear nor quadratic R^2 values were statistically significant. Leaf number per vine was significantly reduced by increasing PLH infestation severity; a linear regression between these two terms was significant at the 0.1% level with an R^2 of 0.70.

For Experiment II, both leaves/vine and average leaf size showed significant reductions due to infestation at Time-2 and both are likely involved in the reduced leaf area observed for this study. By Time-3, treatment differences for leaf area, leaf size and leaves/vine were less pronounced than at Time-2. As with shoot measurements at Time-3, leaf measurements showed that low infestation levels resulted in larger leaves, greater numbers of leaves/vine and higher overall leaf area per vine. This indicates that there was a recovery from growth reductions and possibly some form of compensation as well since vines experiencing mild infestation actually grew to be larger than the Control.

Taking these effects together suggests that canopy morphology of potted Pinot gris grapevines was significantly altered by PLH infestation in the short term (Time-2) but in the long term (Time-3), infestation by low levels of PLH might actually have stimulated growth of the vines in these studies (Tables 15 and 16). Infestation resulted in a general reduction in shoot length and leaf area of the vines leading to stunted growth. Thus, the size of carbohydrate source tissues was reduced. In the absence of any compensatory mechanisms such as enhanced photosynthetic rates of the remaining leaf area, this could translate into a reduction in carbohydrate source strength of infested vines. Reducing the carbohydrate source could potentially limit the vine's ability to meet the growth and productivity requirements of the carbohydrate sink tissues, depending on the strength of those sinks.

If the observations from this study were consistent in a field situation, a reduction in carbohydrate source would require that growers accept lower yields or yields of reduced fruit maturity as assessed by fruit composition values (Mansfield and Howell 1981, Petrie *et al.* 2000a). However, at infestation levels ranging from 0.5-1.5 PLH/leaf for seven days, shoot and leaf growth might actually be stimulated if other stresses are absent. However, in this case there was no fruit stress since the vines were fruitless.

Trends for the biomass data from these experiments were not as clear as those from the leaf symptoms, shoot growth or leaf growth data. For Experiment I, this might have been due to herbicide exposure of these vines toward the end of the study. It is also possible that the biomass data simply show that the vines can compensate for PLH infestations within the levels used for these studies.

Experiment II showed trends that were somewhat clearer, but still no strong trends in biomass existed. One serious concern is the decline in root mass of the 4.5 PLH/leaf vines in response to infestation (Table 17). This data suggests that recovery in growth by leaves and shoots at Time-3 might have been fueled by remobilized storage carbohydrates from the roots, or because the vines were allocating fewer resources to the roots in order to recover from leaf area loss due to infestation. Such a phenomenon has been noted in other studies of source/sink relations for grapevines (Candolfi-Vasconcelos *et al.* 1994, Edson *et al.* 1995b, Miller *et al.* 1996b).

The results of Experiment I indicate that threshold levels exist for the relationship between grapevine growth and PLH infestation (Table 7). Relative to the Control, there was no significant increase in the number or in the percentage of cupped leaves per vine until infestation severity reached 50 PLH/vine. The average score per cupped leaf was also not different from the Control until an infestation level of 50 PLH/vine. Likewise, there was no significant difference in the number or the percentage of leaves discolored per vine until an infestation level of 50 PLH/vine, although the average percent discoloration per discolored leaf was not different from the Control until an infestation level of 100 PLH/vine. Thus, the threshold level for leaf cupping and leaf discoloration was between 25 and 50 PLH per vine, or 0.6 and 1.2 PLH per leaf. The threshold for leaf area per vine occurred at the same level. Relative to the Control, vines infested with 25 PLH were not significantly different from the Control in terms of leaf area per vine, but those infested with 50 PLH had less leaf area than the Control. However, it seems that the threshold for shoot length, leaves per vine and internodes per vine occurred at a lower infestation level as even the 25 PLH per vine treatment was significantly different than the Control. If a threshold exists for these variables, the data indicate that it lies between 0 and 25 PLH per vine, or between 0.0 and 0.6 PLH/leaf.

The results from Experiment II also indicate that damage thresholds for infestation severity exist (Table 18), but additionally that compensatory mechanisms also can occur in the form of recovery from growth reductions. Threshold levels for leaf symptoms all occurred around 0.5 and 1.0 PLH/leaf. Cupped leaves/vine, the percentage of leaves that were cupped and the average discoloration per discolored leaf became significantly higher than the Control by 0.5 PLH/leaf, indicating that the threshold level was just below this infestation severity. The average score per cupped leaf, total number of discolored leaves and percentage of leaves that were discolored became significantly higher than the Control by 1.0 PLH/leaf indicating that the damage threshold for these variables occurred just below this infestation level. Leaf and shoot growth also had thresholds, but the range was larger than for the leaf symptoms. Shoot length, average internode length, average leaf size and leaf area per vine all decreased significantly relative to the Control by 0.5 PLH/leaf; leaves/vine decreased by 1.0 PLH/leaf and internodes per vine did not significantly decrease until a stress level of 3.0 PLH/leaf.

The data for Time-3 of Experiment II showed a very different trend than those previously described. The leaf and shoot growth data showed that mild infestations in the range of 0.5-1.5 PLH/leaf stimulated growth. The highest values for most leaf and shoot growth variables always occurred at an infestation level between the Control and the highest severity of 4.5 PLH/leaf. This trend occurred for internodes/vine, shoot length, leaves/vine, leaf size and leaf area per vine. However, the data for leaf size showed no significant differences.

Although these data show that PLH infestations reduced vine vegetative growth, it is not clear why this occurred. Reductions in shoot and leaf growth were likely the result of disruptions in leaf photosynthesis. There are many studies which show that PLH infestations affect the photosynthetic rates of alfalfa, potato and other field crops. In addition, the grape leafhopper (*Empoasca vitis* Goethe), a close relative of the PLH, has been shown to reduce the rates of assimilation, transpiration, mesophyll conductance and stomatal conductance of Merlot (*Vitis vinifera* L.) leaves (Candolfi *et al.* 1993). It seems likely that grapevine leaf photosynthesis is also inhibited in response to PLH infestations.

Such impacts on photosynthesis by PLH infestations might have resulted in temporary reductions in source strength, ultimately causing the differences in shoot and leaf growth observed.

Although leaf and shoot growth were inhibited in response to infestations, by Time-3 of Experiment II, leaf and shoot growth of the Control were among the smallest of the treatments indicating that some sort of compensation occurred in response to the treatments. The energy required for accelerated growth likely came from either increased assimilation rates of undamaged leaves, remobilization of stored carbohydrates or a combination of both mechanisms. Compensatory increases in photosynthetic rates for grapevine leaves in response to PLH infestation have not been documented, but studies on grapevines and cherry have demonstrated that partial defoliation can result in increased rates of assimilation for the remaining leaves (Layne and Flore 1992, Layne and Flore 1995, Petrie *et al.* 2000c) by altering the ratio of carbohydrate sources and sinks. Although it is likely that grapevines possess compensatory photosynthetic mechanisms which respond to changing source/sink relationships, the data from this study do not show it. However, the data from Time-3 of Experiment II indicate that growth rates of leaves and shoots were enhanced for some infestation levels relative to the Control (Tables 15 and 16). This indicates that there was a compensatory mechanism for the growth of leaves and shoots. It is likely that remobilization of storage carbohydrates from the roots is part of this mechanism. It has been demonstrated that vines have the ability to remobilize stored carbohydrates in order to meet sink demands (Candolfi-Vasconcelos *et al.* 1994). The biomass data from our studies show that root mass was reduced significantly for the 4.5 PLH/leaf treatment and it is likely that remobilized reserves from the roots fueled the acceleration in leaf and shoot growth by the end of Experiment II, or less photosynthate was allocated to the roots in favor of providing fuel for leaf and shoot growth.

If the decrease in vegetative growth observed in these studies is truly the result of impaired leaf function, then source strength of these vines was reduced. This might have caused an imbalance in the ratio of carbohydrate sources to carbohydrate sinks creating a situation where the vines became increasingly source-limited as infestation severity increased. The vines in this study did not carry any fruit. The addition of a fruiting sink will likely induce a further source/sink imbalance, since the vines would now have an additional carbohydrate sink as well as an impaired ability of the major sources to synthesize carbohydrates. Such a fruiting effect would be especially pronounced at veraison, a time when the fruit becomes the dominant sink (Mullins *et al.* 1992). As sink strength increases relative to source strength, at some point there will be an energy deficit and the vines ability to compensate for loss of leaf function will be compromised.

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Chapter 2

Photosynthetic performance of Pinot gris (*Vitis vinifera* L.) leaves in response to potato leafhopper (*Empoasca fabae* Harris) infestation

Abstract

Two experiments were conducted in order to test the affect of potato leafhopper (PLH) infestations on grapevine photosynthesis. In Experiment I, Pinot gris leaves at four different positions along a shoot were infested with 0, 1, 2, 4 or 8 PLH nymphs for 43 hours in order to determine if photosynthetic rates were impacted by feeding and whether or not damage thresholds exist for the photosynthetic response. In Experiment II, response curves were generated for two separate regions of an individual grapevine leaf in order to determine how light and CO_2 utilization were affected by PLH infestations; one region of the leaf was infested with nine PLH nymphs for 42 hours and a separate portion of the same leaf was used as an uninfested Control. The results of Experiment I show that PLH infestation levels were inversely proportional to carbon assimilation (A), transpiration (E) and stomatal conductance (G_s) and directly proportional to internal CO_2 concentration (C_i). Decreases in A were correlated with decreased G_s and increased C_i and thus reductions in A were due to both stomatal and non-stomatal limitations. Damage thresholds, defined as the number of insects necessary to cause damage to the plant (Pedigo *et al.* 1986) existed for A, E, G_s and C_i at most leaf positions. The results of Experiment II show that decreased A in response to PLH infestations was due to a decreased ability of the leaf tissue to utilize both light and CO_2. Reductions in A were again correlated with decreases in G_s and increases in C_i and thus

were due to both stomatal and non-stomatal limitations. Photosynthetic compensation occurred during the post-infestation period for Experiment II. These studies show that: 1) PLH infestations reduce A for Pinot gris leaves; 2) there are damage thresholds for grapevine photosynthesis in response to PLH infestations; 3) changes in photosynthetic rates are due to stomatal and non-stomatal limitations; and 4) photosynthetic compensation can occur during the post-infestation period.

Introduction

The most fundamental physiological process that affects grapevine productivity is leaf photosynthesis (Kriedemann 1977). It has been estimated that over 90% of plant dry matter is derived from atmospheric carbon fixed during photosynthesis (Flore and Lakso 1989). The primary photosynthetic organ of a vine is the leaf, thus damage to the leaves can result in reduced biomass accumulation (Koblet *et al.* 1994, Petrie *et al.* 2000a, Petrie *et al.* 2000b). Inhibition of photosynthesis thus translates into a food shortage for the vine and growth can become limited. In Michigan and the Great Lakes Region, grapevines often experience infestation by PLH typically resulting in discoloration and morphological changes of the leaves. Although PLH infestations can lead to these symptoms, it is not known how infestations will affect grapevine leaf photosynthesis.

PLH feed by using their stylets to probe and lacerate plant cells and tissues. They then secrete saliva while ingesting the fluids that were flushed out from these cells. This behavior can cause plants to develop 'hopperburn', a general yellowing and wilting of leaf tissues often resulting in stunted growth of the host plant (Medler 1941, Hunter and Backus 1989, Backus and Hunter 1989, Kabrick and Backus 1991, Ecale and Backus 1995a, Ecale and Backus 1995b). PLH infestations of alfalfa (*Medicago sativa* L.) and bean (*Vicia faba* L.) can cause damage to the phloem, xylem, vascular cambium and mesophyll (Medler 1941, Hunter and Backus 1989, Kabrick and Backus 1991, Ecale and Backus 1995a, Zhou and Backus 1999). PLH infestations can lead to reduced rates of carbon assimilation, transpiration, stomatal conductance and sugar translocation in potato (*Solanum tuberosum* L.) and alfalfa (Ladd Jr. and Rawlins 1964, Hibbs *et al.* 1964, Womack 1984, Walgenbach and Wyman 1985, Flinn and Hower 1990, Nielsen *et al.* 1990, Nielsen *et al.* 1999, Lamp *et al.* 2001).

If grapevines respond to PLH infestations as other PLH host plants do, then carbon assimilation will likely decrease. This could lead to a corresponding reduction in source strength and vine productivity if carbohydrate synthesis by the leaves is inhibited. In addition, it seems likely that grapevine leaves are able to tolerate some level of infestation without any measurable changes in carbon assimilation or other components of photosynthesis. Further, if assimilation is reduced by infestations, it is possible that photosynthetic compensation can occur. Photosynthetic compensation occurs when leaf Pn or leaf growth is stimulated by reducing source strength or by increasing sink strength (Flore and Lakso 1989, Wardlaw 1990). In some cases, reducing source strength within a leaf or within the canopy of some plant species can result in enhanced assimilation rates in other portions of the leaf or canopy (Layne and Flore 1992, Petrie *et al.* 2000c).

The objectives of these studies were to determine: 1) how PLH feeding affects leaf photosynthesis of grapevines; 2) whether there are photosynthetic damage thresholds for infested grapevine leaves; 3) whether compensation to photosynthetic decline due to PLH feeding can occur.

Materials of Methods

Two experiments were conducted to test the affects of PLH infestations on the photosynthetic performance of grapevines. This section will describe the materials and methods that both experiments have in common, followed by the unique details for each experiment individually.

Plant material: For both experiments, the vines were grown in black plastic pots with ten liters of steam sterilized soil composed of 50% sandy loam, 30% sphagnum peat and 20% washed sand. The vines were watered as needed, usually three times per week. Lateral shoots and non-count shoots were removed as they appeared. The vines were fertilized monthly with 600ml of a solution made by mixing five tablespoons (about 60 g) of 15-30-15 fertilizer with 19 liters of water. This solution contained approximately 475 mg/l of both nitrogen and potassium, 950 mg/l of phosphorus and trace amounts of boron, copper, iron, manganese, molybdenum and zinc.

Insect Source: Second and third instar PLH nymphs were used to infest the vines for this study. Adult PLH were collected from a field mixed with alfalfa (*Medicago sativa* L.) and clover (*Trifolium pratense* L.) using a canvas sweep net. These insects were placed into collapsible cages that measured 61 cm in length, width and height (BioQuip Products, Inc., Gardena CA, model 1450D) and reared indoors on bean plants (*Vicia faba* L.). The second and third instar nymphal offspring of these adults were used for the experiments.

Clip-Cage Design and Treatment Application: Small cages were used to isolate the PLH to a given region of a leaf. The cages were placed on the leaves such that the most apical end of the midrib bisected the cage. Prior to the initial gas exchange measurements, a marker was used to make a small black dot on the midrib in order to ensure that the CIRAS II cuvette and the cage were consistently placed on the same region of the leaf each time.

See Appendix I for pictures of the cages. Each cage was constructed from one metal hair clip (Goody Products, Inc. Peachtree City, GA., model# 03395), two foam gaskets used for the CIRAS II PLC Broad cuvette (PP Systems, Haverhill, MA), two nylon oil drain-plug gaskets (18 mm internal diameter, 2 mm thick) and one piece of white valais sheer fabric (Jo-Ann Fabrics, Hudson, OH). Each cage had a top portion that went over the adaxial leaf surface and a bottom portion that went over the abaxial leaf surface. Only the bottom portion enclosed the PLH. One foam gasket was glued onto one nylon gasket to make the top of the cage. The bottom portion of the cage was the same except that a piece of fabric was glued between the nylon gasket and the arm of the hair clip. One arm of the hair clip was glued to the nylon gasket of the upper cage and one arm of the hair clip was glued to the nylon gasket of the lower cage. The finished cage could then be easily clipped onto a leaf with only the foam gaskets in contact with the leaf surface.

The PLH were collected from an artificial colony using an aspirator (BioQuip Products, Inc., Gardena CA, model 1135A) that was lined with white valais sheer fabric (Jo-Ann Fabrics, Hudson, OH). The PLH were then rendered temporarily immobile using CO_2 gas. While still immobile, the aspirator was dismantled and the PLH were transferred from the fabric to the lower portion of the cage. The cage was then clipped onto the leaf. During Experiment I, the cages remained on the leaves for 42 hours; during Experiment II, the cages remained on the leaves for 43 hours.

Experiment I

Plant Material and Growing Conditions: This experiment was conducted on potted three-year old Pinot gris (*Vitis vinifera* L.) grafted to rootstock Teleki 5C (*V. berlandieri* Planch. x *V. riparia* Michx). On 23 February 2004, dormant vines were moved from a 4°C cold room to a greenhouse and grown under 400 W high-pressure sodium lamps with a 12:12 photoperiod. After budbreak on 3 March 2004 the vines were pruned, thinned to six shoots and later thinned to one flower cluster per shoot.

Measurements: Gas exchange measurements were performed using a CIRAS II Infra-red gas analyzer (PP Systems, Haverhill MA). Gas exchange was measured two times per leaf: prior to treatment application and immediately after treatment removal. All gas exchange measurements were taken between 10:30 am and 1:30 pm under saturating light levels (1,000 μmol photons$\cdot$m$^{-2}\cdot$s^{-1} PAR) which were provided by the CIRAS II cuvette lamp.

Experimental Design and Statistical Analysis: This experiment was a split plot design with repeated measures in time and covariance over space. The whole-plot factor was the shoot and the sub-plot factor was leaf position. The treatment factor was PLH nymphs (2nd and 3rd instars), and the levels were 0 PLH/ -cage, 0 PLH/ +cage, 1 PLH/ +cage, 2 PLH/ + cage, 4 PLH/ +cage and 8 PLH/ +cage. These will be referred to as: 0/-, 0/+, 1/+, 2/+, 4/+ and 8/+ respectively. There were three replicates and the data were analyzed using ANCOVA with the initial pre-treatment data used as the covariate.

Within a vine, the treatments were randomly assigned to the shoots. For each shoot, four leaves were selected based upon phenophase and the same treatment level was applied to each leaf within a shoot. The four leaf phenophases used were: the most apical leaf that was large enough to support a clip-cage (apical), the first leaf basal to the most apical leaf (apical-1), the most recently fully expanded leaf (MRFE) and the leaf opposite the cluster (cluster).

Experiment II

Plant Material and Growing Conditions: The vines used for this study were two-year old Pinot gris (*Vitis vinifera* L.) grafted to rootstock 1103 Paulsen (*V. berlandieri* Planch. x *V. rupestris* Scheele). The vines were potted on 29 June 2005. One 1.5 m length of bamboo was then inserted into the soil at the edge of each pot for shoot training. Each vine was trained to one shoot and all flower clusters were removed. The vines were grown in a greenhouse under natural light.

Measurements and Treatment Levels: After regions of a given leaf were selected for use as experimental units, they were measured for day-1 light response curves (LR) and assimilation/internal [CO_2] response curves (AC_i). The day-1 data comprised the pre-treatment measurements. Two treatment levels were used: a cage with 0 PLH, and a cage with 9 PLH nymphs. The treatments were randomly assigned to leaf sides, then applied and removed after 42 hours. The day-3 response curve data were then collected. Seven days later, the day-10 data were collected. All response curves were measured using a CIRAS II Infrared gas analyzer (PP Systems, Haverhill MA) fitted with the PLC Broad cuvette using the internal light source.

The curves were used to estimate the light compensation points (cp) and CO_2 compensation points (Γ). The raw data was used to designate the linear portion of the curves, which were defined as the first four data points. These linear portions of the curves were used to estimate dark respiration rates (R_d), quantum efficiency (Φ) and carboxylation efficiency (k). Φ was estimated as the slope of the linear portion of the LR curves, R_d was the value of y at x = 0 for the LR curves, and k was estimated as the slope of the linear portion of the AC_i curves. Maximum assimilation at saturating levels of photosynthetically active radiation (A_{max} 1400 PAR) and maximum A at saturating C_i (A_{max} CO_2) were estimated by the maximum value of A for each respective curve based upon the raw data. All parameters were estimated for each replicate, then entered into SAS for statistical analysis using a mixed model with treatment, time and treatment by time as fixed effects, replicate as a random effect using repeated measures across time. In order to normalize the residuals, the data for light compensation points (cp) and A_{max} 1400 PAR were log transformed. The data for rep 4 on day-10 were outliers and were not used for analysis of k, A_{max} CO_2 and Γ (best fit function produced illogical values; cannot calculate the natural log of a negative number).

Light response curves were created by using the following light levels (μmol photons$\cdot m^{-2}\cdot s^{-1}$ PAR): 0, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200 and 1400. The curve began with the lowest light level and proceeded through all levels ending with 1400 μmol photons$\cdot m^{-2}\cdot s^{-1}$. The reference CO_2 concentration was 375 parts per million. A cotton cloth was used to shade the cuvette from ambient light in order to attain the first four light levels. The linear portion of the light response curves will be designated as the first four light levels, ranging from approximately 0 – 150 PAR.

AC_i curves were created by using the following reference CO_2 levels measured in parts per million by volume (ppm): 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200 and 1400. The curve began with the lowest CO_2 level and proceeded through all levels ending with 1400 ppm. Light levels were maintained at 1000 PAR. The linear portion of the AC_i curves will be designated as the first four levels of C_i; the range in C_i over all curves at these levels was 39 – 180 ppm.

Experimental Design and Statistical Analysis: This experiment was a completely randomized block design with repeated measures over time and four replicate leaves, each from a separate vine. The leaves used for experimental units in this study were the youngest leaves on a shoot that were large enough to fit two cages; the average midrib length was 52.3 mm and the average leaf size was 33.7 cm^2. These leaves were chosen because PLH tend to feed on the younger tissues of a grapevine (Isaacs *et al.* 2003). Prior to infestation, the leaves were all uniformly of a normal green color with no mechanical damage such as tears or holes and none of them were fully expanded.

SigmaPlot was used to fit the data with trend curves. This program uses the Marquardt-Levenberg algorithm to find a best fit function that fits the data. The general equation of each trend curve was: $y = y_0 + a(1-e^{-bx})$, where x is PAR for the light response curves and $x = C_i$ for the AC_i curves; in both cases $y = A$ and a is the asymptotic value of the curve. This equation was selected because its shape was representative of the data and it could be used to directly estimate parameters of the data that are indicative of specific photosynthetic components.

Results

Experiment I

Cage Affect and Tissue Discoloration: An uncaged Control (0/-) was used in order to determine whether the cages have a significant impact on leaf photosynthesis. There were no significant differences between the 0/- and the 0/+ treatment for any leaf position or for any measured variable of photosynthetic rates in this study. This indicates that there was no cage effect under these experimental conditions. For this reason, all treatment comparisons will be made relative to the 0/+ values. The appearance of all leaf tissue used in this study was initially a normal green color and all midribs appeared undamaged. After the PLH stress period, leaf tissue within the caged regions that were exposed to 4/+ or 8/+ typically showed a brown and somewhat translucent spot with portions of the midrib appearing brown and shriveled.

Assimilation Rates: All leaf positions showed a significant treatment effect for assimilation (A) rates, but the infestation levels where this occurred differed by leaf position (Tables 19-22). In general, the treatment effect was less pronounced for older leaves than for younger leaves. Significant reductions in A occurred at 1/+ for the apical leaves (Table 19), 2/+ for apical-1 leaves (Table 20), 2/+ for MRFE leaves (Table 21) and not until 8/+ for the cluster leaves (Table 22). The 8/+ level resulted in dramatic reductions in A for all leaves. There was a significant linear relationship between PLH infestation level and A at all leaf positions. This shows that PLH infestations result in an inversely proportional decrease in A for Pinot gris leaves.

Transpiration Rates and Stomatal Conductance: The treatment effects on transpiration (E) and stomatal conductance (G_s) were nearly identical, with the exception of the cluster leaf. The cluster leaf was not significantly affected by any level of infestation for E, but did show a significant reduction in G_s when exposed to the 8/+ level (Table 22). Aside from that exception, there were significant reductions in both G_s and E in response to infestation at all leaf positions. As with A, the data show that younger leaves were more susceptible to infestation than older leaves. The apical and apical-1 leaves showed a decline in both E and G_s after exposure to 1/+. The MRFE leaves did not show decreased rates of E and G_s until the infestation severity reached 2/+. The cluster leaves were not significantly affected by any level of infestation for E and G_s was significantly reduced only by the 8/+ level. Linear regressions of PLH/cage against both G_s and E were significant for all leaf positions. This shows that there was an inverse relationship between treatment level and either variable in response to infestation.

Internal [CO_2]: Internal CO_2 concentration (C_i) tended to be directly proportional to infestation level, with the exception of the apical leaves which showed no clear pattern and no significant differences for any level when compared to the 0/+ Control (Table 19). For the other three leaf positions, C_i tended to increase with increasing infestation severity. The apical-1 and MRFE leaves showed significantly higher C_i at 4/+ and 8/+ levels; the cluster leaves did not show significant increases in C_i until an infestation severity of 8/+. Except for the apical leaves, significant linear regressions occurred for all leaf positions, indicating a direct relationship between infestation severity and C_i.

Thresholds: Table 23 shows damage threshold values in terms of PLH/leaf and PLH-hrs per leaf. Damage thresholds for A occurred at 8/+ for the cluster leaves and 2/+ for both the MRFE and apical-1 leaves. If a damage threshold for A occurred with the apical leaves, then it occurred below the 1/+ level. Damage thresholds for E and G_s occurred at 1/+ for the apical and apical-1 leaves and 2/+ for the MRFE leaves. The cluster leaves showed a damage threshold of 8/+ for G_s, but there seems not to have been a threshold for E. Damage thresholds for C_i occurred at 4/+ for both the apical-1 and MRFE leaves, the cluster leaves showed a damage threshold of 8/+ and there was no threshold for the apical leaves. These thresholds indicate that the Pinot gris leaves used for this study were able to tolerate some degree of PLH infestation and that younger leaves are more sensitive to PLH infestation than older leaves.

Table 19. Gas exchange of Pinot gris leaves in response to infestation by potato leafhoppers (PLH): I. apical leaf position.

I. apical

PLH/cage	A	E	G_s	C_i
0/-	6.8 ab	2.28 ab	84.7 ab	195.2 b
0/+	7.3 a	2.57 a	96.7 a	203.2 ab
1/+	4.9 b	1.73 b	62.4 b	206.7 ab
2/+	2.6 c	0.92 c	29.7 c	192.9 b
4/+	2.2 c	0.94 c	31.6 c	257.8 a
8/+	2.0 c	1.01 c	32.7 c	211.6 ab
F-values	***	***	***	*
linear R^2	0.60**	0.47**	0.50**	ns
quadratic R^2	0.89***	0.84***	0.85***	ns

A=assimilation, E=transpiration, G_s=stomatal conductance, C_i=internal [CO_2].

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/cage.

[c] PLH/cage refers to the number of insects per cage (+) or uncaged Control (-).

Table 20. Gas exchange of Pinot gris leaves in response to infestation by potato leafhoppers (PLH): II. apical-1 leaf position.

II. Apical -1

PLH/cage	**A**	**E**	**G_s**	**C_i**
0/-	7.3 a	2.41 ab	82.1 ab	188.5 c
0/+	7.5 a	2.62 a	94.7 a	189.8 c
1/+	5.4 a	1.84 b	63.0 b	189.8 c
2/+	2.9 b	1.18 c	38.1 c	201.4 c
4/+	2.2 bc	0.97 c	30.7 c	232.2 b
8/+	0.4 c	0.80 c	21.5 c	268.0 a
F-values	***	***	***	***
linear R^2	0.70***	0.62***	0.61***	0.88***
quadratic R^2	0.83*	0.82**	0.81**	ns

A=assimilation, E=transpiration, G_s=stomatal conductance, C_i=internal [CO_2].

[a]Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b]Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/cage.

[c]PLH/cage refers to the number of insects per cage (+) or uncaged Control (-).

Table 21. Gas exchange of Pinot gris leaves in response to infestation by potato leafhoppers (PLH): III. MRFE leaf position.

III. MRFE

PLH/cage	A	E	G_s	C_i
0/-	6.6 a	2.58 ab	85.7 ab	194.6 b
0/+	7.0 a	2.92 a	100.6 a	197.0 b
1/+	7.6 a	2.88 a	103.9 a	200.2 b
2/+	3.7 b	1.75 bc	51.6 bc	199.4 b
4/+	2.2 b	1.19 c	34.9 c	237.9 a
8/+	1.7 b	0.99 c	27.1 c	239.1 a
F-values	***	***	***	***
linear R^2	0.78***	0.74***	0.69***	0.82***
quadratic R^2	0.86*	ns	ns	ns

A=assimilation, E=transpiration, G_s=stomatal conductance, C_i=internal [CO_2].

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/cage.

[c] PLH/cage refers to the number of insects per cage (+) or uncaged Control (-).

Table 22. Gas exchange of Pinot gris leaves in response to infestation by potato leafhoppers (PLH): IV. cluster leaf position.

IV. Cluster

PLH/cage	**A**	**E**	**G_s**	**C_i**
0/-	6.1 a	2.40 a	75.9 a	187.6 b
0/+	5.6 ab	2.17 ab	70.8 a	200.6 b
1/+	4.6 ab	2.14 ab	60.6 ab	189.1 b
2/+	3.7 bc	1.32 ab	42.3 ab	204.2 b
4/+	3.6 bc	1.54 ab	46.9 ab	213.4 ab
8/+	2.4 c	1.08 b	33.9 b	245.9 a
F-values	**	**	*	**
linear R^2	0.74**	0.57*	0.53*	0.82***
quadratic R^2	ns	ns	ns	ns

A=assimilation, E=transpiration, G_s=stomatal conductance, C_i=internal [CO_2].

[a] Means were separated by LSD; values within a column followed by the same letter are not significantly different at the 5% level.

[b] Significant F-values and R^2 values shown at the 5% (*), 1% (**), 0.1% (***) or not significant (ns); regressions for each parameter were plotted against PLH/cage.

[c] PLH/cage refers to the number of insects per cage (+) or uncaged Control (-).

Table 23. Damage thresholds for gas exchange of Pinot gris in response to infestation by potato leafhoppers (PLH) in terms of: I. PLH/leaf, II. PLH-hours/leaf.

I.

	PLH per leaf			
Leaf Position	**A**	**E**	**G_s**	**C_i**
apical	1	1	1	ns
apical-1	2	1	1	4
MRFE	2	2	2	4
cluster	8	ns	8	8

II.

	PLH-hrs per leaf			
Leaf Position	**A**	**E**	**G_s**	**C_i**
apical	43	43	43	ns
apical-1	86	43	43	172
MRFE	86	86	86	172
cluster	344	ns	344	344

A=assimilation, E=transpiration, G_s=stomatal conductance, C_i=internal [CO_2].

[a]Values are based on mean separations from Table 19.

[b]Leaf positions along a shoot are the apical-most leaf (apical), first leaf basal to the apical (apical-1), most recently fully expanded (MRFE) and the leaf opposite the cluster (cluster).

Experiment II

General observations: Prior to treatment application, all leaves used for this study were of a normal green color, normal morphology and lacking any holes, tears or other mechanical damage. The range in midrib lengths for the four leaves over all days was 46-57 mm; based upon a regression between midrib length and leaf area from a previous study (Figure 4), this equates to a leaf area range of 26-40 cm^2. The cages each encompass approximately 2.5 cm^2. Therefore, each cage covered about 15% of the leaf half that it was clipped onto. After the treatments were removed, the leaf area that had been infested with PLH usually had a discolored region in the middle that was about one-third to one-half of the total area. On day-3, this discolored region was pale green to light brown in color, somewhat translucent and the enclosed length of the vein appeared shriveled or wilted as if it had collapsed to some degree. By day-10, the discolored region was a reddish brown and the tissue was opaque and appeared desiccated (personal observation, data not shown).

Light Response Curves

Day-1 (prior to the infestation period): Prior to the infestation period, the light response curves for the Control and PLH sides of the leaves were very similar and showed no statistically significant differences. Although the Control averages showed slightly higher values for R_d and cp, and slightly lower values for Φ and A_{max}, there were no statistically significant differences for any of these variables (Table 24). The amount of PAR received by the Control and PLH sides at any level were not significantly different. The adjusted R^2 values for the curves were 0.99 and 0.98 for the Control and PLH sides respectively.

Day-3 (immediately after the infestation period): Immediately after the 42 hour infestation period ended, there were significant differences in light utilization between the Control and PLH sides of the leaf. The data indicate that the PLH side was assimilating less CO_2 per quantum PAR than the Control. Relative to the Control side, the PLH side had a higher R_d, higher cp and lower A_{max} at 1400 PAR (Table 24). A_{max} of the PLH side was only $^1/_3$ that of the Control. The amount of PAR received by the Control and PLH sides at any level were not significantly different. The adjusted R^2 values for the curves were 0.98 and 0.91 for the Control and PLH sides respectively.

Day-10 (seven days after the infestation period): Although the trend curves at this time were very similar to the day-3 trend curves, the standard errors for the Control side were much higher by day-10 and overlapped with the PLH side at every level. Due to the large amount of variability, it is difficult to draw any conclusions from the actual curves on day-10. However, there were statistically significant differences between treatments in terms of Φ and A_{max} (Table 24). PLH infestation caused a decrease in Φ and A_{max} indicating that light utilization was still impaired by the infestation. No significant differences existed in terms of R_d and cp. PAR values at every level were again similar between the Control and PLH sides of the leaves. The adjusted R^2 values were 0.98 and 0.97 for the Control and PLH sides respectively.

AC_i Curves

Day-1 (prior to the infestation period): Prior to the infestation period, the AC_i curves for the Control and PLH leaf sides were very similar. Adjusted R^2 values were 0.98 and 0.99 for the Control and PLH sides respectively and the standard error bars overlapped at every point. Based upon these R^2 values and the standard errors, the best-fit curves for the data were not statistically different between treatments. Likewise, no significant differences existed between treatments in terms of k, A_{max} CO_2 and Γ (Table 24). For all levels of C_i, taking into account standard errors, there were also no significant differences in A between treatments at any level of C_i.

Day-3 (immediately after the infestation period): Immediately after the PLH infestation period, the PLH side showed significantly lower values of A at nearly every level of C_i. Adjusted R^2 values were 0.97 and 0.88 for the Control and PLH sides respectively, and with the exception of the lowest C_i level, there was no overlap in standard error bars at any point in the curves. Infestation also caused a reduction in k and A_{max}, but did not significantly affect Γ (Table 24). In addition, the PLH side of the leaves attained a C_i value of 845 whereas the Control side only reached a C_i value of 535. This indicates a non-stomatal limitation to A.

Day-10 (seven days after the infestation period): By day-10, differences between the curves were less pronounced than on day-3. The adjusted R^2 values for the best-fit curves were 0.96 for both the Control and PLH leaf sides, indicating that the best fit functions were still sufficient to describe the data. However, even though the PLH side was still operating at lower A than the Control at any level of C_i, there was more variability than on day-3 and more overlap in standard error bars. Therefore, it is difficult to draw conclusions in regards to the AC_i curves on day-10. Values of *k* and A_{max} were still significantly lower for the PLH side indicating that PLH infestations reduced the ability of the infested leaf tissue to utilize CO_2 throughout a range of C_i levels (Table 24).

Day 1

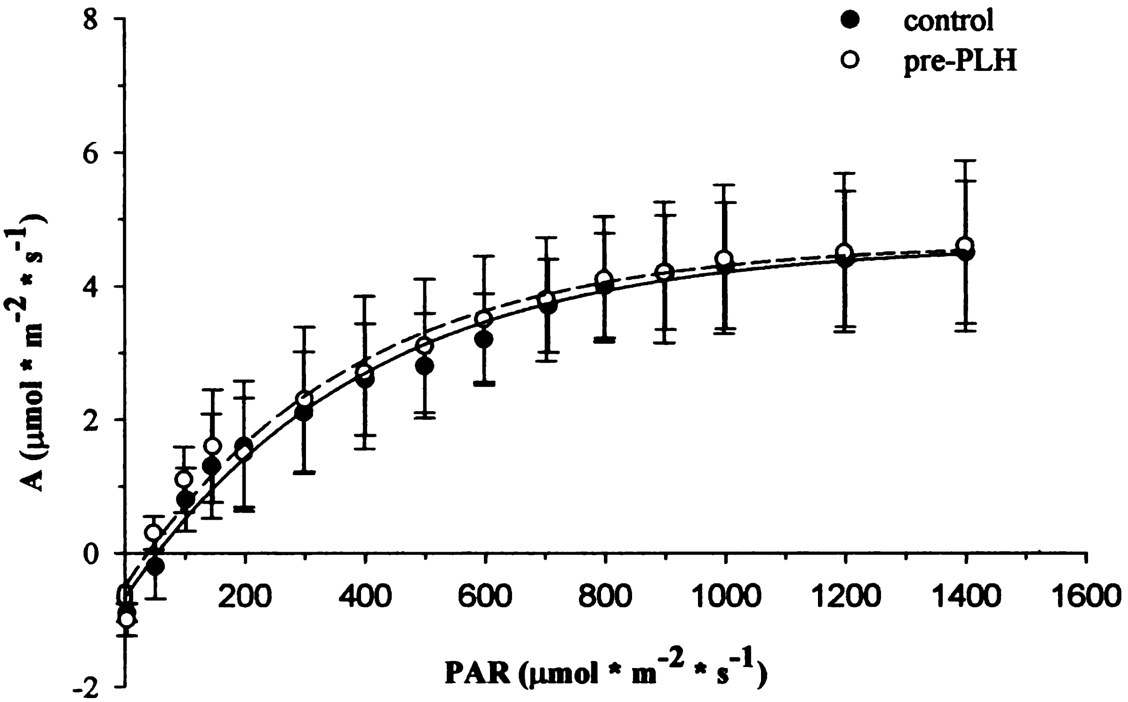

Figure 7: Light utilization of Pinot gris leaves prior to the PLH infestation period (day-1). Each value in the light response curves is the mean of four replicates. Standard errors of the means occur about each data point.

Day 3

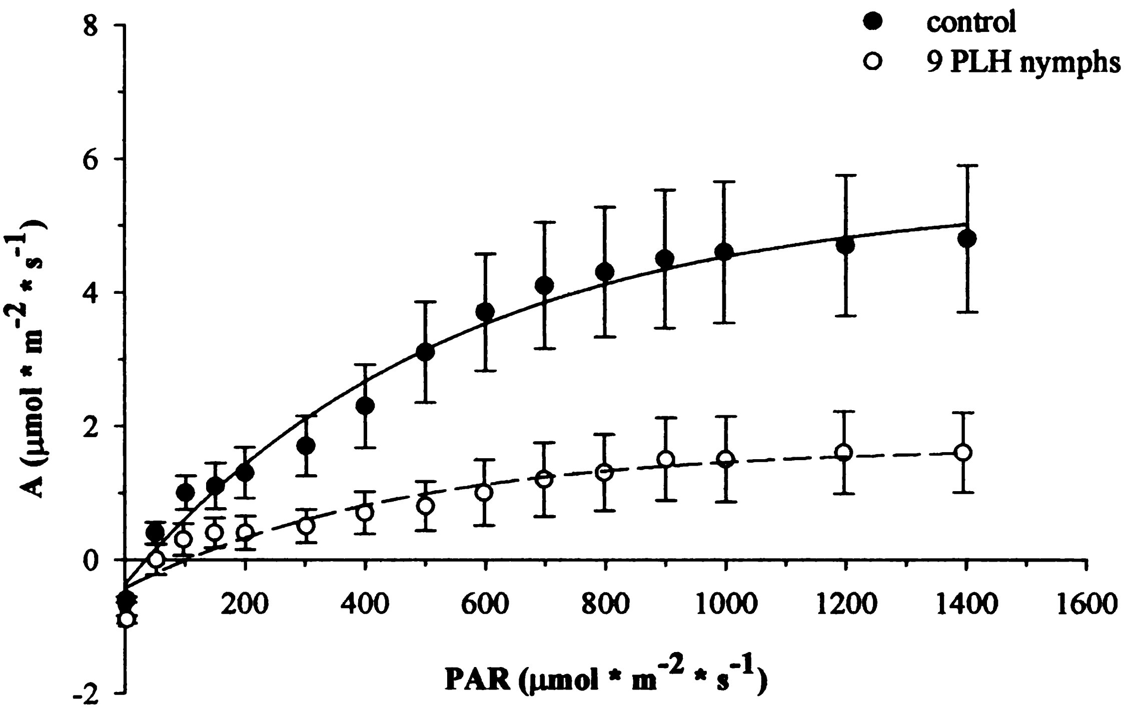

Figure 8: Light utilization of Pinot gris leaves immediately after 42 hours of PLH infestation (day-3). Each value in the light response curves is the mean of four replicates. Standard error of the means occur about each data point.

Day 10

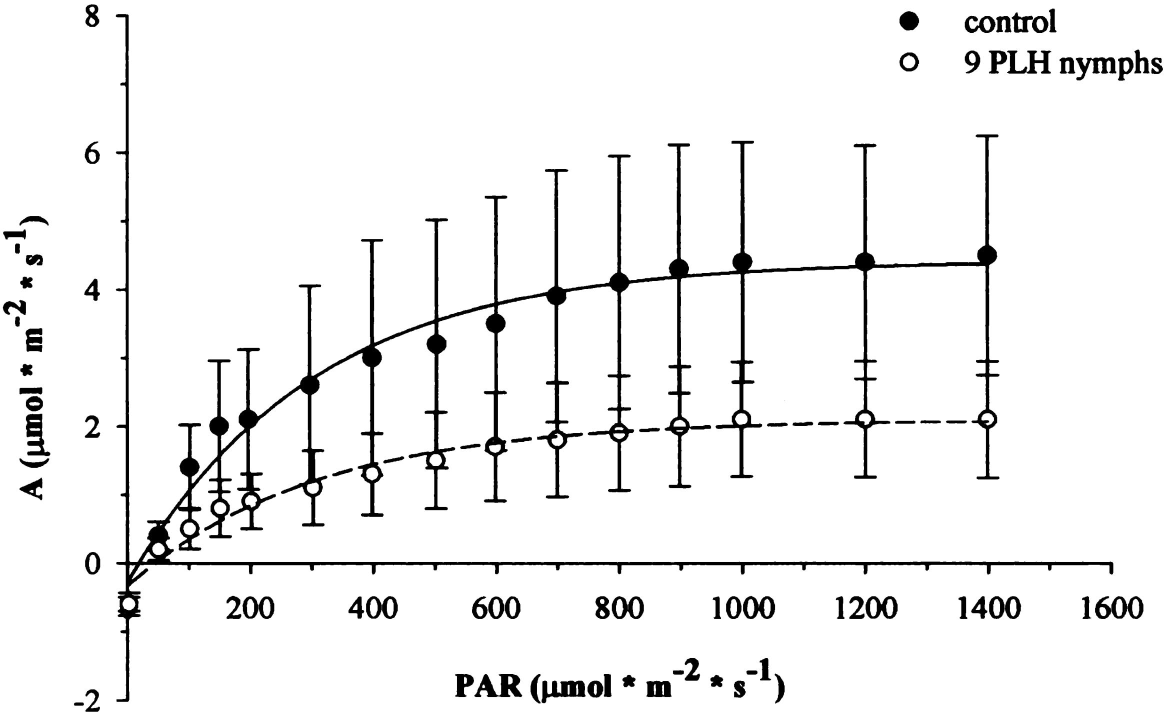

Figure 9: Light utilization of Pinot gris leaves seven days after a PLH infestation period of 42 hours (day-10). Each point in the light response curves is the mean of four replicates. Standard errors of the means occur about each data point.

Day 1

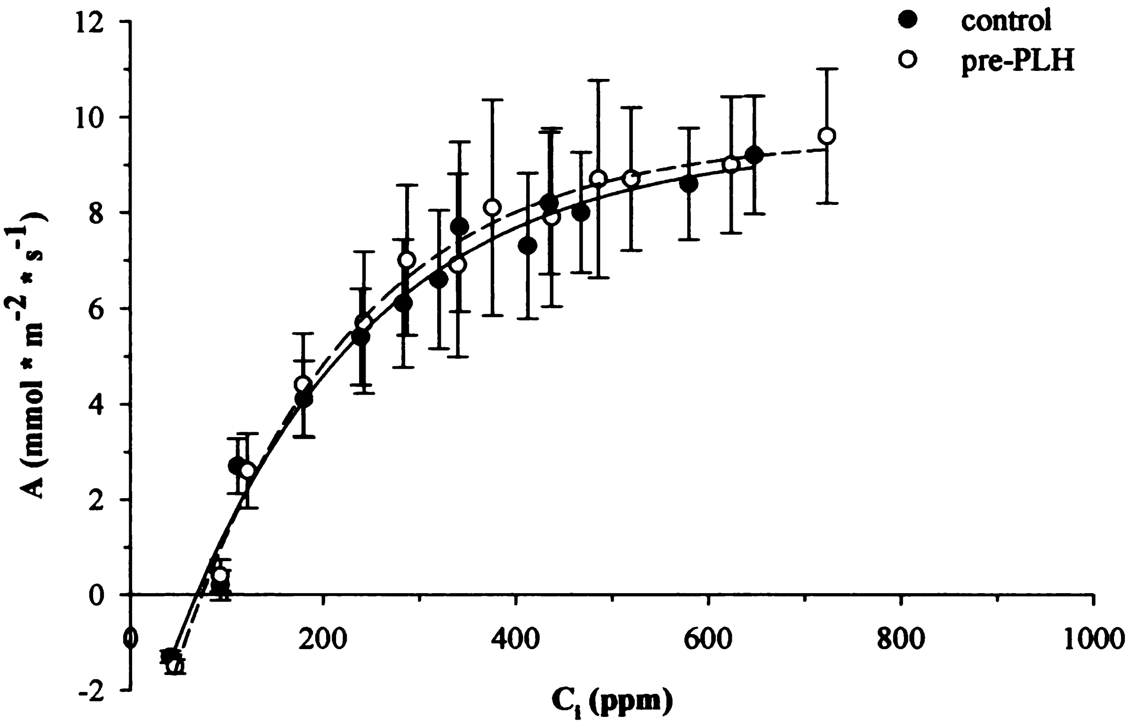

Figure 10: CO_2 utilization of Pinot gris leaves prior to the PLH infestation period (day-1). Each point in the AC_i curves is the mean of four replicates. Standard errors of the means occur about each data point.

Day 3

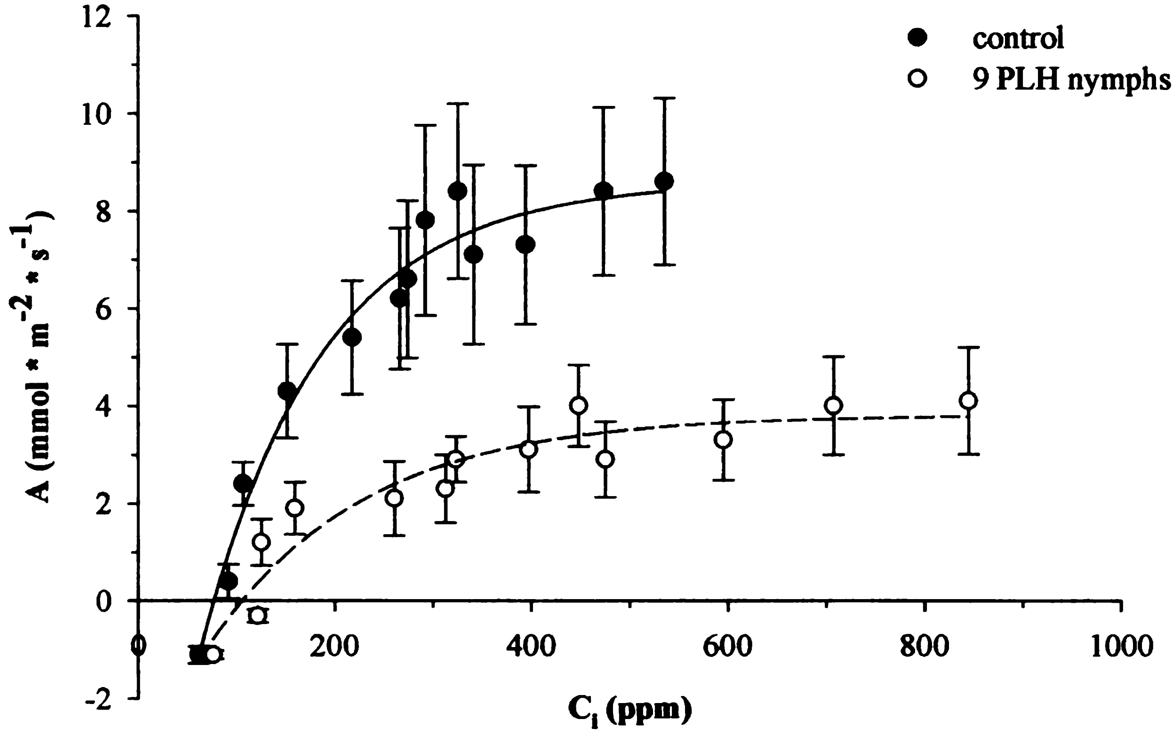

Figure 11: CO_2 utilization of Pinot gris leaves immediately after the PLH infestation period (day-3). Each point in the AC$_i$ curves is the mean of four replicates. Standard errors of the means occur about each data point.

Day 10

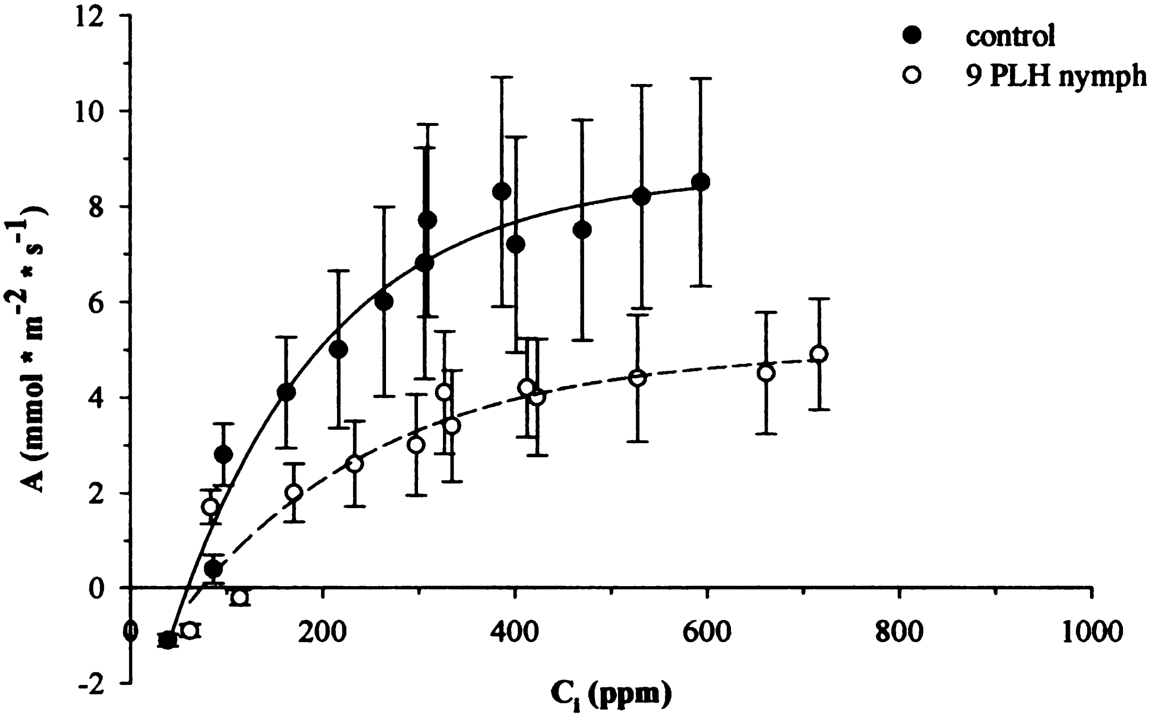

Figure 12: CO$_2$ utilization of Pinot gris leaves seven days after the PLH infestation period (day-10). Each point in the AC$_i$ curves is the mean of four replicates. Standard errors of the means occur about each data point.

Table 24. The effect of PLH infestations on dark respiration rate (R_d), quantum efficiency (Φ), light compensation point (cp), A_{max} at 1400 $\mu mol*m^{-2}*s^{-1}$ PAR, carboxylation efficiency (k), A_{max} at saturating ppm CO_2, and CO_2 compensation point (Γ) of Pinot gris leaves. Treatments are: 9 nymphs (PLH) or 0 (Control).

Treatment x Time	R_d	Φ	cp	A_{max} PAR	k	A_{max} CO_2	Γ
Day-1							
Control	-0.98	0.016	82.2	4.5	0.034	9.1	68.8
PLH (pre-stress)	-0.83	0.018	65.0	4.6	0.036	9.7	75.8
	ns	ns	ns	ns	ns	ns	ns
Day-3							
Control	-0.39	0.011	50.4	4.8	0.036	8.7	66.1
PLH (post-stress)	-0.68	0.008	173.9	1.6	0.014	4.1	85.4
	*	ns	*	**	**	***	ns
Day-10							
Control	-0.53	0.018	55.2	4.5	0.031	8.5	64.5
PLH (post-stress)	-0.43	0.009	133.5	2.1	0.014	4.9	71.8
	ns	*	ns	*	*	**	ns

[a] F-values significant at the 5% (*), 1% (*), 0.1% (***) or not significant (ns).

Discussion

The results of these studies indicate: 1) PLH infestations can reduce the rates of CO_2 assimilation of infested Pinot gris leaf tissue; 2) lowered A occurred as a result of stomatal and non-stomatal limitation; and 3) non-stomatal limitations were due to decreased efficiency of light and CO_2 utilization. In addition, Experiment I indicates that there were damage thresholds to PLH infestation for grapevine leaves and Experiment II shows some evidence that photosynthetic compensation of uninfested leaf tissue can occur when a separate portion of the same leaf is infested with PLH.

Reduced A in response to PLH infestations were observed for both Experiment I and Experiment II. For both experiments, this result was correlated with decreased G_s and increased C_i which indicates both stomatal and non-stomatal limitations to A in response to infestation. Stomatal limitations could be due to stomatal closure or to tissue destruction by the PLH within the region of leaf that was infested by PLH. There have been many studies which document cell and tissue destruction due to stylet probing by the PLH while feeding (Hunter and Backus 1989, Kabrick and Backus 1991, Ecale and Backus 1995a, Zhou and Backus 1999). It is likely that such mechanical damage due to stylet probing rendered at least some of the tissue physiologically inactive. PLH saliva can cause changes in tissue anatomy and function such as abnormal cell enlargement, abnormal cell divisions and vascular blockage (Ecale and Backus 1995b). It is possible that PLH saliva could cause stomatal closure.

In addition to stomatal limitations, the data for both experiments indicate a non-stomatal limitation to A as well. The results from Experiment II indicate that non-stomatal limitations to A were due to impaired ability of light and CO_2 utilization by the leaves. Prior to the infestation period (day-1), there were no significant differences in A_{max} PAR, A_{max} CO_2, Φ or k. After the infestation period, both Φ and k were reduced to half the Control values indicating that A per unit PAR and A per unit C_i decreased within the linear portion of the response curves. Outside the linear portion of the curves, A per unit PAR and A per unit C_i were also reduced as evidenced by decreases in A_{max} PAR and A_{max} CO_2 of over 50% by day-3. It is likely that this was due to tissue destruction by stylet probing or some form of physiological impairment in response to PLH saliva. If the latter, it is possible that decreased A was due to reduced Rubisco activity as a result of limited ATP and NADPH availability from the light reactions.

Thresholds

Experiment I shows that low levels of PLH infestations were tolerated by the leaves. The infestation level at which significant changes in a measured variable occurred will be referred to as the damage threshold. In most cases, for any given combination of leaf position and response variable there seems to have been a damage threshold (Table 23). Although the infestation severities where damage thresholds occurred in this study will likely differ in a field situation, the important point is that these thresholds exist. These thresholds indicate that the Pinot gris leaves used for this study were able to tolerate some degree of PLH infestation and that younger leaves are more sensitive to PLH infestation than older leaves.

Compensation

Compensatory mechanisms have been observed in cases where a plant experiences sudden decreases in source strength. Reports by Layne and Flore (1992, 1993, 1995) show that partial defoliation of cherry can result in enhanced rates of carbon assimilation for the remaining leaf tissue. Petrie *et al.* (2000c) demonstrated that partial defoliation of non-fruiting grapevines resulted in higher A for the remaining leaves. PLH infestation of grapevine leaves can reduce carbon assimilation which will reduce source strength of the infested leaf tissue. It is likely that a PLH infestation of only a portion of the canopy or a portion of a leaf could result in enhanced A for the uninfested portion of the canopy or leaf.

Walgenbach *et al.* (1985) observed partial recovery of assimilation rates for potato (*Solanum tuberosum* L.) following PLH infestations. Studies of alfalfa by Zhou and Backus (1999) showed eventual phloem regrowth whereby new sieve tubes circumvented damaged areas of phloem (Zhou and Backus 1999). These studies indicate that plants can also compensate for damage that occurs in response to PLH infestations. In a previous study (Chapter 1, Experiment II) using whole-vines infested with varying numbers of PLH nymphs, vegetative growth was reduced in the short-term, but ten weeks after the infestation period, vines subjected to relatively low levels of PLH were the largest. This observation led us to speculate that there was a mechanism of recovery involved.

The results of Experiment II provide some evidence that photosynthetic compensation occurred, but further research should be conducted in order to verify this result. R_d and cp were not significantly different prior to the infestation period. On day-3, both variables were significantly higher for the PLH side relative to the Control. On day-10, there were no longer any significant differences between treatments in terms of R_d and cp. It seems that the infested leaf tissue was able to recover from some of the negative impacts of PLH infestation by day-10. This indicates that compensatory mechanisms might have occurred. The data for A_{max} PAR also provide some evidence for compensation. As A_{max} PAR decreased for the PLH side in response to infestation (day-3), the Control side increased. On day-10, the PLH side showed a slight increase in A_{max} PAR relative to the day-3 data, and the Control side showed a slight decrease in A_{max} PAR from day-3. It seems as if the Control side was acting as a counter-weight to balance reductions in A that were experienced by the PLH side.

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Conclusions and Future Work

The studies presented in this dissertation show that PLH infestations reduce vegetative growth of potted non-fruiting Pinot gris grapevines. Infestation severity was directly related to leaf cupping and leaf discoloration and inversely related to leaf and shoot production. Although infestations could cause severe stunting of leaf and shoot growth, the vines tolerated some low levels of short-term infestation and thus there are damage thresholds to infestations by the non-fruiting grapevines used in this study. In addition to damage thresholds, the vines were able to recover for some degree of stunting during the post-infestation period. Such recovery occurred seemingly by means of enhanced growth rates and likely due in part to remobilization of storage carbohydrates from the roots to the shoot and leaves.

Reduced vegetative growth in response to PLH infestation is due, at least partly, to reduced rates of carbon assimilation by the infested leaf tissue. Thus, growth stunting in response to PLH infestation is the result of a source strength reduction of the vine. Source strength reductions were temporary at least, and likely only occurred for infested leaves. Inhibition of carbon assimilation was due to both stomatal and non-stomatal limitations. Stomatal limitations will reduce the flow of CO_2-laden air into the leaf thus limiting the amount of CO_2 that is available for fixation. Non-stomatal limitations will further confound this situation by reducing the physiological ability of the leaves to fix carbon. Taken together, these limitations to CO_2 availability and fixation will limit the ability of the leaf to synthesize sugars, a consequence that can result in less available energy and building materials for growth and metabolism.

Like with growth stunting, the impact that PLH infestations have on grapevine photosynthesis also have damage thresholds. The leaves were able to tolerate low levels of PLH infestations without any significant reductions in the components of photosynthesis. In addition, there was some evidence for photosynthetic compensation of grapevine leaves during the post-infestation period (Table 24).

Caution should be exercised when extrapolating the results of these studies to a field situation where vines are in full production. However, our findings would be useful for the period of vineyard establishment. During the establishment period, fruit is typically removed from the vines for the first several years in order to allow vegetative growth to dominate thus hastening the ability of the vine to produce shoots and wood and thus fill the trellis. Such a situation where non-fruiting vines are growing in the field would be relevant to studies such as those presented here.

Another practical application of our studies would be to refine management strategies for PLH and other pests in the vineyard. These results suggest that relatively small populations of PLH might not cause any significant reductions in vine growth or productivity. Thus, rather than applying pesticides for all cases of PLH infestations of a vineyard, it could be possible to abstain from pesticide use in cases of mild infestations. Thus, quantifying the severity of PLH infestations in the vineyard prior to increasing vineyard management costs through pesticide applications seems to be warranted.

Although the results presented here provide data that can be used for making rational control decisions for PLH infestations, the vines used for these studies were potted vines that were grown in a greenhouse. It will be necessary to conduct similar experiments on field-grown vines in order to ensure that the same patterns occur in a vineyard situation. The vines used in these studies did not have fruit. Repeating these experiments on potted vines with varying levels of fruit in a greenhouse is also important in order to better understand how PLH infestations affect grapevine source/sink relations and to learn more about how grapevines respond to infestations by this insect.

Appendix I: Pictures of cages used during single-leaf studies (Chapter 2). A. side view, a quarter is shown for comparison; B. Top view, a quarter is shown for comparison; C. held in the open position, note: fabric on bottom half of cage only.

A. **B.** **C.**

Appendix II: Trend curve equations and R^2 values for LR curves and ACi curves.

Light response curves

Day 1 (prior to infestation)

Control	$y = -0.671 + 5.302(1.0 - e^{-0.0025x})$	adj. $R^2 = 0.99$
PLH	$y = -0.504 + 5.155(1.0 - e^{-0.0027x})$	adj. $R^2 = 0.98$

Day 3 (immediately after the infestation period ended)

Control	$y = -0.369 + 5.818(1.0 - e^{-0.0019x})$	adj. $R^2 = 0.98$
PLH	$y = -0.430 + 2.118(1.0 - e^{-0.0022x})$	adj. $R^2 = 0.91$

Day 10 (seven days after the infestation period ended)

Control	$y = -0.280 + 4.698(1.0 - e^{-0.0033x})$	adj. $R^2 = 0.98$
PLH	$y = -0.334 + 2.427(1.0 - e^{-0.0033x})$	adj. $R^2 = 0.97$

AC_i curves

Day 1 (prior to infestation)

Control	$y = -3.904 + 13.362(1.0 - e^{-0.0050x})$	adj. $R^2 = 0.98$
PLH	$y = -4.887 + 14.465(1.0 - e^{-0.0055x})$	adj. $R^2 = 0.99$

Day 3 (immediately after the infestation period ended)

Control	$y = -7.413 + 16.018(1.0 - e^{-0.0081x})$	adj. $R^2 = 0.97$
PLH	$y = -3.640 + 7.455(1.0 - e^{-0.0064x})$	adj. $R^2 = 0.88$

Day 10 (seven days after the infestation period ended)

Control	$y = -3.919 + 12.603(1.0 - e^{-0.0063x})$	adj. $R^2 = 0.96$
PLH	$y = -2.113 + 7.136(1.0 - e^{-0.0048x})$	adj. $R^2 = 0.96$