This is to certify that the
dissertation entitled

EFFECTS OF POWDERY MILDEW ON CARBON ASSIMILATION
OF POTTED CHARDONNAY GRAPEVINES.

presented by

WILLIAM ROGERS NAIL IV

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Horticulture

G. Stanley Howell
Major Professor's Signature

30 April 2003

Date

EFFECTS OF POWDERY MILDEW ON CARBON ASSIMILATION OF POTTED CHARDONNAY GRAPEVINES.

By

William Rogers Nail IV

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

2003

ABSTRACT

EFFECTS OF POWDERY MILDEW ON CARBON ASSIMILATION OF POTTED CHARDONNAY GRAPEVINES.

By

William Rogers Nail IV

Potted Chardonnay (*Vitis vinifera* L.) grapevines were inoculated with conidial suspensions of powdery mildew of grape (*Uncinula necator* (Schw.) Burr.) (GPM), and the effects of GPM infection were studied over two seasons. In Season 1, grapevines infected with GPM had reduced CO_2 assimilation rates (A) compared to noninfected vines. Vines inoculated prior to bloom (Early) showed declines in A throughout the growing season and had reduced fresh and dry weight at the end of the season compared to other treatments. Plants inoculated after the 5mm berry stage (Late) showed subsequent declines in A, with no significant reduction in fresh or dry matter compared to control vines. Leaves on both infected treatments senesced earlier than those of control vines. Reductions in A were correlated with reductions in stomatal conductivity (g_s) and transpiration (E), and increased internal CO_2 concentration (C_i). The effects were more pronounced in Season 2. Plants not destructively harvested in Season 1 were grown a second season in a greenhouse and had no GPM infection. Destructively harvested and partitioned plants after Season 2 that had been infected with GPM in season 1 showed reduced fresh and dry weights, shoot lengths, and estimated leaf area compared to control plants. The amounts of the reductions were related to the length of infection time in Season 1. Leaves of infected and noninfected plants were studied for the effects of varying light (PAR) and CO_2 concentrations. Infection by GPM reduced carboxylation efficiency (k), A, g_s, and C_i under ambient CO_2, A_{max} at >900ppm CO_2, stomatal

limitations to A (l_g), and photochemical efficiency (φ), while having no effect on the CO_2 compensation point (Γ) or the light compensation point (cp). Infection by GPM had no effect on chlorophyll fluorescence (F_v/F_m).

DEDICATION

To all the artists, especially musicians, who have provided perspective for my scientific endeavors. Thanks!

ACKNOWLEDGMENTS

Thanks to all the people at Michigan State who have made my time here so rewarding: Stan Howell, for his mentorship and friendship; George Bird, Jim Flore, Annemiek Schilder, and Bob Schutzki, for their help as committee members; and Gerry Adams, who was originally a committee member and who taught a great course in mycology. I appreciate the help and friendship of all the people who have been in the Viticulture and Enology program here: Dan, Leah, Jon, Marcel, Kasey, Melissa, Todd, David, and, especially, Jen and Hongying, with whom I spent so much time in the office and in the field; and Erik Brasher and Stephanie Jones, who helped me so much with my field experiments. Other friends, many of whom have gone on to bigger and better things, include Dave and Sandy, Sarah and Mark, Andy and Carmen, Russell, and Erik Runkle. Thanks to Adriana, Costanza, Marlene, Leo, and Rita, for their help and advice with my research, and Paul, Adam, and Mike, who spent time with us while far away from their homes in New Zealand. And special thanks to Carol, for her love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Key to Symbols and Abbreviations

A	CO_2 assimilation
A_{360}	CO_2 assimilation at ambient CO_2
A_{max}	CO_2 assimilation at $\geq$900 ppm CO_2
C_i	Internal CO_2 concentration
C_{i360}	Internal CO_2 concentration at ambient CO_2
cp	Light compensation point
E	Transpiration
FEL	The most recently fully expanded leaf on a shoot at time of measurement
F_m	Maximum fluorescence
F_v	Variable fluorescence
GDD	Growing degree days
GPM	Powdery mildew of grape
g_s	Stomatal conductance
g_{s360}	Stomatal conductance at ambient CO_2
k	Carboxylation efficiency
l_g	Stomatal limitations to CO_2 assimilation
ORFEL	Original most recently fully expanded leaf at bloom (=FEL at bloom)
PAR	Photosynthetically active radiation
Pn	Net photosynthesis
Γ	CO_2 compensation point
Φ	Photochemical efficiency

Literature Review

The grapevine is one of the oldest cultivated crops in human history. Culture of the grapevine probably originated in Asia Minor (Winkler et al. 1974) which is also the presumed origin of *Vitis vinifera* (L.), the most widely cultivated grape species in the world (ibid.). Other *Vitis* species flourish in many other parts of the world as wild and/or cultivated species, especially in the Americas (Hedrick 1908; Munson 1909; Perold 1927). Human movement has resulted in the spreading of grapevine species all over the world. Most of the spread of grapevine species has been to introduce *V. vinifera* into non-native regions, although small amounts of American species were imported to Europe in the nineteenth century as museum specimens (Mullins et al. 1992) or by horticultural hobbyists (Pearson and Gadoury 1992). These importations resulted in widespread epidemics of disease and arthropod infestation, as *V. vinifera* species were susceptible to damage by many organisms to which native American species were resistant. The most famous of these is phylloxera (*Daktulospharia vitifoliae* Fitch), a root-feeding arthropod, which almost caused the destruction of European viticulture in the mid-nineteenth century. Powdery mildew of grape (GPM), caused by the fungus *Uncinula necator* (Schw.) Burr., was also presumably introduced into Europe at this time. The fungus was first described in North America in 1834 by Schweinitz, and its anamorph was first described in England as *Oidium tuckeri* in 1847 (Pearson and Gadoury 1992). By 1850, GPM had caused crop losses in most of the major grape-growing regions of Europe (Bulit and Lafon 1978), and is today the most widespread and destructive disease of grapevines worldwide (Pearson and Gadoury 1992). It is also the most widespread pest problem in California vineyards (Sall and Teviotdale 1981).

Uncinula necator is an obligate parasite that can infect all green tissues of the grapevine (Bulit and Lafon 1978; Sall and Teviotdale 1981). There are two sources of inoculum. The most common source is conidia produced on the surface of infected tissues (Pearson and Gadoury 1992). These conidia can be produced throughout the growing season, and are responsible for the "powdery" appearance of infected tissues. Ascospores produced in cleistothecia form the other source of inoculum (Pearson and Gadoury 1987, 1992; Pearson and Goheen 1988). These sexual spores are generally released early in the growing season. The fungus can overwinter as cleistothecia and/or by perennation as mycelia in dormant buds (Pearson and Gärtel 1985; Pearson and Goheen 1988; Sall and Wryzinski 1982; Ypema and Gubler 2000). Infected shoots arising from the latter are commonly called "flag shoots".

Powdery mildew of grape has long been known to result in inferior fruit quality (Gadoury et al. 2001; Ough and Berg 1979; Pool et al. 1984). Early season fruit infection may result in decreased fruit set, and may cause berry splitting and tissue scarring (Chellemi and Marois 1992). Infected fruit is unsuitable for fresh market use and may be unsuitable for the production of high quality wine (Ough and Berg 1979; Pool et al. 1984). Infection of fruit by *U. necator* may also predispose berries to secondary infection by *Botrytis cinerea* Pers. and spoilage microorganisms (Ficke et al. 2002).

Grapevine species and cultivars differ in their susceptibility to GPM. The disease is believed to be native to North America, as that is where it was first described, and most native American grapevine species are relatively resistant, while Eurasiatic species such as *V. vinifera*, *V. betulifolia* Diels & Gilg., *V. pubescens* Schltdl., *V. davidii* (Carr.)Foex., and *V. piasezkii* Maxim. are highly susceptible (Pearson and Gadoury 1992). Cultivars within a species may also show differences in susceptibility (Doster and Schnathorst 1985; Pearson and Gadoury 1992).

Grapevine berries (Ficke et al. 2002; Gadoury et al. 2001) and leaves (Doster and Schnathorst 1985) have demonstrated ontogenic resistance to GPM infection, although rachises have a more protracted period of susceptibility (Gadoury et al. 2001). Therefore it is possible that infections later in the season would be less severe. Berries of *V. vinifera* cultivars showed resistance to infection three weeks after bloom (Ficke et al. 2002), while Concord berries became mostly resistant to infection within two weeks after fruit set (Gadoury et al. 2001). The youngest leaves on individual shoots showed increased conidial germination rates compared to leaves two and four nodes proximal to the youngest leaf.

Powdery mildews and gas exchange in plants

Powdery mildews constitute a diverse group of ascomycotal fungi. All are genus-specific obligate parasites of their host plants, and may affect plant growth by reducing photosynthesis (Pn), increasing respiration and/or transpiration, with subsequent growth impairment and reduced yields (Agrios 1997). There is relatively little scientific literature quantifying the specific effects of powdery mildew infection on carbon assimilation in plants. Powdery mildews have been shown to reduce net CO_2 assimilation (*A*) in apple (Ellis et al. 1981), pecan (Gottwald and Wood 1984), barley (Hibberd et al. 1996; Holloway et al. 1992; Williams and Ayers 1981), pepper (Shtienberg 1992), *Prunus* spp. (Layne and Flore 1995), sour cherry (Layne and Flore 1992), winter wheat (Rabbinge et al. 1985; Shtienberg 1992), pea (Ayers 1981) and sugar beet (Magyarosy et al. 1976), as well as grape (Lakso 1982; Shtienberg 1992). Studies of specific effects of powdery mildews on host plant *A* showed that powdery mildew of barley (*Blumeria* (syn. *Erysiphe*) *graminis* D.C. ex Merat f.sp. *hordei* Marchal) resulted in decreases in chlorophyll content after four days of infection and loss of electron transport activity, with no loss of electron carrier concentration in remaining chlorophyll (Holloway et al. 1992). Powdery mildew of sugar beet (*Erysiphe polygoni* DC) inhibited electron transport in noncyclic proteins, accompanied by alterations in chloroplast ultrastructure and reduction of enzyme activity (Magyrarosy et al. 1976). Carboxylation resistance increased in winter wheat infected by powdery mildew (*Blumeria* (syn. *Erysiphe*) *graminis* D.C. ex Merat f.sp. *tritici*), with consequent negative effects on stomatal resistance, boundary layer resistance, and transport resistance (Rabbinge et al. 1985.)

Powdery mildew of grape and gas exchange

Grapevine leaves infected with GPM have shown declines in net Pn (Lakso et al. 1982). Infected vines have demonstrated negative growth patterns, compared to noninfected vines, consistent with reduction in Pn, both in the susceptible hybrid variety Rosette (Seibel 1000) (Pool et al. 1984), and the relatively resistant variety Concord (Gadoury and Seem 2001). Inhibition of Pn can be detrimental to plant health, as ≥90% of plant dry matter is derived from C fixed through Pn (Flore and Lakso 1989).

Reduction in functional leaf area, whether from physical damage (lacerations due to wind, rain, hail, etc.), arthropod predation, infection by pathogens, or deliberate leaf removal as a cultural practice, may negatively affect plant carbon assimilation. Such reductions operate by simply reducing the photosynthetically active leaf area of a plant, and do not alter any specific biochemical pathways as, for instance, herbicide-induced *A* reduction might cause. Experiments attempting to approximate arthropod damage on a single-leaf or whole-plant basis by removing portions of leaves, usually with a paper punch, have been largely successful in mimicking *A* reduction caused by predation (Boucher et al. 1987; Layne and Flore 1992; Poston et al. 1976), although care must be taken to ensure that hole punching position with respect to the midrib be consistent with typical arthropod feeding behavior (Layne and Flore 1992; Poston et al. 1976).

Many plants have demonstrated photosynthetic compensation for loss of functional leaf area. Photosynthetic compensation has been demonstrated for apple (Flore and Irwin 1983; Hall and Ferree 1976), bean (von Caemmerer and Farquhar 1984), lucerne (Hodgkinson 1974), mulberry (Satoh et al. 1977), and soybean (Proctor et al. 1982), as well as grape (Boucher et al. 1987; Candolfi-Vasconcelos and Koblet 1991; Hofäcker 1978; Intrieri et al. 1997; Petrie et al. 2000). Photosynthetic compensation has also been demonstrated in the case of powdery mildew infection of pea (Ayers 1981). Therefore, reductions in functional leaf area may not reflect actual reductions in total plant *A*. The proposed mechanism for photosynthetic compensation is through feedback inhibition caused by carbohydrate buildup in vines which are not source-limited (Layne and Flore 1995; Petrie et al. 2000), implying that leaves of non-source-limited plants typically operate at less than their optimum photosynthetic rate (Edson et al. 1993; Edson et al. 1995; Petrie et al. 2000). It is also possible that grapevines may compensate for reductions in functional leaf area by the production of new leaves, especially on lateral shoots (Koblet et al. 1994).

Estimating photosynthesis

Photosynthesis has most commonly been estimated by measuring gas exchange parameters on a section of an individual leaf. Advances in technology have made measurement of whole-plant Pn more practical (Garcia et al. 1990; Intrieri et al. 1998; Miller et al 1996; Peña and Tarara 2002; Poni et al. 1997; Wünsche and Palmer 1997). Measurement of Pn of individual leaves may not be an accurate measure of whole-vine Pn (Edson et al. 1995; Miller et al. 1996). Edson et al. (1995) found that Pn on the most recently fully expanded leaf on a shoot was more highly correlated with whole-vine Pn than measurements taken at other leaf positions; however, the relationship was quite variable (r^2=0.59, p=0.003). Single leaf Pn was correlated with whole vine Pn early in the season in another experiment, but the relationship was weaker later in the season as the canopy density increased (Miller et al. 1996).

The experiments conducted to determine the effects of GPM on *A* in potted Chardonnay grapevines are described in three chapters. The experiments in the first chapter were designed to test the hypotheses that foliar infection by GPM inhibits single leaf and whole plant *A*, and that photosynthetic compensation for reduction in *A* may occur. The experiments in the second chapter were designed to test the hypotheses that grapevines vary in susceptibility to GPM infection at different phenophases, that effects of GPM infection may be cumulative over a growing season, and that reductions in *A* as a result of GPM infection may have consequences in subsequent growing seasons. The experiments in the third chapter were designed to determine the mechanisms by with GPM might affect *A* in grapevines.

Literature Cited

Agrios, G.N. 1997. Plant Pathology. Academic Press, San Diego, CA.

Ayers, P.G. 1981. Powdery mildew stimulates photosynthesis in uninfected leaves of pea plants. Phytopathologische Zeitschrift 100:312-318.

Boucher, T.J., D. G. Pfeiffer, J.A. Barden, and J.M. Williams. 1987. Effects of simulated insect injury on net photosynthesis of potted grapevines. HortScience 22:927-928.

Bulit, J. amd R. Lafon. 1978. Powdery mildew of the vine. *In* The Powdery Mildews. D.M. Spencer (Ed.), pp. 525-548. Academic Press, New York.

Candolfi-Vasconcelos, M.C. and W. Koblet. 1991. Influence of partial defoliation on gas exchange parameters and chlorophyll content of field-grown grapevines. Mechanisms and limitations of the compensation capacity. Vitis 30:129-141.

Chellemi, D.O. and J.J. Marois. 1992. Influence of leaf removal, fungicide applications, and fruit maturity on incidence and severity of grape powdery mildew. Am. J. Enol. Vitic. 43:53-57.

Doster, M.A. and W.C. Schnathorst. 1985. Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus *Uncinula necator*. Am. J. Enol. Vitic. 36:101-104.

Edson, C.E., G.S. Howell, and J.A. Flore. 1993. Influence of crop load on photosynthesis and dry matter partitioning of Seyval grapevines. I. Single leaf and whole vine response pre- and post-harvest. Am. J. Enol. Vitic. 44:139-147.

Edson, C.E., G.S. Howell, and J.A. Flore. 1995. Influence of crop load on photosynthesis and dry matter partitioning of Seyval grapevines. II. Seasonal changes in single leaf and whole vine photosynthesis. Am. J. Enol. Vitic. 46:469-477.

Ellis, M.A., D.C. Ferree, and D.E. Spring. 1981. Photosynthesis, transpiration, and carbohydrate content of apple leaves infected by *Podosphaera leucotricha*. Phytopathology 71:392-395.

Ficke, A., D.M. Gadoury, and R.C. Seem. 2002. Ontogenic resistance and plant disease management: A case study of grape powdery mildew. Phytopathology 92:671-675.

Flore, J.A. and C. Irwin. 1983. The influence of defoliation and leaf injury on leaf photosynthetic rate, diffusive resistance, and whole tree dry matter accumulation in apple. HortScience 18:72 (abstr.).

Flore, J.A. and A.N. Lakso. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *In* Horticultural Reviews Vol. II. J. Janick (Ed.), pp. 111-157. Timber Press, Portland, OR.

Gadoury, D.M., R.C. Seem, R.C. Pearson, W.F. Wilcox. 2001. Effects of powdery mildew on vine growth, yield, and quality of Concord grapes. Plant Dis. 85:137-140.

Garcia, R.L., J.M. Norman, and D.K. McDermitt. 1990. Measurements of canopy gas exchange using an open chamber system. Remote Sensing Rev. 5(1):141-162.

Gottwald, T.R. and B.W. Wood. 1984. Effect of powdery mildew on net photosynthesis, dark respiration, and kernel composition of pecan. Plant Dis. 86:519-521.

Hall, F.R. and D.C. Ferree. 1976. Effects of insect injury simulation on photosynthesis of apple leaves. J. Econ. Entomol. 69:245-248.

Hedrick, U.P. 1908. The Grapes of New York. J.B. Lyon Co., Albany, NY.

Hibbert, J.M., P. Richardson, R. Whitbread, and J.F. Farrar. Effects of leaf age, basal meristem and infection with powdery mildew on photosynthis in barley grown in 700 micromoles·mol^{-1} CO_2. New Phytol. 134:317-325.

Hodgkinson, K.C. 1974. Influence of partial defoliation on photosynthesis, photorespiration and transpiration by lucerne leaves of different ages. Austral. J. Plant. Physiol. 1:561-578.

Hofäcker, W. 1978. Investigations on the photosynthesis of vines. Influence of defoliation, topping, girdling and removal of the grapes. Vitis 17:10-22.

Holloway, P.J., D.J. Maclean, and K.J. Scott. 1992. Electron transport in thylakoids isolated from barley leaves infected by the powdery mildew fungus (*Erysiphe graminis* DC. Ex Merat f.sp. *hordei* Marchal). New Phytol. 120:145-151.

Intrieri, C., S. Poni, B. Rebucci, and E. Magnanini. 1997. Effects of canopy manipulations on whole-vine photosynthesis: Results from pot and field experiments. Vitis 36:167-173.

Intrieri, C., S. Poni, B. Rebucci, and E. Magnanini. 1998. Row orientation effects on whole-canopy gas exchange of potted and field-grown grapevines. Vitis 37:147-154.

Janoudi, A.K., I.E. Widders, and J.A. Flore. 1993. Water deficits and environmental factors affect photosynthesis in leaves of cucumber (*Cucumis sativus*). J. Amer. Soc. Hort. Sci. 118:366-370.

Koblet, W., M. C. Candolfi-Vasconcelos, W. Zweifel, and G.S. Howell. 1994. Influence of leaf removal, rootstock, and training system on yield and fruit composition of Pinot noir grapevines. Am. J. Enol. Vitic. 45:181-187.

Lakso, A.M., C. Pratt, R.C. Pearson, R.M. Pool, and M.J. Welser. 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (powdery mildew). Phytopathology 72:232-236.

Layne, D.R. and J.A. Flore. 1992. Photosynthetic compensation to partial leaf area reduction in sour cherry. J. Amer. Soc. Hort. Sci. 117:279-286.

Layne, D.R. and J.A. Flore. 1995. End-product inhibition of photosynthesis in *Prunus cerasus* L. in response to whole-plant source-sink manipulation. J. Amer. Soc. Hort. Sci. 120:583-599.

Magyarosy, A.C., P. Schürmann, and B.B. Buchanan. 1976. Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. Plant Physiol. 57:486-489.

Miller, D.P., G.S. Howell, and J.A. Flore. 1996. A whole-plant, open, gas-exchange system for measuring net photosynthesis of potted woody plants. HortScience 31:944-946.

Mullins, M.G., A. Bouquet, and L.E.Williams. 1992. Biology of the Grapevine. Cambridge University Press, Cambridge.

Munson, T.V. 1909. Foundations of American Grape Culture. T.V. Munson & Son, Denison, TX.

Ough, C.S. and H.W. Berg. 1979. Powdery mildew sensory effect on wine. Am. J. Enol. Vitic. 30:321.

Pearson, R.C. and Gadoury, D.M. 1987. Cleistothecia, the source of primary inoculum for powdery mildew in New York. Phytopathology 77:1509-1514.

Pearson, R.C. and Gadoury, D.M. 1992. Grape powdery mildew. *In* Plant Diseases of International Importance. Vol. III, Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh, and A.N. Mukhopadhyay (Eds.), pp. 129-146. Prentice Hall, Englewood Cliffs, NJ.

Pearson, R.C. and Gärtel. 1985. Occurrence of hyphae of *Uncinula necator* in buds of grapevine. Plant Dis. 69:149-151.

Pearson, R.C. and A.C. Goheen. 1988. Compendium of Grape Diseases, APS Press St. Paul, MN.

Peña, J.P. and J. Tarara. 2002. Chamber for measuring whole vine photosynthesis in the vineyard. Abstr. ASEV 53rd Annu. Meet. Am. J. Enol. Vitic. 53:65.

Perold, A.I. 1927. A Treatise on Viticulture. Macmillan and Co., Ltd, London.

Petrie, P.R., M.C.T. Trought, and G.S. Howell. 2000. Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. Vitis 39:31-36.

Poni, S., E. Magnanini, and B. Rebucci. 1997. An automated chamber system for measurements of whole-vine gas exchange. HortScience 32:64-67.

Pool. R.M., R.C. Pearson, M.J. Welser, A.N. Lakso, and R.C. Seem. 1984. Influence of powdery mildew on yield and growth of Rosette grapevines. Plant Dis. 68:590-593.

Poston, F.L., L.P. Pedigo, R.B. Pearce, and R.B. Hammond. 1976. Effects of artificial and insect defoliation on soybean net photosynthesis. J. Econ. Entomol. 69:109-112.

Proctor, J.T.A., J.M. Bodnar, W.J. Blackburn, and R. L. Wilson. 1982. Analysis of the effects of the spotted tentiform leaf miner (*Phyllonorycter blancardella*) on the photosynthetic characteristics of apple leaves. Can. J. Bot. 60:2734-2740.

Rabbinge, R., I.T.M. Jorritsma, and J. Schans. 1985. Damage components of powdery mildew in winter wheat. Neth. J. Plant Pathol. 91:235-247.

Sall, M.M. and B.L. Teviotdale. 1981. Powdery mildew. *In* Grape Pest Management (D.L. Flaherty, F.L. Jensen, A.N. Kasimatis, H. Kido, and W.J. Moller (Eds.) pp. 46-50. Univ. Calif Publ. 4105.

Sall, M.A. and Wryzinski. 1982. Perennation of powdery mildew in buds of grapevines. Plant Dis. 66:678-679.

Satoh, M., P.E. Kriedemann, and B.R. Loveys. 1977. Changes in photosynthetic activity and related processes following decapitation in mulberry trees. Physiol. Plant. 41:203-210.

Shtienberg, D. 1992. Effects of foliar diseases on gas exchange processes: A comparative study. Phytopathology 82:760-765.

von Caemmerer, S. and G.D. Farquhar. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced p(CO_2) on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. Planta 160:320-329.

Williams, G.M. and P. G. Ayers. 1981. Effects of powdery mildew and water stress on CO_2 exchange in uninfected leaves of barley. Plant Physiol. 68:527-530.

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. 1974. General Viticulture. University of California Press, Berkeley, CA.

Wünsche, J.N. and J.W. Palmer. 1997. Portable through-flow cuvette system for measuring whole-canopy gas exchange of apple trees in the field. HortScience 32:653-658.

Ypema, H.L. and W.D. Gubler. 2000. The distribution of early season grapevine shoots infected by *Uncinula necator* from year to year: A case study in two California vineyards. Am. J. Enol. Vitic. 51:1-6.

Chapter 1

Effects of Powdery Mildew Infection on Carbon Assimilation of Potted Chardonnay (*Vitis vinifera* L.) Grapevines

ABSTRACT

Potted Chardonnay (*Vitis vinifera* L.) grapevines were inoculated with conidial suspensions of powdery mildew of grape (*Uncinula necator* (Schw.) Burr.) (GPM), and the effects of GPM on infection on CO_2 assimilation (A) were studied over two seasons. Vines infected with GPM had reduced single leaf and whole vine A compared to noninfected plants in both years. Reductions in A were correlated with reductions in stomatal conductivity (g_s) and transpiration (E), and increased internal CO_2 concentration (C_i). The effects were more pronounced in Season 2. Leaves on infected vines senesced earlier than those on noninfected vines. Infected vines had reduced fresh and dry weights at the end of the season compared to noninfected vines.

Introduction

Biotic and abiotic stresses on plants frequently result in reductions in plant growth and productivity. Knowledge of specific plant physiological responses to stress, and combinations of stresses, is becoming increasingly important as integrated crop management systems are being developed and improved. Many interactions between plants and biotic stress factors are incompletely understood.

Foliar injury caused by biotic and abiotic factors can reduce the ability of a plant to assimilate CO_2. Powdery mildews, which are species-specific foliar fungal pathogens, have been associated with reductions in photosynthesis (Pn) and transpiration (E) in a variety of crops, including barley (Williams and Ayers 1981), apple (Ellis et al. 1981), and grape (Lakso et al. 1982, Shtienberg 1992). Powdery mildew of grape, caused by *Uncinula necator* (Schw.) Burr. (GPM) is the most widespread and destructive disease of grapevines worldwide (Pearson and Gadoury 1992), and has long been known to result in inferior fruit quality (Gadoury et al. 2001a; Ough and Berg 1979; Pool et al. 1984). Early-season fruit infection may result in decreased fruit set, and may cause berry splitting and tissue scarring (Chellemi and Marois 1992). Infected fruit is unsuitable for fresh market use and may be unsuitable for the production of high quality wine (Ough and Berg 1979; Pool et al. 1984).

GPM infections have been associated with reduced vine size (as determined by cane pruning weights) and yield in susceptible varieties (Pool et al. 1984), or only with vine size in relatively resistant varieties (Gadoury et al. 2001b). Infections have also been demonstrated to cause reductions in C metabolism, but not *E*, of individual leaves of susceptible grape species (Lakso et al. 1982). Reduction of net CO_2 assimilation (*A*) caused by GPM infection may be caused by a reduction in photosynthetically active leaf area, although Shtienberg (1992) found that visual assessments of foliar pathogen damage frequently underestimate a foliar pathogen's effect on gas exchange. Lakso et al. (1982) found that leaf necrosis associated with GPM infection was primarily associated with palisade layer destruction in infected grape leaves.

Reductions in CO_2 assimilation have been associated with delayed ripening and/or decreased yields. Many plants have demonstrated photosynthetic compensation for losses in functional leaf area (Boucher et al. 1987; Intrieri et al. 1997; Layne and Flore 1992; Poston et al. 1976; Proctor et al. 1982; van Caemmerer and Farquhar 1984). Defoliation experiments have sometimes been used to mimic functional leaf area reduction caused by biotic stresses. Results from several studies indicate that grapevines can compensate photosynthetically for some degree of leaf area loss (Candolfi-Vasconcelos and Koblet 1991; Hofäcker 1978; Intrieri et al. 1997), although, in another experiment, removal of entire leaves of grapevines (*Vitis vinifera* cv. Pinot noir) did not result in increased *A* in remaining leaves (Candolfi-Vasconcelos et al. 1994).

Grapevine species and cultivars have demonstrated variable susceptibility to GPM (Doster and Schnathorst 1985). Only members of the Vitaceae are susceptible to GPM (Pearson and Goheen 1988); however, this includes almost all of the economically important grapes in the world. The fungus is presumably native to North America (ibid.); consequently, *V. vinifera* L. species are relatively susceptible, while native American species, especially *V. labruscana* Bail., are considered relatively resistant, although they can also be negatively affected by GPM infection (Gadoury et al. 2001a; Gadoury et al. 2001b). There is also a large degree of within-species variability in susceptibility to GPM (Gut et al. 2002).

The goal of these experiments was to evaluate the effects of GPM on grapevine C status using single leaf and whole plant gas exchange measurements and its influence on seasonal C sequestration and partitioning.

Materials and Methods

Plant material. *Experiment 1.* Two-year-old dormant grapevines (*V. vinifera* cv. Chardonnay, Dijon clone 96 grafted to C. 3309 rootstock) were planted in 19L pots in a pasteurized medium of 45% sand, 45% loam, and 10% sand, and grown and maintained in a greenhouse on the campus of Michigan State University, East Lansing, MI, USA, during the spring of 2001. Minimum and maximum temperatures were maintained at

23°C and 32°C, respectively. Plants were thinned to two shoots per vine and defruited at bloom. Vines were watered regularly and fertilized at bloom and monthly thereafter with a soluble fertilizer at a rate of 0.38g N, 0.17g P, and 0.32g K per pot (Peter's 20-20-20).

Experiment 2. Two-year-old dormant grapevines (*V. vinifera* cv. Chardonnay, Dijon clone 96 grafted to C.3309 rootstock) were planted in 19L pots in a medium of 70% loam, 20% sand, and 10% peat, and grown and maintained on a gravel pad outdoors at the Horticultural Teaching and Research Center, Michigan State University, East Lansing, MI, USA during the 2001 and 2002 growing seasons. Plants were thinned shortly after full bud burst to three shoots per vine. Vines were watered regularly and fertilized monthly with Peter's 20-20-20 solution as above. Plants were largely fruitless; the fruit on a few plants, not used in the experiment, were retained to determine phenological stages during the growing season. Fruit was removed from all treatment plants prior to bloom. Laterals were removed as they appeared throughout the growing season. Two applications of Sevin (1-naphthyl N-methylcarbamate (carbaryl), Aventis, Bridgewater, NJ) liquid were made as needed to control Japanese beetle (*Popillia japonica* Newman) infestations. All applications were made at least seven days prior to gas exchange measurements.

Experimental design and treatments. *Experiment 1.* Eighteen plants were arranged in a completely randomized design and inoculated with a conidial suspension of *U. necator* (produced by soaking infected leaves of Marechal Foch (Kuhlmann 188-2) grapevines for $\approx$10min and agitating to dislodge conidia) when three leaves had appeared on most shoots. Each plant constituted an individual experimental unit.

Experiment 2. Plants were blocked according to fresh weight of the dormant, unpotted vines and arranged in a completely randomized block designs as follows: each block contained vines of similar initial fresh weight, and all phenological stages based on fruit development were determined based on observations of the fruited, non-experimental vines:

Year 1: Plants were arranged in six blocks, with seven subsamples per treatment randomly arranged within each block to allow for three sequential destructive harvests, each consisting of one plant per treatment per block. Four plants per treatment per block were not destructively harvested at the end of the season, and were retained for another experiment. Treatments were assigned randomly within blocks and were:

1. Plants inoculated with a conidial suspension of *U. necator* in distilled water as described above just prior to bloom (as determined from the non-treatment, fruited vines), using a hand sprayer and sprayed to runoff. This treatment was designated "Early".

2. Plants were sprayed with myclobutanil (α-butyl-α-(4-chlorophenyl)-1*H*-1,2,4, triazole-1-propanenitrile (NOVA), Rohm and Haas, Philadelphia, PA) at bloom and inoculated with a conidial suspension of *U. necator* as above between the 5mm berry stage and 1200 growing degree days (GDD) (base 50°F), which was 35 days after Early inoculation. This treatment was designated "Late".

3. Plants were protected from GPM infection with myclobutanil at bloom, between 5mm berry size and 1200GDD, and at veraison. This treatment was designated as "Control".

Year 2: Plants were arranged in 32 blocks with one vine of each treatment per block. Treatments were identical to those of Year 1, although there was very little inoculum available for imposing the Early treatment; Early plants were reinoculated along with the Late plants, and a single Late inoculation was assumed for analysis purposes.

Plants sprayed with myclobutanil were separated from inoculated plants by ≈10m for 48h to help eliminate the potential effects of drift and/or volatiles from affecting inoculated plants in both years.

Gas exchange measurements. Single leaf measurements were conducted using a portable infrared gas analyzer (IRGA) (CIRAS-2, PP Systems, Amesbury, MA) fitted with a leaf cuvette with light source (PLC6, ibid.). Measurements were taken between 900 and 1500hr at 1000 PAR and 27°C (±3°C).

Experiment 1. Single leaf measurements were taken on the most recent fully expanded leaf (FEL) on each shoot beginning 23 days post-inoculation, by which time symptoms of GPM were evident on many leaves, and thereafter at two-week intervals for the next 28 days. Prior to each leaf measurement, the leaf to be measured was evaluated for GPM disease severity, expressed as the percentage of the leaf area with visible GPM symptoms. Each leaf was measured twice, in case there was significant variability within the leaf, and the results were averaged.

Experiment 2. Single leaf measurements.

Year 1. Single leaf measurements were conducted at bloom, the 5mm berry stage, midseason (≈1200GDD), and ≈17 days post-veraison. At bloom, a representative shoot was selected on each plant, and the most recent FEL on that shoot was measured and marked. Subsequent measurements were conducted on the same, original, leaf (ORFEL), and also on the current most FEL on the same shoot. GPM infection severity was determined on each leaf prior to each gas exchange measurement, and expressed as the percentage of the leaf area which showed GPM symptoms. On leaves having ≥20% PM infection, two measurements per leaf were taken and the values averaged, in case infection caused significant variances across the leaf surface.

Year 2. Leaves were selected as in Year 1. Single leaf measurements were conducted at bloom, 5mm berry stage, midseason (≈1200GDD base 50°C), veraison, and harvest. Only one measurement was taken on each leaf, as data from Year 1 showed no significant differences between taking one or two measurements on infected leaves, as determined by analysis of variance.

Whole vine measurements. Whole vine gas exchange measurements were conducted using an open gas exchange system as described by Miller et al. (1996). Mylar M-30 film (polyethylene terephthalate, polyvinylidene chloride coated; DuPont, Wilmington, DE) was formed into a cylinder with a 4.0cm interior diameter (i.d.) piece of polyvinylchloride (PVC) pipe at the top, and attached to a wooden base with elastic ("bungee") cord. The wooden base had holes drilled into it to allow for the grape trunk (3.8cm diameter) and air inlet (4.0cm) to help minimize the effects of soil and root respiration on gas exchange measurements. The 3.8cm hole was further insulated with small strips of foam weather-strip material. Air was supplied using a small shaded pole blower fan (model 4C004, Dayton, Inc, Dayton, OH). The fan was attached to a section of 10.2cm i.d., 2.7m section of PVC pipe. The outlet end consisted of reduction and angled couplings just before the chamber inlet (Figure 1). A small piece of tape was loosely placed over the inlet to diffuse airflow entering the chamber. Airflow and temperature were measured with a thermal anemometer (Tri-Sense model 37000-60, Cole-Parmer, Chicago, IL). Airflow was measured through a hole drilled midway on the inlet pipe; measurements were taken at incremental depths of 2.5cm, and averaging the readings. Volume of air was calculated from the averaged flow measurements by the formula:

$$V = 0.51\left(\frac{\pi r^2 l}{10}\right) - 0.1 \text{ (Miller et al. 1996)}$$

where V=volume of air in L/s, r= radius of the air supply cylinder in cm, and l=the linear flow rate in m/s.

CO_2 measurements were performed using the CIRAS-2 unit as an IRGA only; inlet air was sampled first through a ≈1.3m section of flexible tubing, then the air at the outlet of the chamber. Three pairs of measurements were made, and the average of the values was used for calculating Pn. Whole vine CO_2 assimilation was calculated by the formula:

$$Pn(\mu mol / vine / s) = \left(\frac{((\Delta CO_2)/\mu L)((flow)L/\min)}{(29.2\mu L/\mu mol)(60s/\min)}\right) \text{ (ibid).}$$

Temperature inside and outside the chamber was measured prior to each series of Pn measurements, and airflow was adjusted to maintain the temperature difference inside the chamber to within 2°C of ambient temperature; if the airflow required adjustment to reduce the temperature difference, the chamber was allowed to reequilibrate prior to taking Pn measurements. Prior to enclosing plants in the Mylar chambers, GPM severity was visually determined and expressed as the percentage of plant leaf area showing disease symptoms. Measurements were taken on cloudless days between 900 and 1500h to help ensure uniformity of plant light interception.

Leaf area per vine was estimated by measuring shoot lengths on each measured vine. Shoot length in grapevines is correlated with leaf area (Miller et al. 1996). The relationship between shoot length and leaf area was determined by destructively harvesting 30 non-treatment vines between midseason and veraison and measuring shoot length and actual leaf area as determined by a belt-driven leaf area meter (LI-COR Model LI-3000, LI-3050ASH, LI-COR Inc., Lincoln, NE), and was determined to be ($y=12.01x^{0.9744}$, $r^2=0.916$).

Destructive harvests. *Year 1*. After the completion of each series of gas exchange measurements, one plant from each treatment per block was selected at random and destructively harvested. Plants were cut into component plant parts (roots, trunk, shoots, and leaves), and fresh weights were measured. These plant parts were dried in a forced-air drying oven at 45°C for ≥2 weeks, and dry weights were measured. Fresh shoot lengths were also measured.

Year 2. Plants from 24 blocks were harvested after veraison; shoot lengths and fresh and dry weights were measured as in Year 1.

Statistical analysis. Statistical analysis was performed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC). ANOVA mean separation was performed using Fisher's protected LSD. Regression p-values were obtained using linear regression.

Figure. 1. Whole vine photosynthesis chambers for measuring carbon assimilation on potted Chardonnay grapevines, showing Mylar chambers, blowers with ductwork, and CIRAS-2 infrared gas analyzer (after Miller et al. 1996).

Results

Experiment 1. Carbon assimilation was negatively correlated with disease severity at all three dates of measurement as determined by regression (Figure 2). The relationship was linear in Week 1, becoming more curvilinear in Weeks 3 and 5. Using combined data from all three series of measurements, there was little decrease in *A* from 0-20% GPM severity, and little apparent decrease in *A* with increasing GPM severity over ≈50%, which was confirmed by analysis of variance of the combined data (not shown).

Experiment 2. Year 1. GPM inoculation resulted in decreased single leaf *A* compared to control plants in Year 1 (Figure 3). *A* declined throughout the growing season on both FEL and ORFEL, but the effect was greater on the ORFEL. Differences between infected and noninfected FEL were significant at all measurement times, while differences between ORFEL were not significant after the midseason (pre-veraison) period. Stomatal conductance (g_s) also declined over time (Figure 4), but no significant trends were apparent. Differences in *E* were evident on ORFEL at the 5mm berry stage and post-veraison (Figure 5), in a pattern similar to that of g_s. There were no significant effects of infection on internal CO_2 concentration (C_i) in the experiment (data not shown).

There was no effect of GPM infection on whole vine *A* at the 5mm berry stage. At midseason, whole vine *A* decreased with increasing GPM severity (Figure 6). There were no statistically significant differences in fresh or dry weights at any individual destructive harvest date, probably due to the small sample size. Data from all three destructive harvests were combined; only leaf weights were significantly different among treatments, due to a high degree of senescence after veraison on infected vines (data not shown).

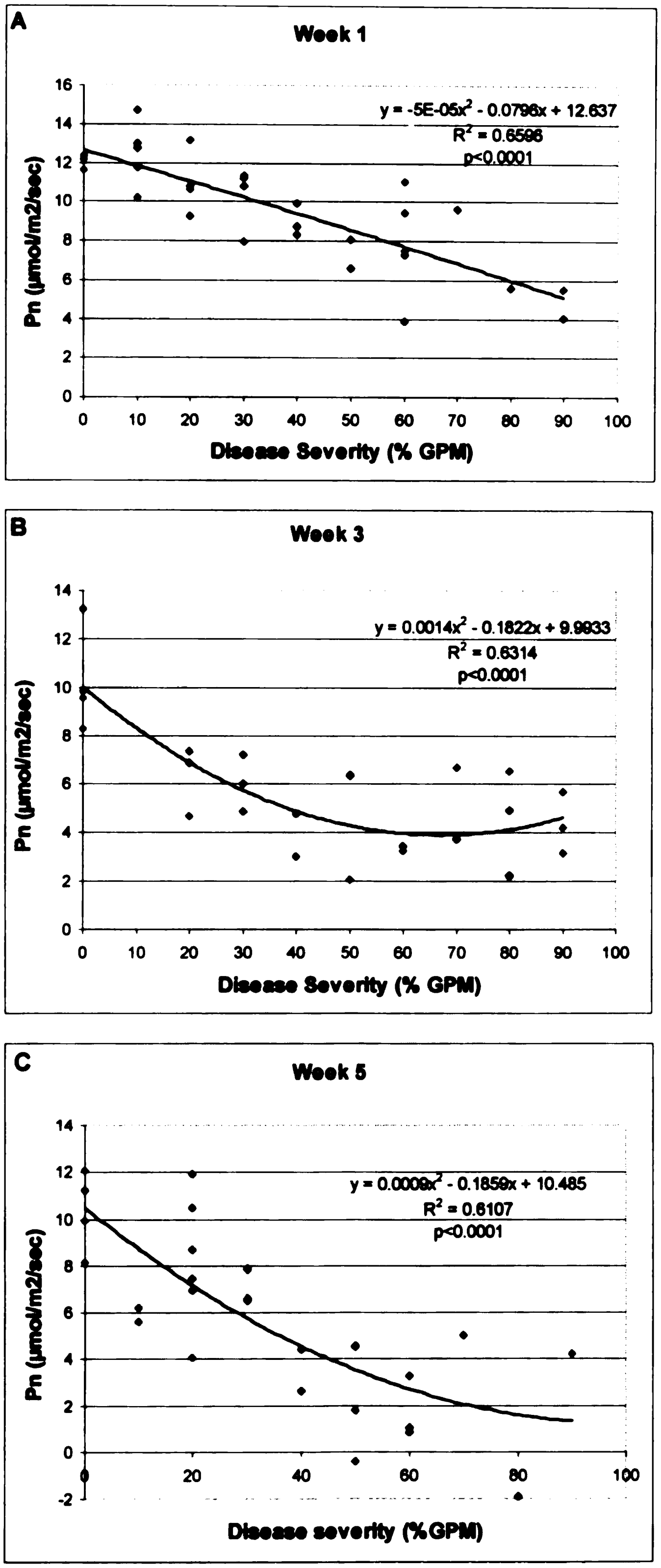


Figure 2. Impact of powdery mildew of grape (GPM) disease severity on single leaf photosynthesis (Pn) rates of greenhouse-grown Chardonnay grapevines. Week 1 measurements (A) were conducted 23 days after inoculation with *Uncinula necator*, and every 14 days thereafter (B and C).

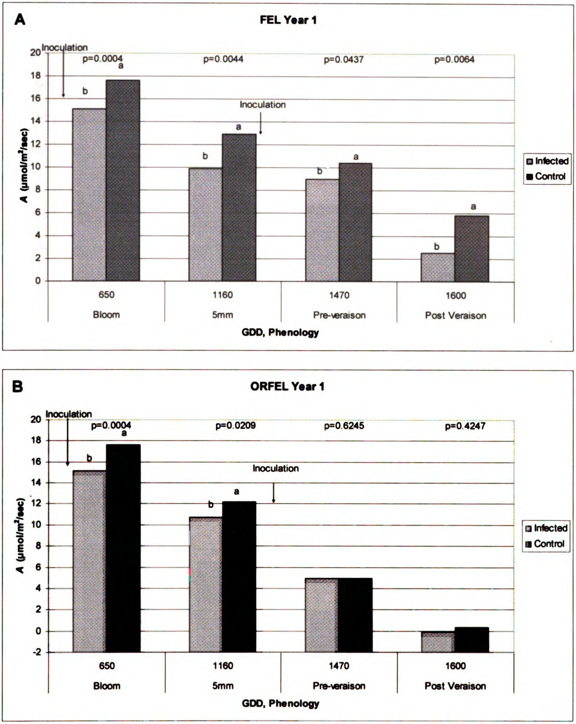
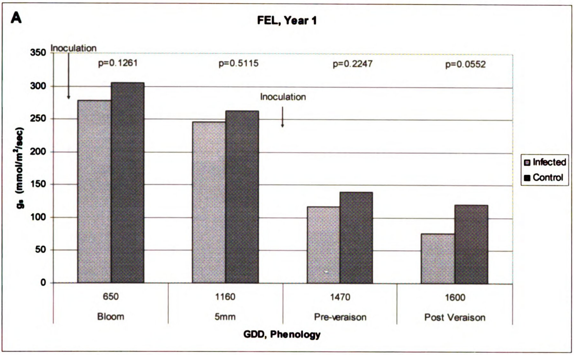


Figure 3. Effect of powdery mildew of grape infection on single leaf CO_2 assimilation (*A*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* twice during the growing season.



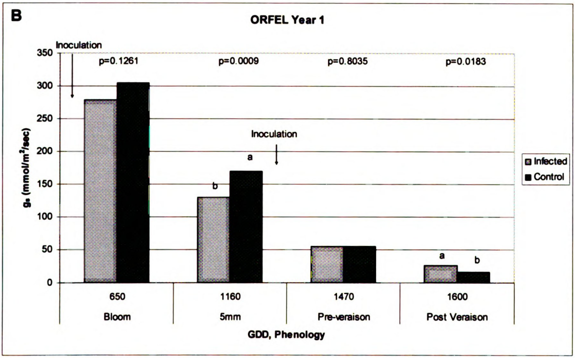
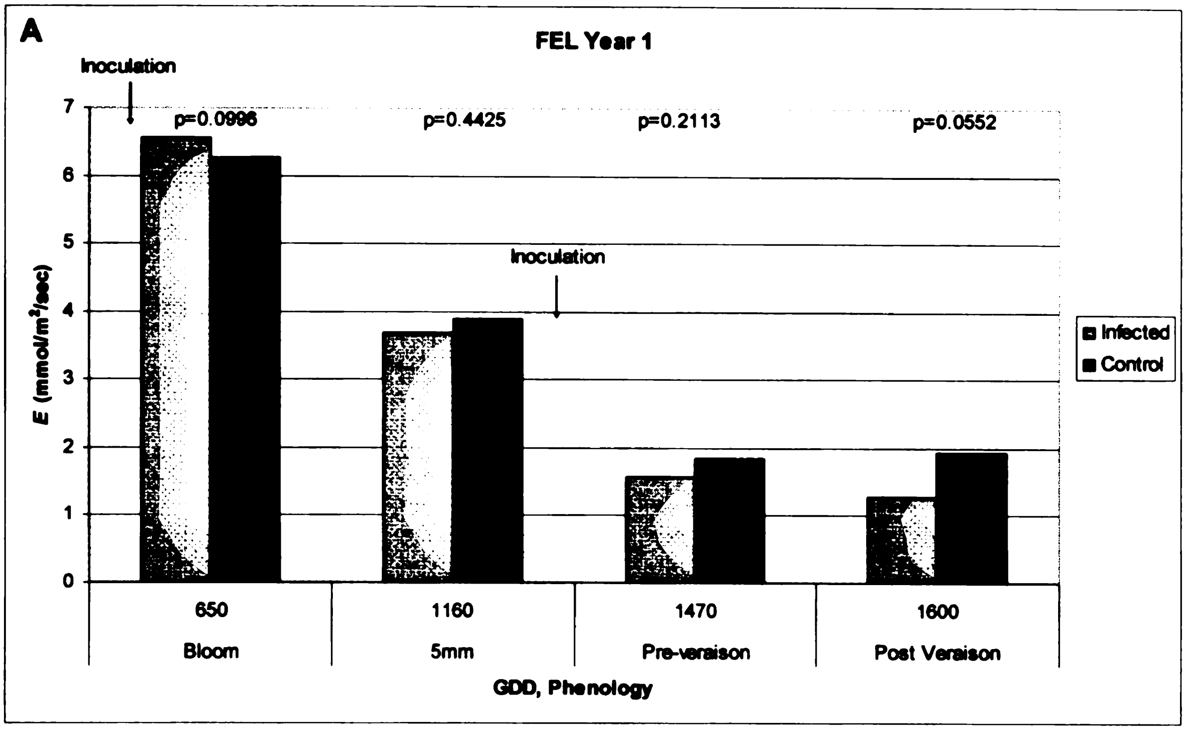


Figure 4. Effect of powdery mildew of grape infection on single leaf stomatal conductance (g_s) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* twice during the growing season.



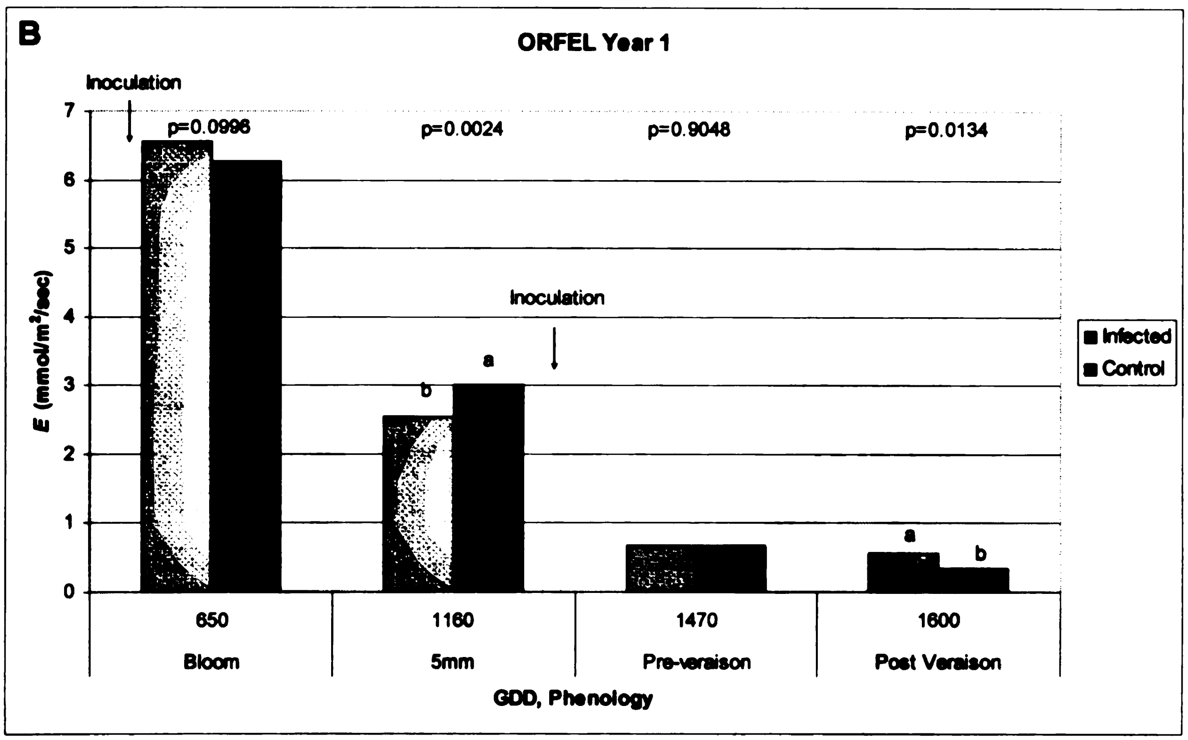


Figure 5. Effect of powdery mildew of grape infection on single leaf transpiration (*E*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* twice during the growing season.

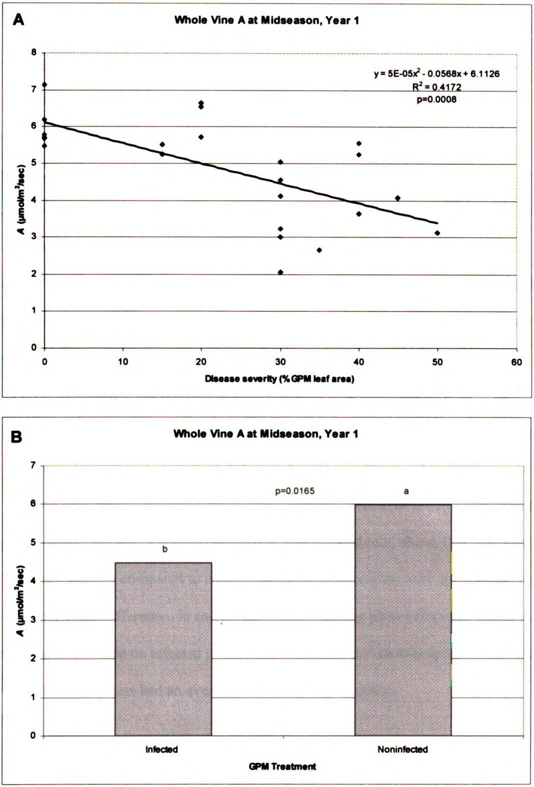


Figure 6. Impact of powdery mildew of grape (GPM) infection on whole vine CO_2 assimilation (*A*) of potted Chardonnay grapevines at midseason ($\approx$1200 growing degree days, base 50°F) (A and B).

Year 2. No infection resulted from the pre-bloom inoculation of *U. necator*; therefore, most leaves became infected at the same time, after being inoculated after the 5mm berry stage. No effects of GPM infection were detected at mid-season (16 days after inoculation) on single leaf measurements, although leaves had begun showing symptoms. Whole vine *A* was negatively affected by infection; infected vines had significantly lower *A* than noninfected vines (Figure 7), although the correlation between disease severity and *A* was not significant at the p=5% level (p=0.0766, r^2=0.1319). By veraison, all single leaf parameters were affected by GPM infection on both FEL and ORFEL (Figures 8-11). *A*, g_s, and *E* were reduced, while C_i was increased on infected vines. These relationships also existed at harvest. There was no statistically significant difference in whole vine *A* between infected and noninfected vinesat veraison at the $p \leq 5\%$ level (p=0.0890), nor correlation between disease severity and *A* (p=0.0863, r^2=0.0693). At harvest, there were differences between infected and noninfected vines, and there was a significant correlation between disease severity and *A* (Figures 7, 12).

Both total fresh weight and dry weight (biomass) were affected by GPM infection (Figures 13-14). All plants infected with GPM had reduced root, shoot, leaf, and total fresh and dry weights compared to control plants. Trunk weights were not affected by GPM. Most of the differences in carbon partitioning among plant parts were due to much greater leaf senescence on infected plants; infected plants had an average of 37 leaves, while noninfected plants had an average of 54 leaves (p<0.0001).

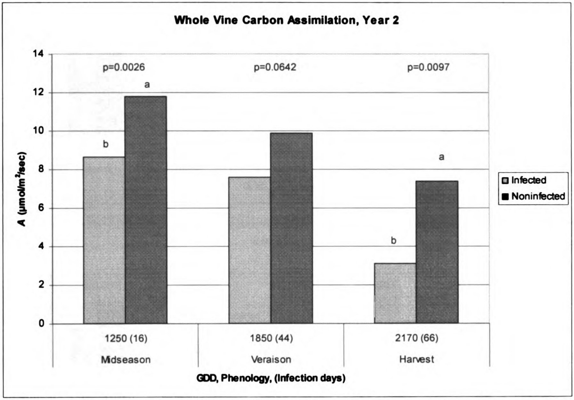


Figure 7. Effect of powdery mildew of grape (GPM) infection on whole-vine CO_2 assimilation (*A*) on potted Chardonnay grapevines as related to stages of vine growth phenology, growing degree days (GDD) (base 50°F), and days after inoculation (infection days) with a conidial suspension of *Uncinula necator*.

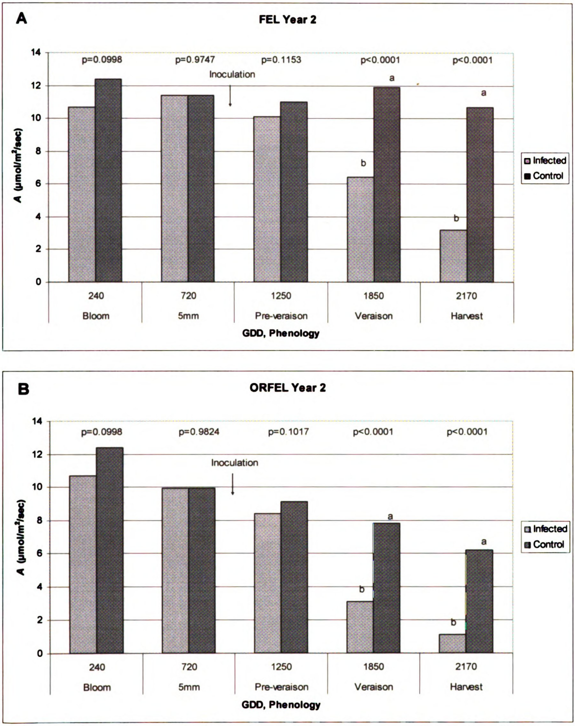


Figure 8. Effect of powdery mildew of grape infection on single leaf CO_2 assimilation (*A*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* once during the growing season.

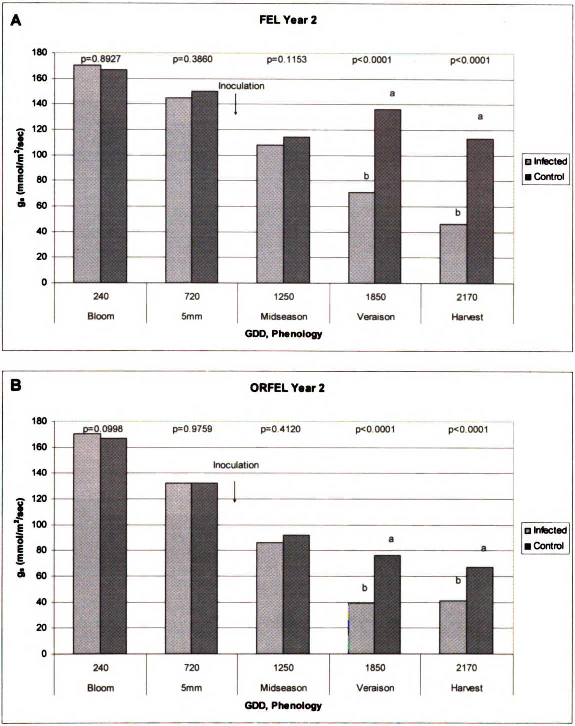
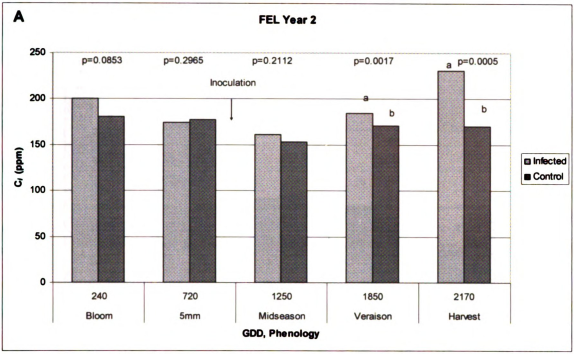


Figure 9. Effect of powdery mildew of grape infection on single leaf stomatal conductance (g_s) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* once during the growing season.



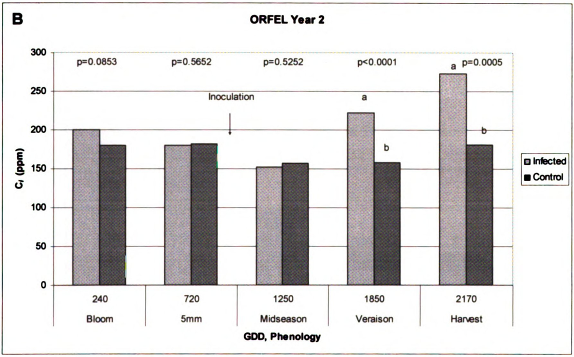


Figure 10. Effect of powdery mildew of grape infection on single leaf internal CO_2 concentration (C_i) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* once during the growing season.

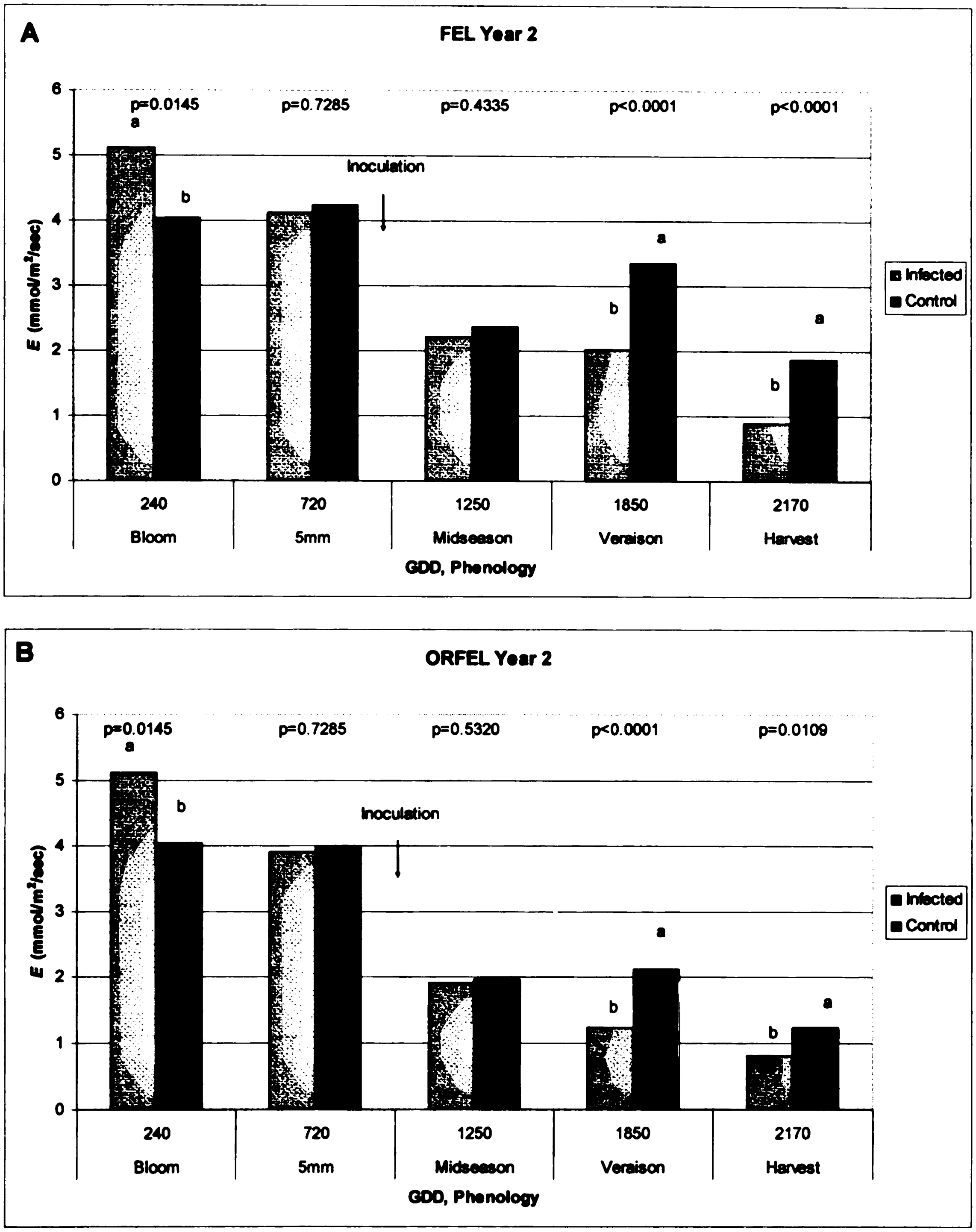


Figure 11. Effect of powdery mildew of grape infection on single leaf transpiration (*E*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). Vines were inoculated with *Uncinula necator* once during the growing season.

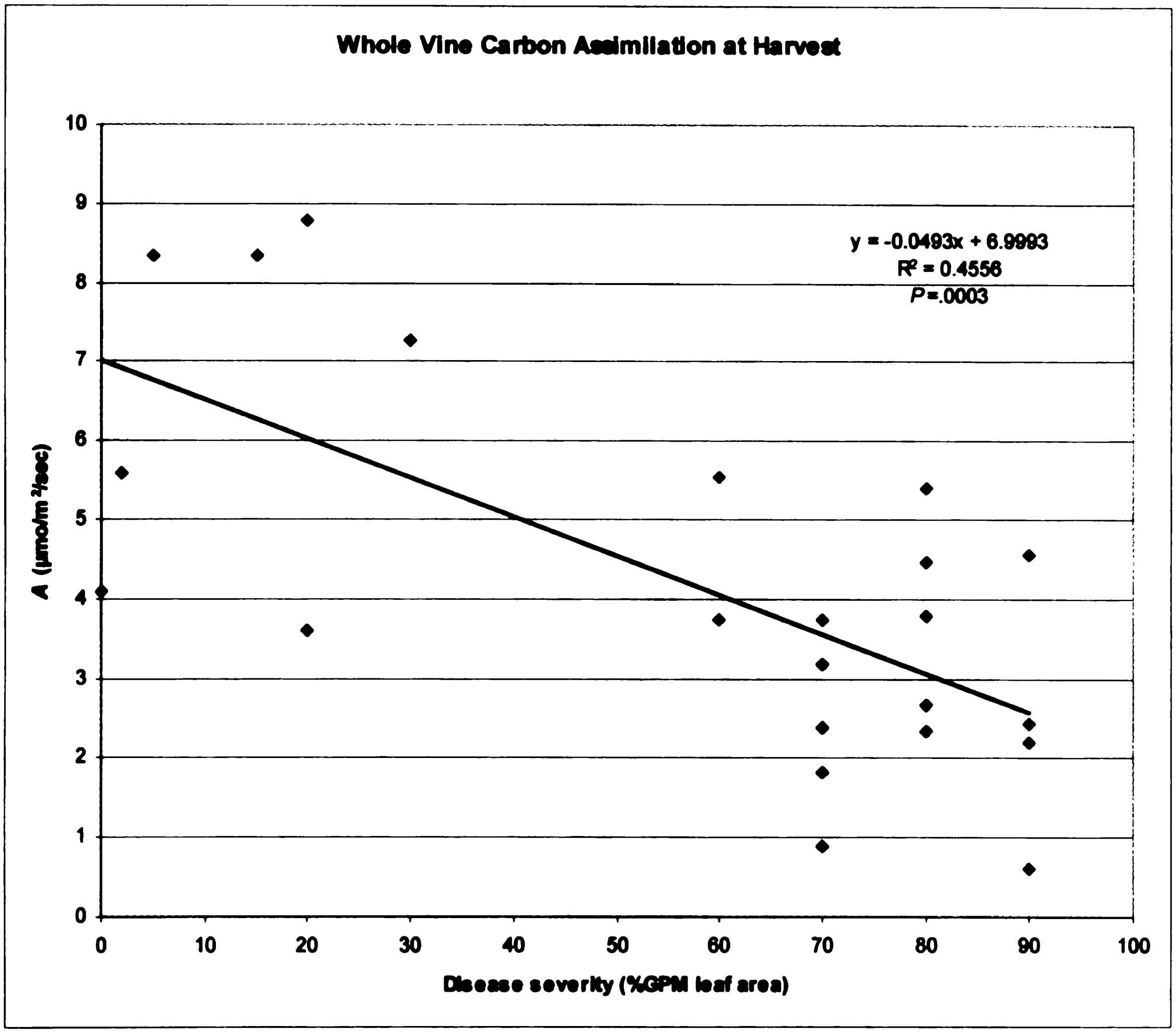


Figure 12. Effect of powdery mildew of grape (GPM) infection on whole-vine CO_2 assimilation (A) on potted Chardonnay grapevines at harvest ($\approx$2170 growing degree days, base 50°F).

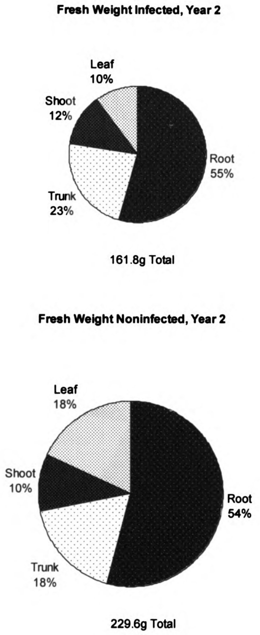


Figure 13. Effect of powdery mildew of grape infection on season-long CO_2 assimilation and carbon partitioning of potted Chardonnay grapevines.

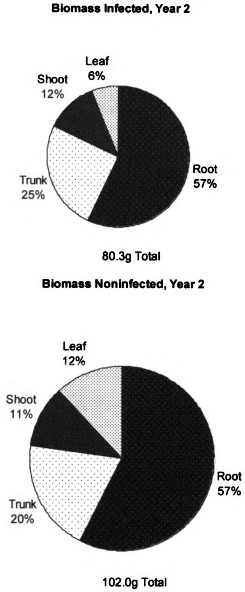


Figure 14. Effect of powdery mildew of grape infection on season-long CO_2 assimilation and carbon partitioning of potted Chardonnay grapevines.

Discussion

Infection with GPM negatively affected carbon assimilation whenever it was present in these experiments. The degree of damage as a consequence of disease severity was extremely variable at low (≤20%) GPM severity, among different single leaf measurements. Consequently, evidence of photosynthetic compensation for GPM infection was variable and inconclusive. Results from Experiment 1 suggest that infected leaves may tolerate up to 20% disease severity. Results from Experiment 2 sometimes showed tolerances to GPM between 0 and 20%, but at other times showed that any GPM infection may be detrimental to single leaf *A*. This was most apparent in the Year 1 single leaf assessment of *A* at bloom, when there was a significant decrease in *A* on inoculated leaves in the absence of GPM symptoms. At other times, there were no measured leaves with infection rates between 0 and 20%, so no valid inferences could be made on these degrees of disease severity. An approximate damage threshold level of 20% leaf damage is consistent with the estimated compensation levels for other perennial fruit crops (Flore and Irwin 1983; Layne and Flore 1992). Whole vine results suggest that grapevines may photosynthetically compensate on a whole plant level up to approximately a 20% infection level. If single leaf compensation does not occur, grapevines may compensate for GPM foliar damage through their ability to continue to produce new leaves and/or lateral shoots throughout the growing season.

The effect of GPM infection on g_s, C_i, and *E* was much more pronounced in Year 2. There are no obvious climatological explanations for this. Grapes grown in Year 1 were potted later in the season (mid-June, vs. mid-May in Year 2), but both growing seasons were fairly typical for mid-Michigan, and temperatures were never high or low enough to inhibit gas exchange until late in the season.

The effects of GPM on *E* in Year 2 indicated that water use efficiency was not compromised in leaves infected with GPM, since infected vines also had reduced *E* rates. These data are in agreement with Shtienberg (1992), who found decreases in *E* associated with GPM infection, but in contrast to the findings of Lakso et al. (1982), who found no reduction in *E* of grape leaves infected with GPM. However, there was a great degree of variability in their study on the susceptible *V. vinifera* cultivar White Riesling (r=0.11), whose susceptibility to GPM is similar to that of Chardonnay (Gut et al. 2002), while there was relatively little variability in the resistant variety Concord. Such variability in crops infected with powdery mildews has been widely reported by Shtienberg (1992). There was no perceptible pattern to the *E* differences observed in Year 1.

Plants showing reductions in *A* also showed reduced g_s, indicating that stomatal conductance is a barrier to carbon assimilation. This disagrees with Clearwater's findings on 'Riesling', in which the carbon assimilation mechanism had relatively little association with decreases in *A* associated with GPM (Clearwater et al. 2002). The association between reduced *A* and g_s was particularly strong after GPM caused declines in *A* in Year 2 (r^2=0.8655, p<0.0001), indicating a strong stomatal limitation to *A* in infected plants. Infected plants showing reduced *A* also had greater C_i in Year 2, further indicating that the carbon assimilation mechanism was compromised by GPM.

The reductions in *A* due to GPM infection resulted in decreased seasonal biomass accumulation. This may have implications for future seasons, as the amount of perennial wood on grapevines has been associated with subsequent vine growth, yield, and fruit quality (Koblet et al. 1994). This association was primarily concerned with above-ground wood (trunks), which, in the potted vine study, was the only plant part whose biomass was not significantly affected by GPM infection. This is probably due to the architecture of the potted vines, which have much less relative trunk area and weight than do mature, field-grown vines. Since all woody tissues of the plant contribute to carbohydrate storage during the dormant season, it is probable that the reduced root biomass as a consequence of GPM infection would also result in similar negative effects. Reduction in woody tissue biomass would also explain the gradual vine size declines on vines infected with GPM as reported by Gadoury et al. (2001a) and Pool et al. (1984).

These data suggest that GPM reduces *A* in infected grapevines, and that infected plants may be able to compensate for disease severities $\leq$20%. The observed reductions in *A* were probably a consequence of reductions in stomatal functions. Reductions in biomass accumulation as a result of decreased *A* in infected plants may have negative implications for future plant growth and development. Further research is needed to more accurately quantify photosynthetic compensation, so that damage thresholds can be established for vineyard management.

Literature Cited

Boucher, T.J., D.G. Pfeiffer, J.A. Barden, and J.M. Williams. 1987. Effects of simulated insect injury on net photosynthesis of potted grapevines. HortScience 22:927-928.

Candolfi-Vasconcelos, M.C. and W. Koblet. 1991. Influence of partial defoliation on gas exchange parameters and chlorophyll content of field-grown grapevines. Mechanisms and limitations of the compensation capacity. Vitis 30:129-141.

Candolfi-Vasconcelos, M.C., W. Koblet, G.S. Howell, and W. Zweifel. 1994. Influence of defoliation, rootstock, training system, and leaf position on gas exchange of Pinot noir grapevines. Am. J. Enol. Vitic. 45:173-180.

Chellemi, D.O. and J.J. Marois. 1992. Influence of leaf removal, fungicide applications, and fruit maturity on incidence and severity of grape powdery mildew. Am. J. Enol. Vitic. 43:53-57.

Clearwater, L., G.S. Howell, and M. Trought. 2002. Impact of powdery mildew infection and fungicide application on grapevine photosynthesis. Abstr. ASEV 53rd Annu. Meet. Am. J. Enol. Vitic. 53:26.

Doster, M.A. and W.C. Schnathorst. 1985. Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus *Uncinular necator*. Am. J. Enol. Vitic. 36:101-104.

Ellis, M.A., D.C. Ferree, and D.E. Spring. 1981. Photosynthesis, transpiration, and carbohydrate content of apple leaves infected by *Podosphaera leucotricha*. Phytopathology 71:392-395.

Flore, J.A. and C. Irwin. 1983. The influence of defoliation and leaf injury on leaf photosynthetic rate, diffusive resistance, and whole tree dry matter accumulation in apple. Abstr. HortScience 18:72.

Gadoury, D.M., R.C. Seem, R.C. Pearson, W.F. Wilcox. 2001a. Effects of powdery mildew on vine growth, yield, and quality of Concord grapes. Plant Dis. 85:137-140.

Gadoury, D.M., R. C. Seem, A. Ficke, and W.W. Wilcox. 2001b. The epidemiology of powdery mildew on Concord grapes. Phytopathology 91:948-955.

Gut, L.J., R. Isaacs, J.C. Wise, A.L. Jones, A.M.C. Schilder, B. Zandstra, and E. Hanson. 2002. 2003 Michigan Fruit Management Guide. Michigan State University Extension, East Lansing, MI.

Hofäcker, W. 1978. Investigations on the photosynthesis of vines. Influence of defoliation, topping, girdling and removal of the grapes. Vitis 17:10-22.

Intrieri, C., S. Poni, B. Rebucci, and E. Magnanini. 1997. Effects of canopy manipulations on whole-vine photosynthesis: Results from pot and field experiments. Vitis 36:167-173.

Koblet, W., M. C. Candolfi-Vasconcelos, W. Zweifel, and G.S. Howell. 1994. Influence of leaf removal, rootstock, and training system on yield and fruit composition of Pinot noir grapevines. Am. J. Enol. Vitic. 45:181-187.

Lakso, A.M., C. Pratt, R.C. Pearson, R.M. Pool, and M.J. Welser. 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (powdery mildew). Phytopathology 72:232-236.

Layne, D.R., and J.A. Flore. 1992. Photosynthetic compensation to partial leaf area reduction in sour cherry. J. Amer. Soc. Hort. Sci. 117:279-286.

Miller, D.P., G.S. Howell, and J.A. Flore. 1996. A whole-plant, open, gas-exchange system for measuring net photosynthesis of potted woody plants. HortScience 31:944-946.

Ough, C.S. and H.W. Berg. 1979. Powdery mildew sensory effect on wine. Am. J. Enol. Vitic. 30:321.

Pearson, R.C. and A.C. Goheen. 1988. Compendium of Grape Diseases, APS Press St. Paul, MN.

Pearson, R.C. and Gadoury, D.M. 1992. Grape powdery mildew. *In* Plant Diseases of International Importance. Vol. III, Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh, and A.N. Mukhopadhyay (Eds.), pp. 129-146. Prentice Hall, Englewood Cliffs, NJ.

Pool. R.M., R.C. Pearson, M.J. Welser, A.N. Lakso, and R.C. Seem. 1984. Influence of powdery mildew on yield and growth of Rosette grapevines. Plant Dis. 68:590-593.

Poston, F.L., L.P. Pedigo, R.B. Pearce, and R.B. Hammond. 1976. Effects of artificial and insect defoliation on soybean net photosynthesis. J. Econ. Entomol. 69:109-112.

Proctor, J.T.A., J.M. Bodnar, W.J. Blackburn, and R. L. Wilson. 1982. Analysis of the effects of the spotted tentiform leaf miner (*Phyllonorycter blancardella*) on the photosynthetic characteristics of apple leaves. Can. J. Bot. 60:2734-2740.

Shtienberg, D. 1992. Effects of foliar diseases on gas exchange processes: A comparative study. Phytopathology 82:760-765.

von Caemmerer, S. and G.D. Farquhar. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. Planta 160:320-329.

Williams, G.M., and P.G. Ayers. 1981. Effects of powdery mildew and water stress on CO_2 exchange in uninfected leaves of barley. Plant Physiol. 68:527-530.

Chapter 2

Effects of Timing of Powdery Mildew Infection on Carbon Assimilation and Subsequent Seasonal Growth of Potted Chardonnay (*Vitis vinifera* L.) Grapevines

ABSTRACT

Potted Chardonnay (*Vitis vinifera* L.) grapevines were inoculated with conidial suspensions of powdery mildew of grape (*Uncinula necator* (Schw.) Burr.) (GPM) just prior to bloom (Early), just after the 5mm berry stage (Late), or not inoculated and treated with myclobutanil fungicide (Control) and the effects of timing of GPM infection were studied over two seasons. Early vines had reduced CO_2 assimilation rates (A) than Late and Control vines until the pre-veraison period, when Late vines also showed reductions in A. Early vines had reduced fresh and dry weights compared to other treatments. Leaves on both Early and Late vines senesced earlier than those on Control vines. During the following growing season, shoot lengths and fresh and dry weights were negatively correlated with the length of infection time in Season 1.

Introduction

Factors that interfere with carbon assimilation in grapevines may have different effects based on the phenological stage of the plant when interferences occur. Powdery mildew of grape (*Uncinula necator* (Schw.) Burr.) (GPM) is a pathogen of all green tissues of the grapevine, and inhibits carbon assimilation (A) when leaves are infected (Lakso et al. 1982). Reductions in A near bloom may cause increased competition for carbohydrates within a grapevine, and may result in decreased fruit set (Smithyman et al. 1998). Between bloom and veraison, grapevines are usually operating at less than optimal photosynthetic capacity, and the fruit is a relatively weak carbohydrate sink, although sink strength increases over time (Edson et al. 1995). Between veraison and harvest, the fruit is the predominant sink of the plant.

Grape species and cultivars differ in their overall susceptibility to GPM (Doster and Schnathorst 1985); only Vitaceae genera are susceptible (Pearson and Goheen 1988), although this family includes almost all of the economically important grapes in the world. *Vitis vinifera* L. species are particularly susceptible (Pearson and Gadoury 1992). Grape berries also have demonstrated the development of ontogenic resistance to GPM as they mature (Gadoury and Seem 1995; Gadoury et al. 2001a), becoming virtually resistant by the 5 to 7mm berry stage (Gadoury and Seem 1995). In contrast, rachises remain susceptible throughout the season in the relatively resistant *V. labruscana* variety Concord (Gadoury et al. 2001a). Grape leaves have also demonstrated ontogenic resistance to GPM infection with increasing age (Doster and Schnathorst 1985; Sall and Teviotdale 1981), generally becoming resistant to new infections after two months (Sall and Teviotdale 1981). The mechanism for this resistance is not known. However, germinated conidia develop hyphae on susceptible leaves, but not on resistant leaves (Doster and Schnathorst 1985).

Grapevines remain somewhat susceptible to GPM infections throughout the season in spite of the resistance of individual leaves, as new leaves are continuously being produced, especially prior to veraison. Pool et al. (1984) found that early-season applications of protectant fungicides controlled GPM on fruit as well as regular, periodic sprays throughout the growing season, while leaves remained susceptible to infection all season, showing no definite patterns of variable susceptibility as a consequence of vine phenology. The effects of timing of the infection of GPM on grape leaves have not been examined with regard to whole plant behavior.

Inhibition of *A* resulting from GPM infection may affect plant growth in subsequent growing seasons (Gadoury et al. 2001b; Pool et al. 1984). Infection of GPM on the relatively susceptible interspecific hybrid variety Rosette (Seibel 1000) resulted in lower cane pruning weights and yield during the seasons following infection compared to noninfected plants, as well as reduced fruit quality due to higher acidity (Pool et al. 1984). Cane maturity (as determined by the number of mature nodes on hardened canes) and winter hardiness of canes were also reduced on infected plants compared to noninfected plants. Bud fertility in the absence of severely cold winter temperatures was lower in vines infected the previous season. GPM infection of the relatively resistant variety Concord showed similar effects in years following infection, except that, even though fewer buds matured, those that did mature were not less cold-hardy, and yield was not reduced in the following growing season (Gadoury et al. 2001b).

These experiments examine the effects of the timing of GPM infection on carbon assimilation of potted Chardonnay grapevines during the growing season, and subsequent effects on vine performance in the following season.

Materials and Methods

Plant material. *Season 1.* Two-year-old dormant grapevines (*V. vinifera* cv. Chardonnay, Dijon clone 96 grafted to 3309 rootstock) were planted in 19L pots in a medium of 60% loam, 25% sand, and 15% peat and grown and maintained on a gravel pad outdoors at the Horticultural Teaching and Research Center, Michigan State University, East Lansing, MI, USA during the 2001 growing season. Plants were thinned shortly after full bud burst to three shoots per vine. Vines were watered regularly and fertilized monthly with a soluble fertilizer at a rate of 0.38g N, 0.17g P, and 0.32 g K per pot (Peter's 20-20-20). Plants were largely fruitless; the fruit on a few plants, not used in the experiment, was retained to determine phenological stages during the growing season. Fruit was removed from all treatment plants prior to bloom. Laterals were removed as they appeared throughout the growing season. Two applications of Sevin (1-naphthyl N-methylcarbamate (carbaryl), Aventis, Bridgewater, NJ) liquid were made as needed to control Japanese beetle (*Popillia japonica* Newman) infestations. All applications were made at least seven days prior to gas exchange measurements.

Season 2. Plants not destructively harvested in Season 1 were left outdoors for two months during dormancy, then pruned to two nodes per cane and moved into an environmentally controlled greenhouse (high and low temperatures 34°C and 20°C, respectively). Plants were thinned to three shoots per plant at bloom and fertilized monthly as in Season 1. Laterals were removed as they appeared.

Experimental design. *Season 1.* Plants were blocked according to the fresh weights of the dormant, unpotted vines and arranged a completely randomized block design with six blocks, and seven subsamples per treatment randomly arranged within each block to allow for three sequential destructive harvests, each consisting of one plant per treatment per block. Four plants per treatment per block were not destructively harvested at the end of the season, and were retained for Season 2. Treatments were assigned randomly within blocks and were:

1. Plants inoculated with a conidial suspension of *U. necator* (produced by soaking infected leaves of Marechal Foch (Kuhlmann 188-2) grapevines for ≈10min and

agitating to dislodge conidia) just prior to bloom (as determined from the non-treatment, fruited vines), using a hand sprayer and sprayed to runoff. This treatment was designated "Early".

2. Plants were sprayed with myclobutanil (α-butyl-α-(4-chlorophenyl)-1*H*-1,2,4, triazole-1-propanenitrile (NOVA), Rohm and Haas, Philadelphia, PA) at bloom and inoculated with a conidial suspension of *U. necator* as above between the 5mm berry stage and 1200 growing degree days (GDD) (base 50°F), which was 35 days after Early inoculation. This treatment was designated "Late".

3. Plants were protected from GPM infection with myclobutanil at bloom, between 5mm berry size and 1200GDD, and at veraison. This treatment was designated as "Control". Plants sprayed with myclobutanil were separated from inoculated plants by ≈10m for 48h to help eliminate the potential effects of drift and/or volatiles from affecting inoculated plants.

Season 2. Plants were arranged in a randomized complete block design, keeping the same blocking arrangement as in Season 1, with four subsamples per block. No plants were inoculated with *U. necator* during the growing season.

Fruitfulness measurements. *Season 2.* Florets were counted on all shoots on all vines in two randomly selected blocks prior to bloom. There were typically two clusters per shoot; third clusters were removed from those shoots on which they occurred. Apical and basal clusters were evaluated separately. Two weeks after fruit set, set berries were counted. Fruit set was calculated as the ratio of the number of set berries to the number of florets on each cluster, and expressed as a percentage.

Gas exchange measurements. Single leaf measurements were conducted using a portable infrared gas analyzer (IRGA) (CIRAS-2, PP Systems, Amesbury, MA) fitted with a leaf cuvette with light source (PLC6, ibid.). Measurements were taken between 900 and 1500hr at 1000 PAR and 27°C (±3°C). GPM infection was determined on each leaf prior to each gas exchange measurement, and expressed as the percentage of the leaf area which showed GPM symptoms. On leaves having ≥20% GPM infection, two measurements per leaf were taken and the values averaged, in case infection caused significant variances across the leaf surface.

Whole vine gas exchange measurements were conducted using an open gas exchange system as described by Miller et al. (1996). Mylar M-30 film (polyethylene terephthalate, polyvinylidene chloride coated; DuPont, Wilmington, DE) was formed into a cylinder with a 4.0cm interior (i.d.) diameter piece of polyvinylchloride (PVC) pipe at the top, and attached to a wooden base with elastic ("bungee") cord. The wooden base had holes drilled into it to allow for the grape trunk (3.8cm diameter) and air inlet (4.0cm) to help eliminate the effects of soil and root respiration on gas exchange measurements. The 3.8cm hole was further insulated with small strips of foam weather-strip material. Air was supplied using a small shaded pole blower fan (model 4C004, Dayton, Inc, Dayton, OH). The fan was attached to a section of 10.2cm i.d., 2.7m-long section of PVC pipe. The outlet end consisted of reduction and angled couplings just before the chamber inlet. A small piece of tape was loosely placed over the inlet to diffuse airflow entering the chamber. Airflow and temperature were measured with a thermal anemometer (Tri-Sense model 37000-60, Cole-Parmer, Chicago, IL), Airflow was measured through a hole drilled midway on the inlet pipe; measurements were taken at incremental depths of 2.5cm, and averaging the readings. Volume of air was calculated from the averaged flow measurements by the formula:

$$V = 0.51\left(\frac{\pi r^2 l}{10}\right) - 0.1 \text{ (Miller et al. 1996)}$$

where V=volume of air in L/s, r= radius of the air supply cylinder in cm, and l=the linear flow rate in m/s.

CO_2 measurements were performed using the CIRAS-2 unit as an IRGA only; inlet air was sampled first through a ≈1.3m section of flexible tubing, then the air at the outlet of the chamber. Three pairs of measurements were made, and the average of the values was used for calculating Pn. Whole vine CO_2 assimilation was calculated by the formula:

$$Pn(\mu mol / vine / s) = \left(\frac{((\Delta CO_2) / \mu L)((flow) L / \min)}{(29.2 \mu L / \mu mol)(60 s / \min)}\right) \text{ (ibid).}$$

Temperature inside and outside the chamber was measured prior to each series of Pn measurements, and airflow was adjusted to maintain the temperature difference inside the chamber to within 2°C of ambient temperature; if the airflow required adjustment to reduce the temperature difference, the chamber was allowed to reequilibrate prior to taking Pn measurements. Prior to enclosing plants in the Mylar chambers, GPM severity was visually determined and expressed as the percentage of plant leaf area showing disease symptoms.

Leaf area per vine was estimated by measuring shoot lengths on each measured vine. Shoot length in grapevines has been correlated with leaf area (Miller et al. 1996). The relationship between shoot length and leaf area was determined by destructively harvesting 30 non-treatment vines between midseason and veraison and measuring shoot length and actual leaf area as determined by a belt-driven leaf area meter (LI-COR Model LI-3000, LI-3050ASH, LI-COR Inc., Lincoln, NE), and was determined to be ($y=12.01x^{0.9744}$, $r^2=0.916$).

Season 1. Single leaf measurements were conducted at bloom, 5mm berry stage, pre-veraison (≈1200GDD base 50°F), and post-veraison. At bloom, a representative shoot was selected on each plant, and the most recent fully expanded leaf (FEL) on that shoot was measured and marked. Subsequent measurements were conducted on the same, original, leaf (ORFEL), and also on the current most FEL on the same shoot. Whole vine measurements were conducted at the 5mm berry size and pre-veraison stages.

Season 2. Leaves were selected as in Year 1. Single leaf measurements were conducted at bloom, 5mm berry stage, midseason (≈1200 GDD, base 50°F), veraison, and harvest. Since plants were not inoculated with *U. necator*, any treatment effects measured were from infection in Season 1. Treatments were designated "Early(2°)", "Late(2°)", and "Control(2°)" according to their treatments in Season 1.

Destructive harvest and shoot length measurements. *Season 1.* After the completion of the series of gas exchange measurements at the 5mm berry stage, pre-, and post-veraison, one plant from each treatment per block was selected at random and destructively harvested. Plants were cut into component plant parts (roots, trunk, shoots, and leaves), and fresh weights were measured. These plant parts were dried in a forced-air drying oven at 45°C for ≥2 weeks, and dry weights were determined.

Season 2. Shoot lengths were measured prior to each series of single leaf gas exchange measurements. Plants were destructively harvested after harvest, and fresh and dry weights were determined as in Season 1.

Statistical analysis. Statistical analysis was performed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC). ANOVA mean separation was performed using Fisher's protected LSD. Regression *p*-values were obtained using linear regression.

Results

Season 1. Early inoculated plants showed reductions in *A* seven days after inoculation compared to noninoculated plants, although GPM symptoms were not present (Figure 1). Early FEL continued to have lower *A* than noninfected FEL throughout the season. FEL of Late plants showed reductions in single leaf *A* 21 days after inoculation, at which point *A* was statistically the same as for Early FEL. *A* declined throughout the season in all treatments. *A* of infected FEL was lower than that of noninfected FEL throughout the growing season; by post-veraison, *A* on infected FEL was approximately half that of Control FEL.

Only Early ORFEL showed effects of GPM infection at the 5mm berry stage, as Late plants had not yet been inoculated. After pre-veraison, there was no difference between treatments on ORFEL. By the post-veraison period, OFREL were practically nonfunctional, and many infected leaves had senesced.

Differences in stomatal conductance (g_s) among treatments were only apparent on FEL at post-veraison, when Early and Late FEL had reduced g_s compared to Control plants (Figure 2). Reductions in g_s occurred on ORFEL of infected leaves at the 5mm berry stage; there were also significant differences between treatments at pre- and post-veraison; although Late ORFEL g_s was slightly higher than other treatments, no trends were apparent. Differences in internal CO_2 concentration (C_i) were only apparent on FEL at post-veraison, where Early and Late plants had slightly higher C_i than Control plants (Figure 3). Transpiration (E) was only affected on FEL at post-veraison, when it was reduced in infected leaves. Early ORFEL showed reduced E at the 5mm berry stage and, to a lesser extent, at pre-veraison (Figure 4). There were no differences between treatments in whole vine A at either the 5mm berry stage or pre-veraison. There were also no significant differences between treatments on fresh weights and component biomass when evaluating each harvest date separately, presumably due to the small sample size. Data from the three destructive harvests were combined to make a composite sample for the growing season. Early infected plants had lower fresh and dry root, shoot, and total plant weights than Late and Control plants, which were not statistically different (Figures 5-6). Total plant fresh and dry weights of Early plants were 86% and 81% of maximum, respectively, while Late and Control plants differed by only 1% and were statistically identical. Early and Late plants had much greater leaf senescence during the post-veraison period compared to Control plants (Figure 7). Fresh and dry weights of individual leaves were statistically identical (data not shown); the relative lack of leaves on infected plants is responsible for the large differences.

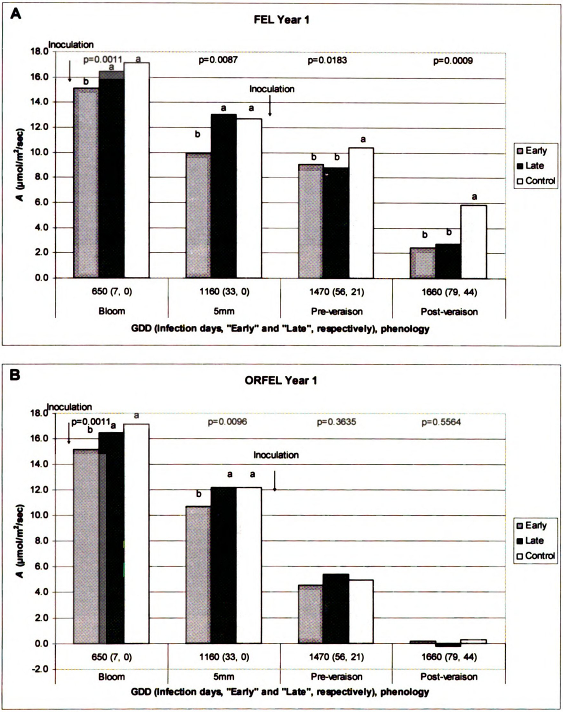


Figure 1. Effect of powdery mildew of grape infection on single leaf CO_2 assimilation (*A*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology, growing degree days (GDD) (base 50°F), and days from inoculation (infection days). Vines were inoculated with *Uncinula necator* twice during the growing season.

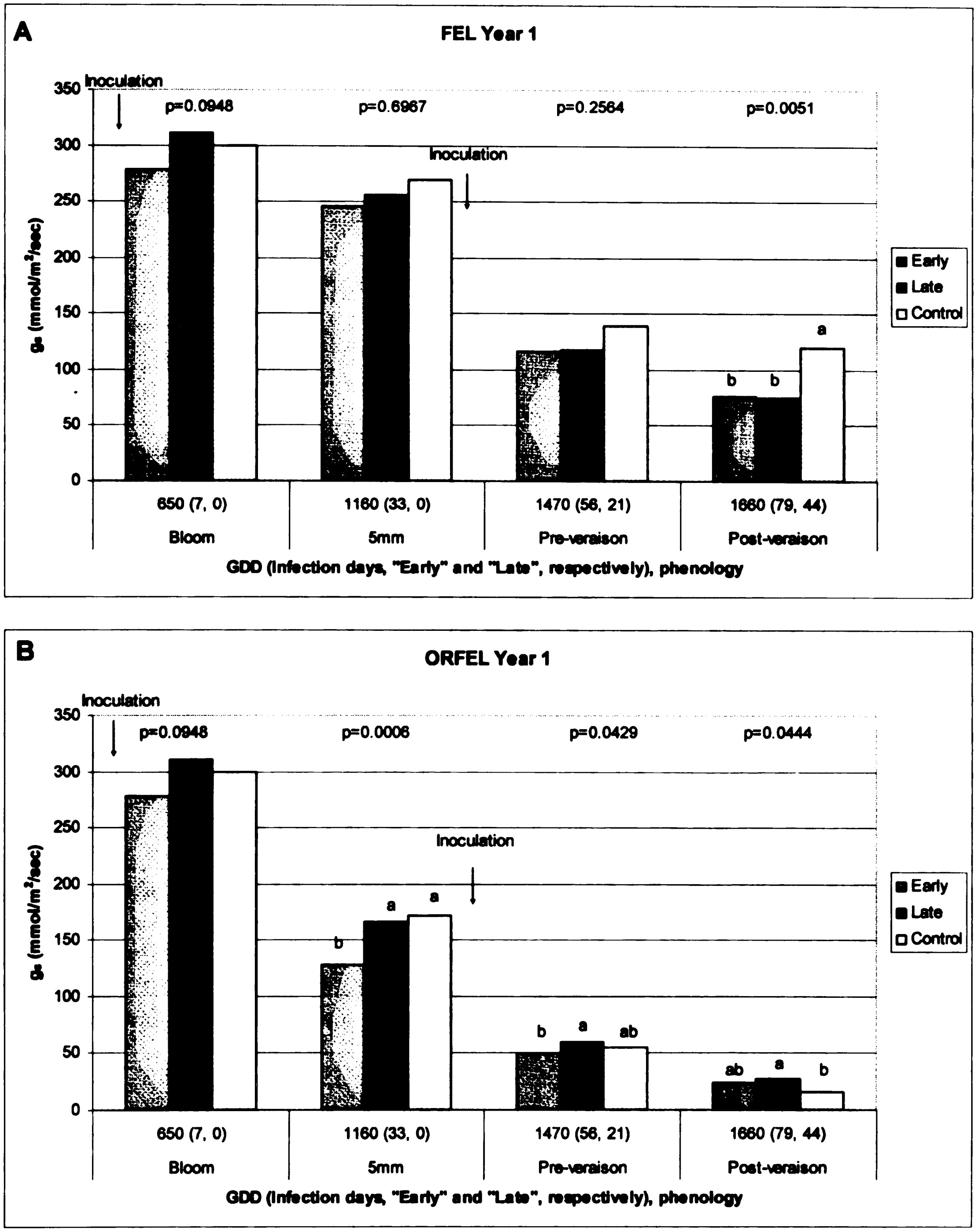


Figure 2. Effect of powdery mildew of grape infection on single leaf stomatal conductance (g_s) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology, growing degree days (GDD) (base 50°F), and days from inoculation (infection days). Vines were inoculated with *Uncinula necator* twice during the growing season.

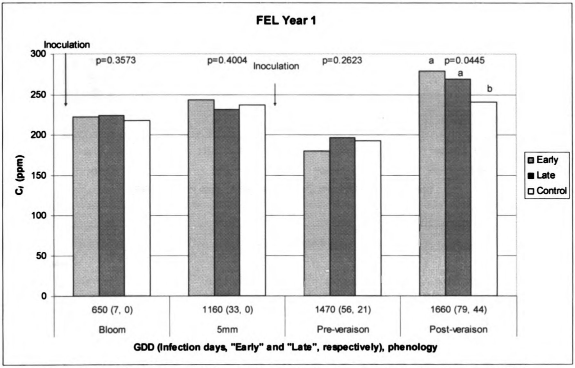


Figure 3. Effect of powdery mildew of grape infection on single leaf internal CO_2 concentration (C_i) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) at different stages of vine growth phenology, growing degree days (GDD) (base 50°F), and days from inoculation (infection days). Vines were inoculated with *Uncinula necator* twice during the growing season..

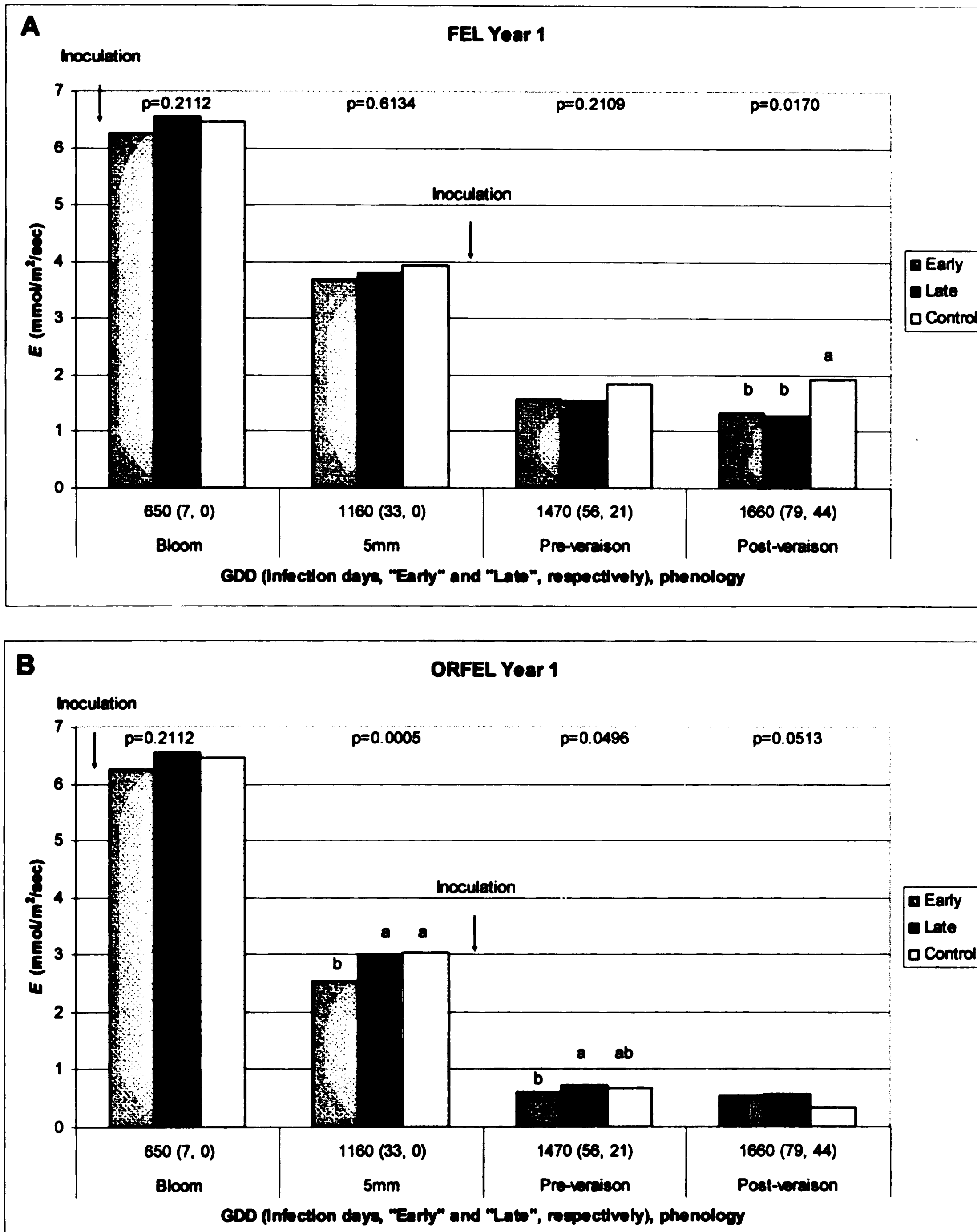


Figure 4. Effect of powdery mildew of grape infection on single leaf transpiration (*E*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) at different stages of vine growth phenology, growing degree days (GDD) (base 50°F), and days from inoculation (infection days). Vines were inoculated with *Uncinula necator* twice during the growing season.

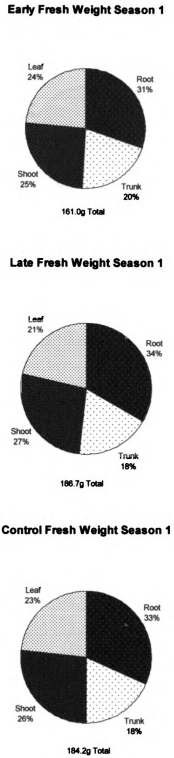
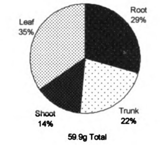
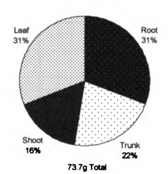


Figure 5. Effect of powdery mildew of grape infection on CO_2 assimilation and carbon partitioning of potted Chardonnay grapevines. "Early" plants were inoculated with *Uncinula necator* seven days pre-bloom; and "Late" vine were inoculated three days after the 5mm berry stage. Data were combined from three sequential destructive harvests.

Early Dry Weight Season 1



Late Dry Weight Season 1



Control Dry Weight Season 1

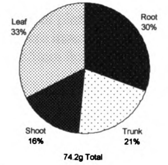


Figure 6. Effect of powdery mildew of grape infection on CO_2 assimilation and carbon partitioning of potted Chardonnay grapevines. "Early" plants were inoculated with *Uncinula necator* seven days pre-bloom; and "Late" vine were inoculated three days after the 5mm berry stage. Data were combined from three sequential destructive harvests.

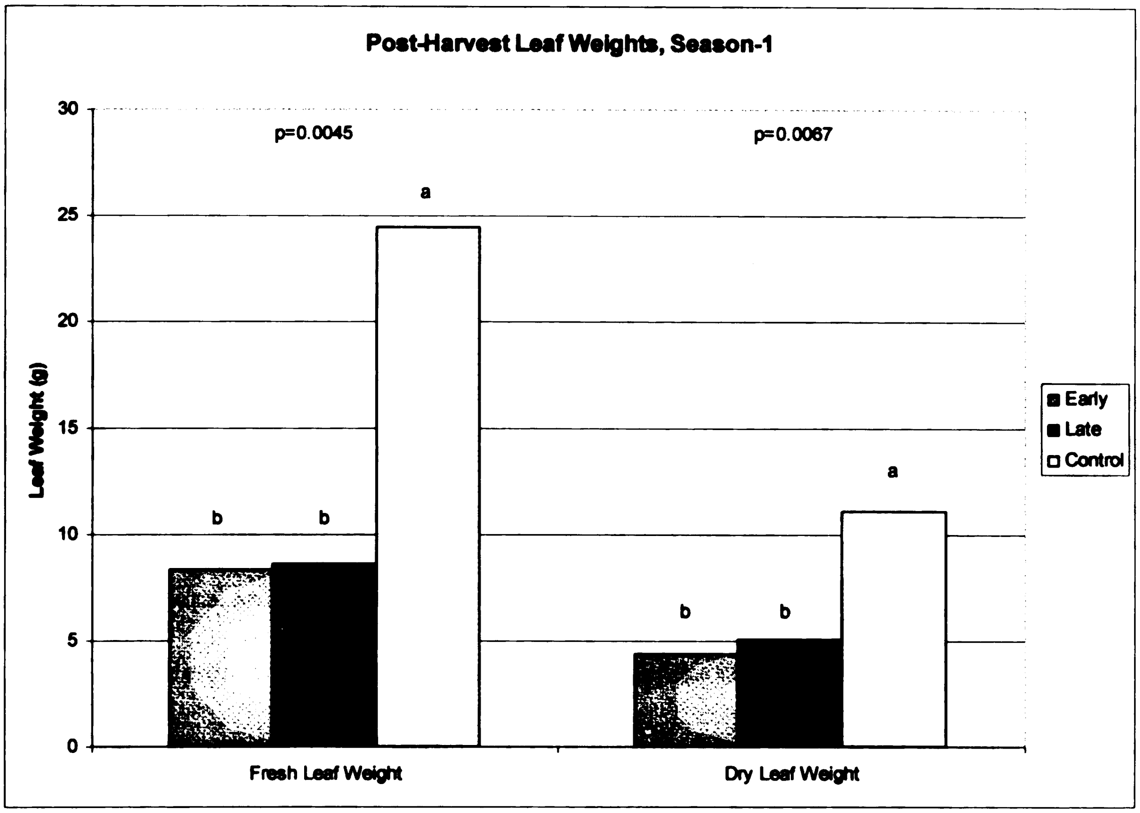
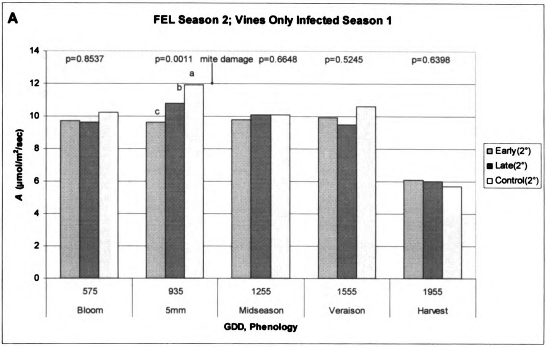


Figure 7. Fresh and dry leaf weights of plants infected at two inoculation times with *Uncinula necator*. "Early" plants were inoculated seven days pre-bloom; "Late" vine were inoculated three days after the 5mm berry stage.

Season 2. There were no differences in number of florets, number of set berries, or percentage fruit set among treatments (data not shown). No GPM was observed on any leaves throughout the growing season, so any treatment effects were only ascribed to infection in Year 1. Gas exchange parameters were not affected by the previous year's infection by GPM at bloom. At the 5mm berry stage, there were differences in single leaf *A* on both FEL and ORFEL (Figure 8). *A* was negatively correlated with the previous season's infection duration on FEL, and ORFEL leaves infected the previous season had reduced *A* compared to Control(2°) plants. Between the 5mm berry stage and midseason, an outbreak of mites occurred on most plants; early signs of damage were on leaves above eye level, which was also the general area of the FEL. By the time the degree of damage was assessed and treatment for mites was applied, significant foliar damage had occurred, and no further differences were observed between treatments, presumably as a consequence of mite damage. The distribution of mite damage was consistent over individual blocks, so no treatment should have been affected more than any other. The only other differences between treatments were a reduction in g_s on Early(2°) plants at midseason compared to Late(2°) and Control(2°) treatments (Figure 9), and elevated C_i in treatments infected the previous year compared to Control(2°) plants (Figure 10). No significant differences in *E* were observed during the growing season.

Shoot lengths were highly correlated with treatment effects in Season 1 throughout the season (Figure 11). Shoot lengths did not change much after bloom; there was some apical meristem necrosis, presumably due to excessive handling when taking measurements and moving plants into position for gas exchange measurements. It is also presumed that the relatively heavy fruit crop (Figures 12-13) in Season 2 served as a strong sink, redirecting resources to the cluster at the expense of shoot growth. Most plants had two clusters per shoot, and there were no treatment effects on fruit set, berries per cluster, berry weight, or total cluster weight at harvest (data not shown).

Plant fresh weight and biomass were affected by GPM infection in the previous season (Figures 12-14). Root, shoot, leaf, and total plant fresh and dry weights were negatively impacted by the previous season's infection, while trunk and fruit weights were not affected.



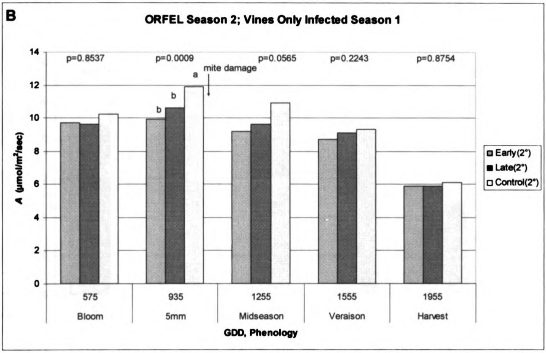
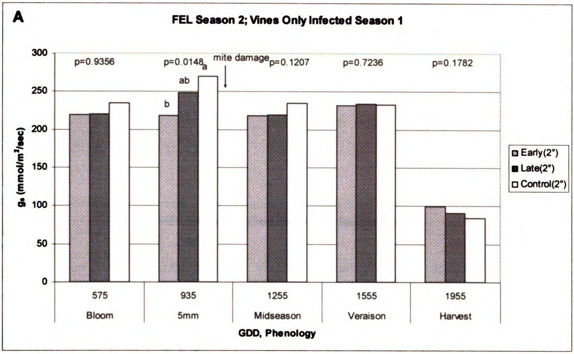


Figure 8. Effect of powdery mildew of grape infection on single leaf CO_2 assimilation (*A*) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFĖL) (B) status at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). "Early(2°)" plants were inoculated with *Uncinula necator* seven days pre-bloom in the previous growing season; "Late(2°)" vine were inoculated three days after the 5mm berry stage in the previous growing season.



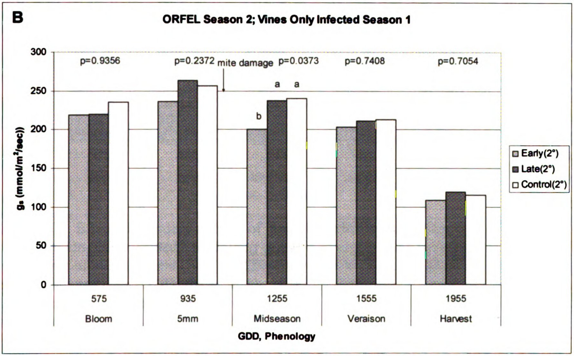


Figure 9. Effect of powdery mildew of grape infection on single leaf stomatal conductance (g_s) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) (A), and the original, initial FEL from the first series of measurements (ORFEL) (B) status at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). "Early(2°)" plants were inoculated with *Uncinula necator* seven days pre-bloom in the previous growing season; "Late(2°)" vine were inoculated three days after the 5mm berry stage in the previous growing season.

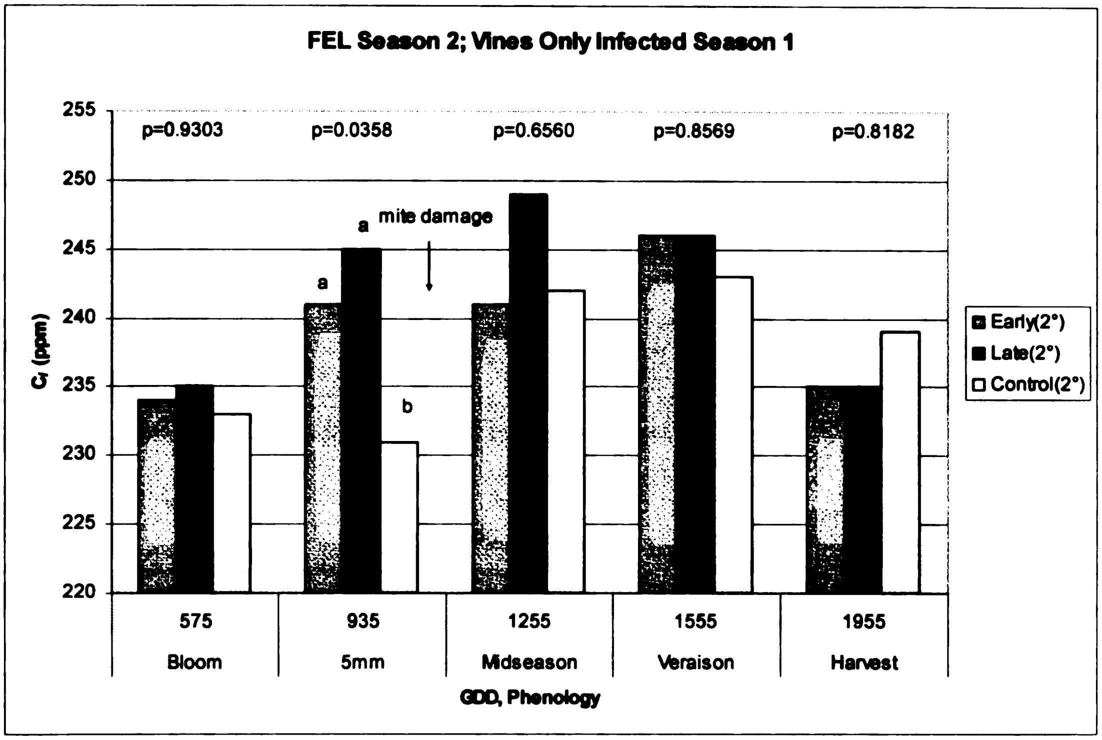


Figure 10. Effect of powdery mildew of grape infection on single leaf internal CO_2 concentration (C_i) on potted Chardonnay grapevines on the most recent fully expanded leaf at time of measurement (FEL) at different stages of vine growth phenology and growing degree days (GDD) (base 50°F). "Early(2°)" plants were inoculated with *Uncinula necator* seven days pre-bloom in the previous growing season; "Late(2°)" vine were inoculated three days after the 5mm berry stage in the previous growing season.

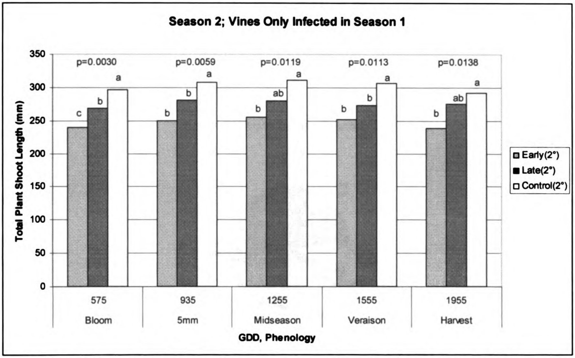
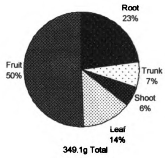
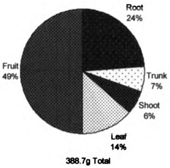


Figure 11. Effects of powdery mildew of grape on shoot length of potted Chardonnay grapevines during the growing season following infection at different stages of vine growth phenology, and growing degree days (GDD) (base 50°F). "Early(2°)" plants were inoculated seven days pre-bloom in the previous growing season; "Late(2°)" vine were inoculated three days after the 5mm berry stage in the previous growing season.

Fresh Weight Season 2, Early(2°)



Fresh Weight Season 2, Late(2°)



Fresh Weight Season 2, Control(2°)

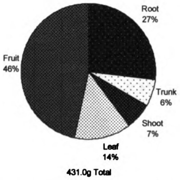
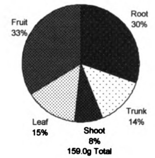
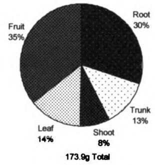


Figure 12. Impact of the previous season's infection by powdery mildew of grape on fresh weight of component plant parts of potted Chardonnay grapevines. "Early(2°)" plants were inoculated with *Uncinula necator* seven days prior to bloom, and "Late(2°)" plants were inoculated three days after the 5mm berry stage in Season 1.

Dry Weight Season 2, Early(2°)



Dry Weight Season 2, Late(2°)



Dry Weight Season 2, Control(2°)

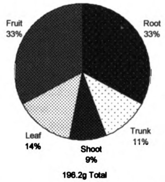


Figure 13. Impact of the previous season's infection by powdery mildew of grape on biomass of component plant parts of potted Chardonnay grapevines. "Early(2°)" plants were inoculated with *Uncinula necator* seven days prior to bloom, and "Late(2°)" plants were inoculated three days after the 5mm berry stage in Season 1.

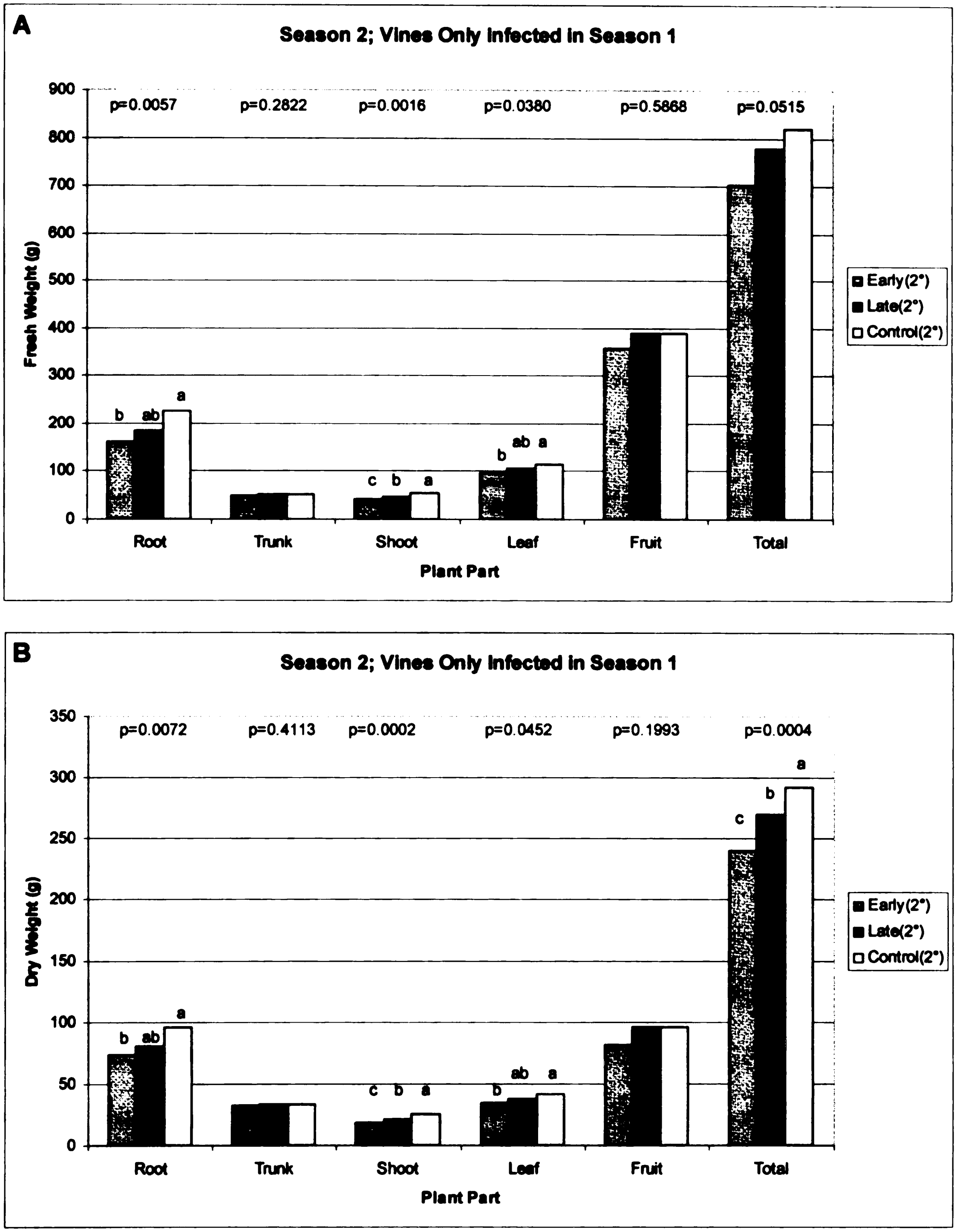


Figure 14. Effects of powdery mildew of grape on subsequent season accumulated fresh weight (A) and biomass (B) of potted Chardonnay grapevines. "Early(2°)" plants were inoculated with *Uncinula necator* seven days prior to bloom, and "Late(2°)" plants were inoculated three days after the 5mm berry stage in Season 1.

Discussion

Grapevine leaves infected with GPM at any time consistently showed reduced *A* compared to controls, with the exception of older leaves from midseason on. The negative effect of leaf aging on Pn has been described by Kriedmann et al. (1970) and Poni et al. (1994) on vines which, like those in this experiment, were not sink-limited. The lack of differences in *A* older leaves between infected and noninfected vines may be due to the equilibrating effects of leaf aging. The consistent negative effect of GPM on the FELs and leaves up to ≈30 days older indicates that GPM can compromise the carbon assimilation capacity of grapevines at any time during the growing season, and implies that the impact may be cumulative over time. This is reflected in the destructive harvest data, as Early plants had reduced average total season fresh weights and biomass compared to Late and Control plants. Even though Late plants had reduced *A* from midseason on, their total fresh weights and biomass were not different from Control plants. Plants infected with GPM continued to develop disease symptoms on newer leaves until late in the season, when temperatures were no longer favorable for conidial germination.

The premature senescence of leaves in Season 1 may have strongly influenced the reduced vine growth in Season 2. The ability to accumulate carbon during the period after harvest is extremely important for subsequent seasonal growth, especially in cooler climates, where there is typically a relatively small period of time between harvest (prior to which the ripening fruit is the strongest sink on the plant for photosynthates), and the onset of either temperatures too cold for carbon assimilation to occur, or leaf senescence due to cold weather. Therefore, anything that will inhibit the ability of a plant to accumulate carbon during this period has the potential to predispose the plant to suboptimal growth in future seasons. Loss of substantial leaf area, even if leaves were performing optimally, is likely to result in decreased carbon accumulation. This effect may be exacerbated by the reduced *A* resulting from GPM infection during this period.

The effects of the previous season's GPM infections were evident in Season 2. The influence of the previous season's GPM infections were inconclusive for single leaf gas exchange parameters; while plants infected in Season 1 began to show differences in *A* at the 5mm berry stage consistent with GPM treatment in the prior season, no other significant trends were observed over the growing season. It is possible that the mite damage obscured Season 1 treatment effects, as the data at 5mm berry size is highly correlated with Season 1 treatments, and differences between treatments were not apparent after the presence of significant mite damage.

Plants infected with GPM in Season 1 showed no signs of compensation for suboptimal Season 1 *A* in Season 2. GPM treatments that reduced *A* in Season 1 showed diminished growth in Season 2 in relation to their duration of GPM infection in Season 1. Early(2°) plants consistently had the lowest shoot length, fresh weight, and dry weight (biomass) accumulation of all treatments. Late(2°) plants, which had the same biomass accumulation as Control plants in Season 1, also had significantly reduced shoot length, fresh weight, and biomass accumulation than Control(2°) plants. These data are in agreement with those of field-grown grapevines which have shown reduced growth in season's following foliar infection by GPM (Pool et al. 1984). Carbon partitioning was not significantly influenced by Season 1 treatments, only total carbon accumulation.

The reduction in Season 2 shoot length in plants infected with GPM in Season 1 corresponded to a reduction in leaf area, as determined by regression in Season 1 ($y=12.01x^{0.9744}$, $r^2=0.916$). The reduction in leaf area would account for the reduced biomass at the end of Season 2 for both Early(2°) and Late (2°) plants.

These data suggest that inhibition of *A* caused by GPM infection can result in both within-season and long-term degradation in vine health. This may apply to other foliar pathogens that inhibit *A* in grapevines. The earlier the onset of infection in the growing season, the greater the plant's ability to assimilate CO_2 is compromised, indicating the need to control the disease early in the growing season. This corresponds to the initial period of infection, whether from cleistothecial ascospores or from conidia. Later infections than those in this study may not result in such significant reductions in season-long biomass accumulation. The determination of the latest phenophase at which GPM ceases to affect leaf senescence deserves further study.

Literature Cited

Doster, M.A. and W.C. Schnathorst. 1985. Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus *Uncinular necator*. Am. J. Enol. Vitic. 36:101-104.

Edson, C.E., G.S. Howell, and J.A. Flore. 1995. Influence of crop load on photosynthesis and dry matter partitioning of Seyvel grapevines. III. Seasonal changes in dry matter partitioning, vine morphology, yield, and fruit composition. Am. J. Enol. Vitic. 46:478-485.

Gadoury, D.M. and R. C. Seem. 1995. Development of ontogenic resistance to powdery mildew (*Uncinula necator*) in fruit of Concord grapevines. Phytopathology 85:1149.

Gadoury, D.M., R. C. Seem, A. Ficke, and W.W. Wilcox. 2001a. The epidemiology of powdery mildew on Concord grapes. Phytopathology 91:948-955.

Gadoury, D.M., R.C. Seem, R.C. Pearson, W.F. Wilcox. 2001b. Effects of powdery mildew on vine growth, yield, and quality of Concord grapes. Plant Dis. 85:137-140.

Kriedemann, P.E. 1968. Photosynthesis in vine leaves as a function of light intensity, temperature, and leaf age. Vitis 7:213-220.

Lakso, A.M., C. Pratt, R.C. Pearson, R.M. Pool, and M.J. Welser. 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (powdery mildew). Phytopathology 72:232-236.

Miller, D.P., G.S. Howell, and J.A. Flore. 1996. A whole-plant, open, gas-exchange system for measuring net photosynthesis of potted woody plants. HortScience 31:944-946.

Pearson, R.C. and D.M. Gadoury. 1992. Grape powdery mildew. *In* Plant Diseases of International Importance. Vol. III, Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh, and A.N. Mukhopadhyay (Eds.), pp. 129-146. Prentice Hall, Englewood Cliffs, NJ.

Pearson, R.C. and A.C. Goheen. 1988. Compendium of Grape Diseases, APS Press St. Paul, MN.

Petrie, P.R., M.C.T. Trought, and G.S. Howell. 2000. Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. Vitis 39:31-36.

Pool, R.M., R.C. Pearson, M.J. Welser, A.N. Lakso, and R.C. Seem. 1984. Influence of powdery mildew on yield and growth of Rosette grapevines. Plant Dis. 68:590-593.

Poni, S., C. Intrieri, and O. Silvestroni. 1994. Interactions of leaf age, fruiting, and exogenous cytokinins in Sangiovese grapevines under non-irrigated conditions. I. Gas exchange. Am. J. Enol. Vitic. 45:71-78.

Sall, M.A. and B.L. Teviotdale. 1981. Powdery mildew. *In* Grape Pest Management. D. Glaherty, F. Jensen, A. Kasimatis, H. Kido, and W. Moller (Eds), pp. 46-50. Agricultural Sciences Publications, University of California, Berkeley, CA.

Smithyman, R.P., G.S. Howell, and D.P. Miller. 1998. The use of competition for carbohydrates among vegetative and reproductive sinks to reduce fruit set and botrytis bunch rot in Seyval blanc grapevines. Am. J. Enol. Vitic. 49:163-170.

Williams, L.E. 1995. Grape. *In* Carbon Partitioning and Source-Sink Interactions in Plants. M.A. Madore and W.J. Lucas (Eds.), pp. 851-881. Am. Soc. Plant Physiol., Rockville, MA.

Chapter 3

Effects of Powdery Mildew of Grape on the Carbon Assimilation Mechanisms of Potted Chardonnay (*Vitis vinifera* L.) Grapevines

ABSTRACT

Potted Chardonnay (*Vitis vinifera* L.) grapevines were inoculated with conidial suspensions of powdery mildew of grape (*Uncinula necator* (Schw.) Burr.) (GPM). Leaves of infected and noninfected plants were studied for the effects of varying light (PAR) and CO_2 concentrations on factors affecting carbon assimilation. Infection by GPM reduced carboxylation efficiency (*k*), net CO_2 assimilation rate (*A*), stomatal conductance (g_s), and internal CO_2 concentration (C_i) under ambient CO_2, A_{max} at >900 ppm CO_2, stomatal limitations to *A* (l_g), and photochemical efficiency (φ), while having no effect on the CO_2 compensation point (Γ) or the light compensation point (cp). Infection by GPM had no effect on chlorophyll fluorescence (F_v/F_m).

Introduction

Plant responses to foliar biotic and abiotic stresses may vary with the nature of the stress. Net CO_2 assimilation (*A*) by foliage is a critical factor influencing plant productivity, since ≥90% of plant dry matter is derived from C fixed through photosynthesis (Pn) (Flore and Lakso 1989). Therefore, factors that inhibit assimilation through photosynthesis may be detrimental to productivity.

Photosynthesis in plants can be limited by biotic stresses in a variety of ways. Johnson (1987) divided the seven categories of pest effects on plants as described by Boote et al. (1983) into two groups: a) those whose major effects are on solar radiation interception (tissue consumers, leaf senescence accelerators, stand reducers, and light stealers), and b) those whose major effects are on relative use efficiency (photosynthetic rate reducers, assimilate sappers, and turgor reducers). Damage to the photosynthetic apparatus may occur by more than one of these effects; reductions in *A* caused by the effects of most foliar pathogens on photosynthetic activity result from a decrease in the photosynthesizing leaf area and/or its reduced efficiency (Goodman et al. 1986; Shtienberg 1992; Yarwood 1967).

Response patterns affecting reductions in Pn and transpiration (*E*) have been related to the general type of trophic relationships involved (Shtienberg 1992); powdery mildews tended to have more similar response patterns as compared to other foliar pathogens, for example. Infections of powdery mildew of barley (*Blumeria* (syn. *Erysiphe*) *graminis* D.C. ex Merat f.sp. *hordei* Marchal) resulted in both decreases in chlorophyll after four days of infection and loss of electron transport activity, with no loss of electron carrier concentration in remaining chlorophyll (Holloway et al. 1992). Infections of powdery mildew of sugar beet (*Erysiphe polygoni* DC) inhibited electron transport in noncyclic proteins, accompanied by alterations in chloroplast ultrastructure and reduction of enzyme activity (Magyrarosy et el. 1976). Carboxylation resistance increased in winter wheat infected by powdery mildew of wheat (*Blumeria* (syn. *Erysiphe*) *graminis* D.C. ex Merat f.sp. *tritici*), with consequent negative effects on stomatal resistance, boundary layer resistance, and transport resistance (Rabbinge et al. 1985). *A* was negatively affected by powdery mildew infection in all three studies. There does not appear to be a relationship between decreases in *A* and *E* among pathosystems; rather, *E* has been shown to increase, decrease, or stay the same in response to foliar pathogens, including powdery mildews (Shtienberg 1992).

Grape leaves infected with powdery mildew of grape (*Uncinula necator* (Schw.) Burr.) (GPM) have demonstrated reduced photosynthetic rates compared to uninfected leaves (Lakso et al. 1982), due to destruction of palisade cells by the fungus. *E* was not affected; consequently, water use efficiency was less in infected leaves. Field experiments have demonstrated negative effects of GPM on grapevine health during the season of infection, including decreased fruit quality (Gadoury et al. 2001; Ough and Berg 1979; Pool et al. 1984) and fruit set (Chellemi and Marois 1992). Multiseasonal effects include reduced vine size (as determined by cane pruning weights) and yield in susceptible varieties (Pool et al. 1984), or only with vine size in relatively resistant varieties (Gadoury et al. 2001).

Defoliation experiments have been conducted on grapevines for a variety of reasons, including manipulation of fruit set, modifying the fruit microclimate, and to simulate pest damage. Grapevine responses to defoliation by removing whole leaves frequently include increased *A* by the remaining leaves (Hofäcker 1978; Candolfi-Vasconcelos and Koblet 1990; Candolfi-Vasconcelos and Koblet 1991; Intrieri et al. 1997), although Candolfi-Vasconcelos et al. (1994) found no increase in photosynthetic rate in the remaining leaves. Punching holes in the leaves of other crop species have been used to simulate the effects of damage by phytophagous arthropods (Boucher et al. 1987; Flore and Irwin 1983; Poston et al. 1976). Stacey (1983) found that leaf removal on tomato plants largely approximated pest damage. Defoliation experiments have been inconsistent in approximating damaged caused by foliar pathogens, as visual estimates of infection do not always adequately indicate the effects of a pathogen on photosynthetic and transpirational activities (Shtienberg 1992).

Measurements of chlorophyll fluorescence have also been employed to determine the health of photosynthetic mechanisms in plants (Buwalda and Noga 1994; Krause and Weis 1991), and have been correlated with end-product inhibition of leaf *A* due to damage to photosystem II (PSII) (Layne and Flore 1993). Depending on the nature of pathogen-induced foliar damage, damaged leaves may exhibit less potential maximal photochemical efficiency than uninfected leaves.

These experiments were designed to determine the physiological effects of GPM infection on individual grape leaves regarding gas exchange and chlorophyll fluorescence.

Materials and Methods

Plant material. Two-year-old dormant grapevines (*V. vinifera* L. cv. Chardonnay, Dijon clone 96, grafted to 3309 rootstock) were planted in 19L pots in a medium of 50% loam, 40% sand, and 10% peat. The plants were grown and maintained on a gravel pad outdoors at the Horticultural Teaching and Research Center, Michigan State University, East Lansing, MI, USA during the 2002 growing season. Plants were thinned shortly after full bud burst to three shoots per vine. Vines were watered regularly and fertilized monthly with a soluble fertilizer at a rate of 0.38g N, 0.17g P, and 0.32 g K per pot (Peter's 20-20-20). Plants were largely fruitless; a few plants which did have fruit were retained to determine phenological stages during the growing season. Flower clusters were removed from all treatment plants prior to bloom. Laterals were removed as they appeared throughout the growing season. Two applications of Sevin (1-naphthyl N-methylcarbamate (carbaryl), Aventis, Bridgewater, NJ) liquid were made as needed to control Japanese beetle (*Popillia japonica* Newman) infestations. All chemical applications were made at least seven days prior to gas exchange measurements.

Experimental design. Plants were blocked according to the fresh weight of the dormant, unpotted vines and arranged in a randomized complete block design with 32 blocks. Treatments were assigned randomly within blocks and were:

(1) Plants inoculated with a conidial suspension of GPM in distilled water (produced by soaking infected leaves of Marechal Foch (Kuhlmann 188-2) grapevines for $\approx$10 minutes and agitating to dislodge conidia) between the 5mm berry (as determined from the non-treatment fruited vines) and 1200 growing degree days (GDD) (base 50°F) stages using a hand sprayer and sprayed to runoff. This treatment was designated "Infected".

(2) Plants were sprayed with myclobutanil (α-butyl-α-(4-chlorophenyl)-1*H*-1,2,4, triazole-1-propanenitrile (NOVA), Rohm and Haas, Philadelphia, PA) at bloom and between the 5mm berry stage and midseason ($\approx$1200GDD). This treatment was designated "Noninfected".

Plants sprayed with myclobutanil were separated from inoculated plants by $\approx$10m for 48h to help eliminate the potential effects of drift and/or volatiles from affecting inoculated plants.

Ten plants from each treatment were selected for gas exchange responses to varying CO_2 concentrations and photosynthetically active radiation (PAR) level measurements by the following criteria: The most recent fully expanded leaves on the longest shoot on each plant were examined just prior to veraison; leaf health was evaluated based on visual ratings of disease severity, expressed as a percentage of the leaf surface with visible GPM infection. The most recent fully expanded leaves from each of the 10 blocks which had both the healthiest Noninfected leaves and an obviously Infected, but otherwise undamaged (by insects, wind laceration, etc.) leaf, were selected for gas exchange measurements. Disease severity on Infected leaves ranged from 50-90% infected leaf area.

Gas exchange measurements. Gas exchange measurements were conducted using a portable infrared gas analyzer (IRGA) (CIRAS-2, PP Systems, Amesbury, MA) fitted with a leaf cuvette with light source (PLC6, ibid.). Effects of CO_2 concentration were determined by gradually increasing CO_2 from 0 to 200ppm at 50ppm increments, and from 200 to 1000ppm at 100ppm increments at photosynthetically active radiation (PAR)=1500, allowing the IRGA to equilibrate between each measurement using the onboard computer (Fujitsu PenCentra 130, Fujitsu PC Corporation, Santa Clara, CA) and software (version 1.0, PP Systems, Amesbury, MA). Responses to changes in PAR were taken immediately afterward, using the same equipment and software, by reducing PAR from 2000 to 200 in 200PAR increments, and from 200 to 0 in 50PAR increments. Measurements were taken between 0900 and 1500hr at 26°C (±2°C). Plants were measured within each block according to their random placement to help alleviate the effects of natural diurnal variances in *A* (Downton et al. 1987).The data were analyzed by applying the Marquardt-Levenberg algorithm for nonlinear regression analysis for curve fitting (Marquardt 1963; Layne and Flore 1992, 1995).

Parameters calculated from plant responses of *A* to variable PAR (light response curves) were: the light compensation point (cp), extrapolated from the data where A=0, and quantum yield (φ), as determined by the slopes of the linear portion of the curve.

Parameters calculated from plant responses of A to variable internal CO_2 concentration (C_i) were the CO_2 compensation point (Γ), extrapolated from the data where A=0; carboxylation efficiency (k), as determined by the slopes of the linear portion of the curve; stomatal limitation to A (l_g,), calculated according to the differential method of Jones (1985); and A_{max}, the maximum A value at saturating CO_2. A, g_s, and C_i at ambient CO_2 concentrations and saturating light conditions were also measured (A_{360}, g_{s360}, and C_{i360}, respectively).

Single leaf measurements were also performed on the most recent fully expanded leaf of the longest shoot on all plants in the plot over a period of two days to determine relationships, if any, between A and g_s and C_i, at PAR=1000 and CO_2=375ppm.

Chlorophyll fluorescence measurements

Three blocks were randomly selected for chlorophyll fluorescence measurements. The longest shoot on each plant, also used for gas exchange measurements, was selected and each leaf evaluated for disease severity, expressed as the percentage of leaf area with visible PM symptoms. A clip with a sliding window to admit or exclude light was attached to each leaf, and the leaf section was allowed to dark acclimate for ≥30 min. Chlorophyll fluorescence was measured with a Hansatech Plant Efficiency Analyzer (model PEA, Hansatech Instruments, Norfolk, England). Fluorescence was expressed as the ratio of variable fluorescence (F_v) to the maximum fluorescence (F_m) (F_v/F_m).

Statistical analysis. Statistical analysis was performed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC). ANOVA mean separation was performed using Fisher's protected LSD. Curve fitting was performed using SigmaPlot software (version 8.01; SPSS Ltd., Chicago, IL).

Results

While A and g_s were negatively affected by GPM infection under ambient CO_2 and saturating light conditions, there was no negative effect of GPM on C_i (Table 1). Values for A_{360} and g_{s360} on Infected plants were 38% and 36% of those of Noninfected plants. k and A_{max} were also negatively affected by GPM infection (37% and 47%, respectively, on Infected compared to Noninfected plants). There were no significant differences in Γ between treatments. l_g was higher in Infected plants compared to Noninfected plants.. There was no decline in A at high CO_2 levels.

Single leaf measurements showed a strong relationship between A and g_s on both Infected and Noninfected plants (Figure 2), although the linear relationships between A and g_s were different for the two treatments. There was a general negative correlation between A and C_i in Infected plants; the relationship between A and C_i in Noninfected plants was not significant at the $p \leq 0.10$ level, but was generally positive.

Infected plants showed reduced (52%) φ compared to Noninfected plants. There were no differences in cp between treatments. There were also no significant differences in chlorophyll fluorescence between treatments (Figure 3) or between different levels of disease severity.

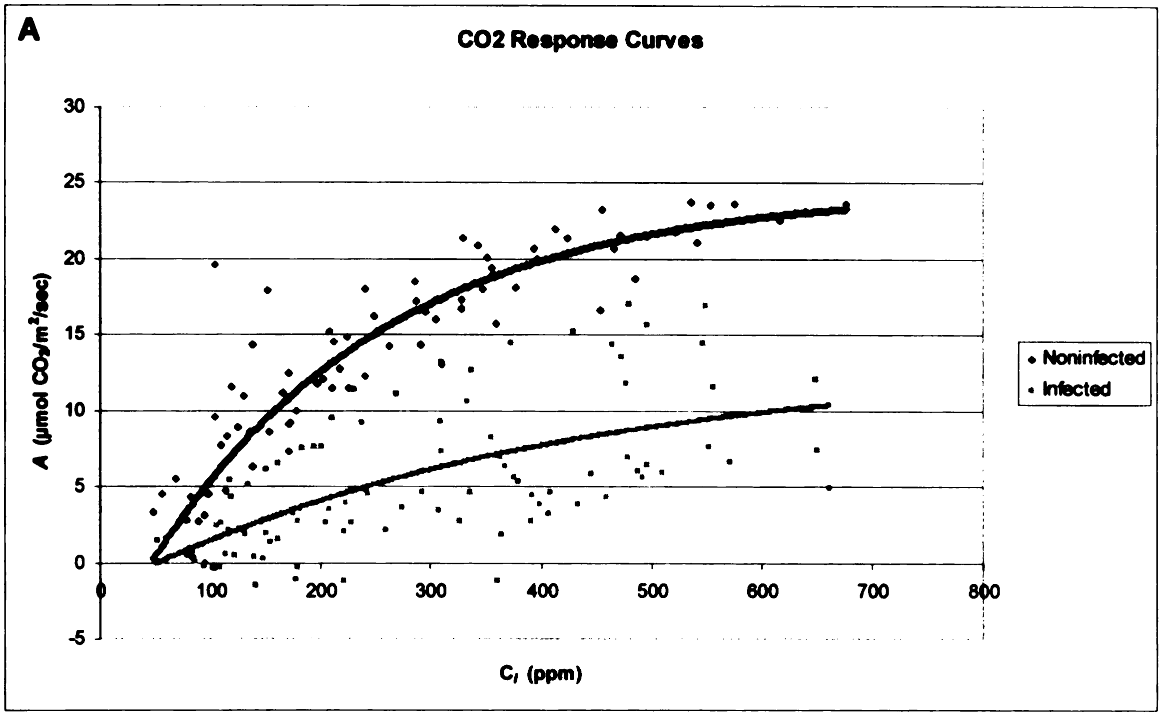
Table 1. The effect of powdery mildew of grape infection on CO_2 compensation point (Γ), carboxylation efficiency (k), CO_2 assimilation rate (A_{360}), stomatal conductance (g_{s360}), and internal CO_2 concentration (C_{i360}) under ambient CO_2, A_{max} at >900 ppm CO_2, stomatal limitations to A (l_g), photochemical efficiency (φ), and light compensation point (cp) on most recently fully expanded grape leaves on potted Chardonnay grapevines.

CO2 Parameters

	Γ (μmol/CO_2/mol)	k (mol/CO_2/m^2/sec)	A_{360} (μmol/CO_2/m^2/sec)	g_{s360} (mmol/CO_2/m^2/sec)	C_{i360} (μmol/CO_2/mol)	A_{max} (μmol CO_2/m^2/sec)	l_g (%)
Infected	65.4	0.037 b	4.1 b	41 b	195	10.4 b	52.2 a
Noninfected	53.7	0.101 a	10.8 a	113 a	176	21.9 a	39.4 b
p-value	0.3519	0.0012	0.0011	0.0048	0.9194	0.0012	0.0028

Light Parameters

	φ (μmol/CO_2/mol/PPF)	cp (μmol/CO_2/m^2/sec)
GPM		
Infected	0.02 b	90
Noninfected	0.04 a	95
p-value	0.0008	0.8216



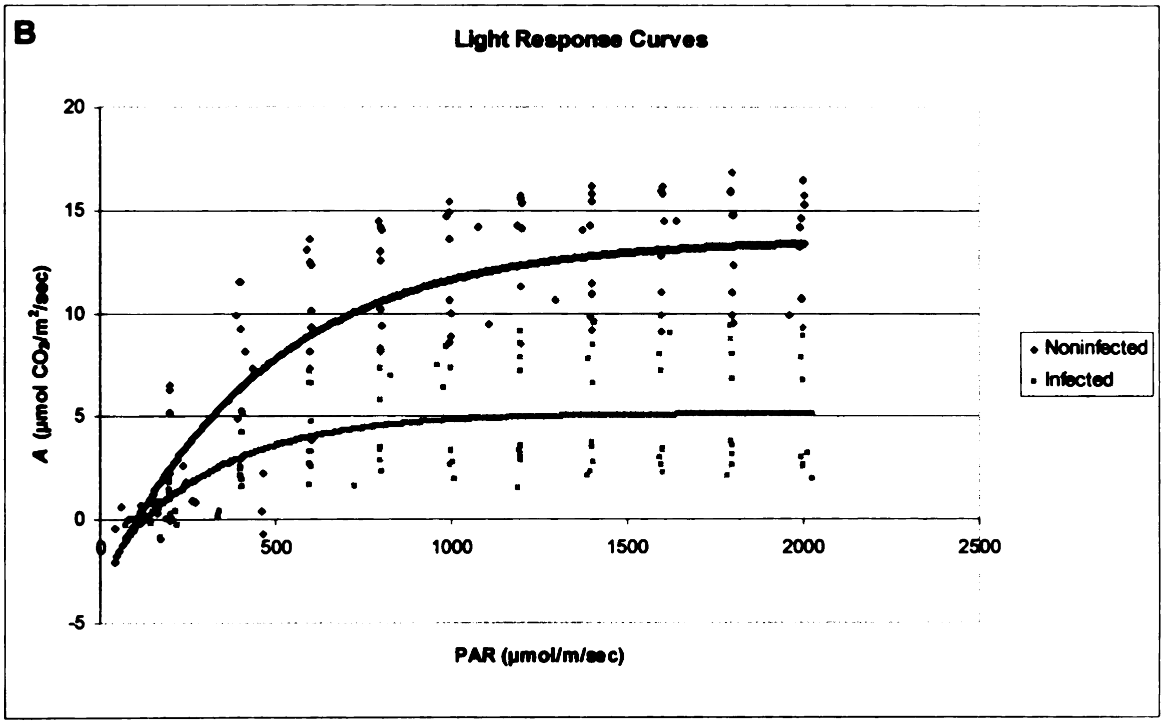


Figure 1. CO_2 (A) and light (B) response curves of single leaves of potted Chardonnay grapevines infected and not infected with powdery mildew of grape.

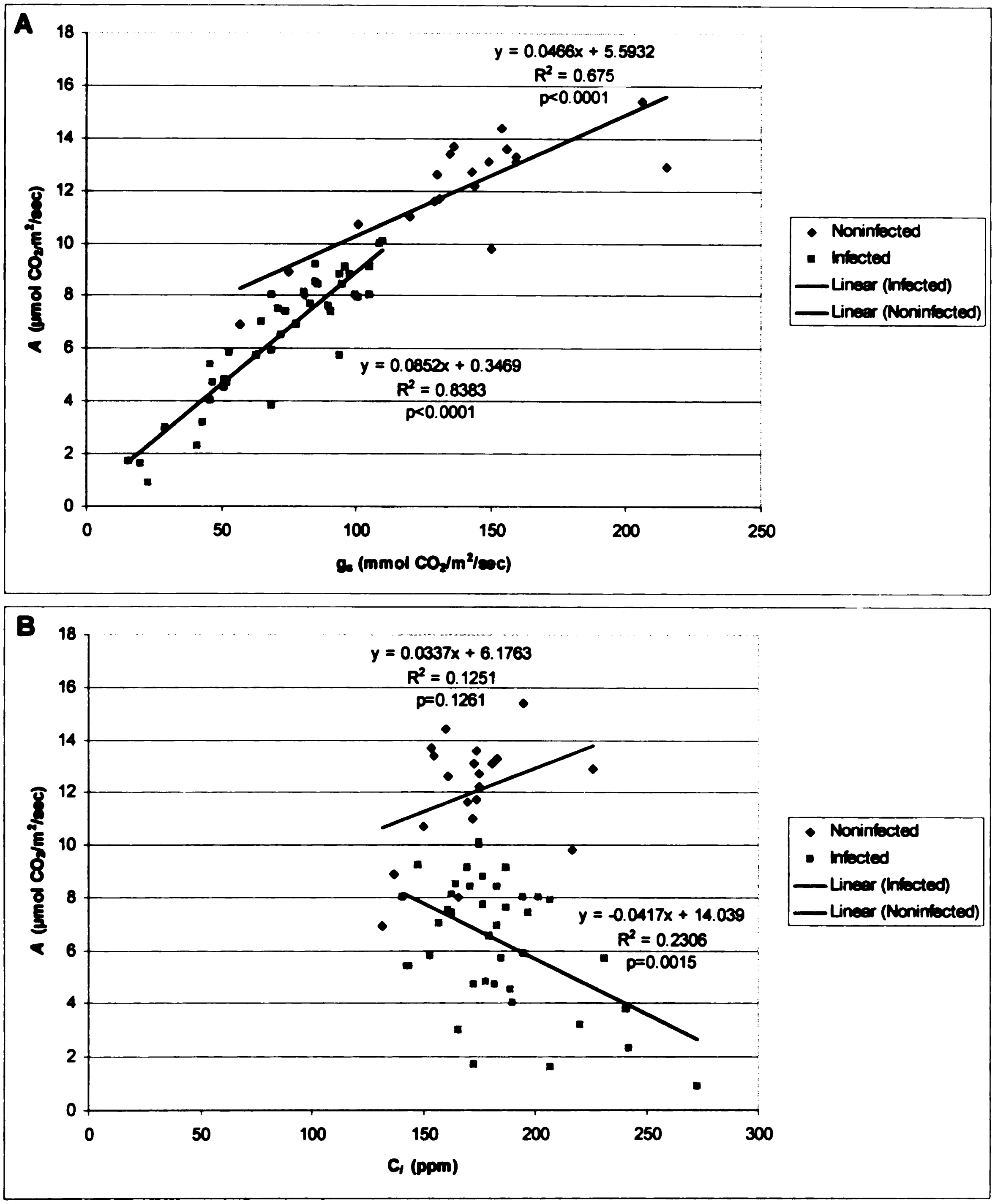


Figure 2. Relationships between single leaf CO_2 assimilation (A) and stomatal conductance (g_s) (A), and single leaf A and internal CO_2 concentration (C_i) (B) in leaves of potted Chardonnay grapevines infected and not infected with powdery mildew of grape.

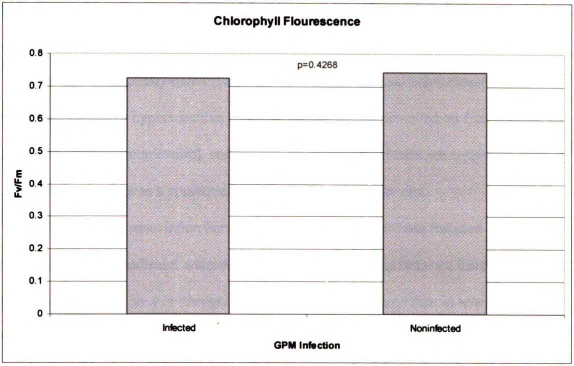


Figure 3. Chlorophyll fluorescence on leaves of potted Chardonnay grapevines infected and not infected with powdery mildew of grape (GPM). Fluorescence is expressed as the ratio between variable fluorescence (F_v) and maximum fluorescence (F_m).

Discussion

GPM infection compromised the carbon assimilation mechanism of grape leaves at several levels. The reduced *k* values for Infected plants indicate that the carboxylation reactions, on a leaf area basis, were negatively affected by GPM infection. Similarly, reductions in φ in Infected plants indicate a reduction in overall quantum efficiency on a leaf area basis. These data are consistent with those of Lakso et al. (1982), who found that GPM damaged the photosynthetic apparatus of grape leaves by causing death of palisade cells. The lack of differences in chlorophyll fluorescence between treatments indicates that there was no significant effect of GPM infection on the specific PSII thylakoid reactions, and that the reduction of *A* as a consequence of GPM infection was not due to disruptions of specific biochemical pathways, but rather to relatively largescale destruction of entire cells. GPM fungi do not actually invade palisade cells, only epidermal cells (Pearson and Goheen 1988). However, the death of adjacent palisade cells has been consistently noted (Lakso et al. 1982; Doster and Schnathorst 1985), presumably due to a hypersensitive response similar to that observed on fruit (Seem, R.C 2000, personal communication), and the results of this experiment are consistent with photosynthetic losses as a consequence of palisade cell destruction.

The positive association between g_s and A in leaves of both Infected and Noninfected plants indicates a strong mechanistic relationship between the two, and that the correlation of g_s on A is stronger in leaves of Infected plants than in leaves of Noninfected plants. This stronger relationship is reflected in the negative relationship between A and C_i in Infected leaves. The relationship between A and C_i was much weaker, but positive, in Noninfected leaves. The correlation between increased g_s and A is similar to that observed in defoliation experiments on grapevines, when remaining leaves demonstrated photosynthetic compensation for reduced leaf area (Hofäcker 1978, Candolfi-Vasconcelos and Koblet 1991, Petrie et al. 2000). However, in this experiment, any possible photosynthetic compensation was apparently overridden by the negative effects of the high levels of GPM infection, as A levels on leaves of Infected plants were consistently lower than those of leaves of Noninfected plants. The lack of compensation was also evident in the reduced k and φ of infected plants; previous studies of photosynthetic compensation for reduction in leaf area on sour cherry showed that k and, to a lesser extent, φ increased after partial (20%) defoliation (Layne and Flore 1992). Disease severity in this experiment was much higher than 20%.

Increased l_g in Infected leaves also shows stomatal influences on A, and implies that the stronger positive relationship between g_s and A on Infected leaves might be partially alleviated by increased stomatal resistance. The lack of decrease in A at saturating PAR for either Infected or Noninfected plants indicates that ribulose-1,5-bisphosphate (RuBP) regeneration capacity is not affected by GPM infection.

Photosynthetic responses of plants in response to infection by foliar pathogens vary with the nature of the infection (Shtienberg 1992). Results from this experiment are consistent with those to be expected from necrosis of palisade cells, with which GPM has been associated (Lakso et al. 1982), but not by interfering with specific metabolic CO_2 assimilation pathways. The reduction in carboxylation efficiency was similar to that observed in winter wheat infected with powdery mildew (Rabbinge et al. 1985). The reduced electron transport in response to powdery mildew of barley (Holloway et al. 1992), attributed to the destruction of chloroplasts and not inhibition of metabolic pathways, also resembled the results of this study. Powdery mildew of sugar beets did alter metabolic pathways by reducing enzyme activity (Magyarosy et al. 1976), indicating that the mechanisms of inhibition of the photosynthetic apparatus vary with the obligate pathogen and/or host plant reaction.

Results from these experiments suggest that GPM inhibits single leaf A in grapevines by quantitatively interfering with the carbon assimilation apparatus of individual leaves. These reductions in A are caused mostly by disruptions of stomatal and photochemical functions. Cultural practices designed to reduce GPM infection of berries in vineyards may have both short- and long-term health benefits for grapevines as a result of a lack of GPM-induced reduction of A in foliage. Additional research should address the impact of lower levels of GPM on the photosynthetic apparatus of individual leaves and whole vines.

Literature Cited

Boote, K.J., J.W. Jones, J.W. Mishoe, and R.D. Berger. 1983. Coupling pest to crop growth simulators to predict yield reductions. Phytopathology 73:1581-1587.

Boucher, T.J., D. G. Pfeiffer, J.A. Barden, and J.M. Williams. 1987. Effects of simulated insect injury on net photosynthesis of potted grapevines. HortScience 22:927-928.

Buwalda, J.G. and G. Noga. 1994. Intra-plant differences in leaf chlorophyll fluorescence parameters in perennial fruiting plants. New Zealand J. Crop Hort. Sci. 22:373-380.

Candolfi-Vasconcelos, M.C. and W. Koblet. 1990. Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*- Evidence of compensation and stress recovering. Vitis 29:199-221.

Candolfi-Vasconcelos, M.C. and W. Koblet. 1991. Influence of partial defoliation on gas exchange parameters and chlorophyll content of field-grown grapevines. Mechanisms and limitations of the compensation capacity. Vitis 30:129-141.

Candolfi-Vasconcelos, M.C., W. Koblet, G.S. Howell, and W. Zweifel. 1994. Influence of defoliation, rootstock, training system, and leaf position on gas exchange of Pinot noir grapevines. Am. J. Enol. Vitic. 45:173-180.

Chellemi, D.O. and J.J. Marois. 1992. Influence of leaf removal, fungicide applications, and fruit maturity on incidence and severity of grape powdery mildew. Am. J. Enol. Vitic. 43:53-57.

Dorster, M.A. and W.C. Schnathorst. 1985. Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus *Uncinula necator*. Am. J. Enol. Vitic. 36:101.

Downton, W.J.S., W.J.R. Grant, and B.R. Loveys. 1987. Diurnal changes in the photosynthesis of field-grown grape vines. New Phytol. 105:71-80.

Flore, J.A. and C. Irwin. 1983. The influence of defoliation and leaf injury on leaf photosynthetic rate, diffusive resistance, and whole tree dry matter accumulation in apple. HortScience 18:72 (abstr.)

Flore, J.A. and A.N. Lakso. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *In* Horticultural Reviews Vol. II. J. Janick (Ed.), pp. 111-157. Timber Press, Portland, OR.

Gadoury, D.M., R.C. Seem, R.C. Pearson, W.F. Wilcox. 2001. Effects of powdery mildew on vine growth, yield, and quality of Concord grapes. Plant Dis. 85:137-140.

Goodman, R.N., Z. Kiraly, and K.R. Wood. 1986. Photosynthesis. *In* The Biochemistry and Physiology of Plant Diseases. R.N. Goodman, (Ed.), pp. 46-74. University of Missouri Press, Columbia, MO.

Hofäcker, W. 1978. Investigations on the photosynthesis of vines. Influence of defoliation, topping, girdling and removal of the grapes. Vitis 17:10-22.

Holloway, P.J., D.J. Maclean, and K.J. Scott. 1992. Electron transport in thylakoids isolated from barley leaves infected by the powdery mildew fungus (*Erysiphe graminis* DC. Ex Merat f.sp. *hordei* Marchal). New Phytol. 120:145-151.

Intrieri, C., S. Poni, B. Rebucci, and E. Magnanini. 1997. Effects of canopy manipulations on whole-vine photosynthesis: Results from pot and field experiments. Vitis 36:167-173.

Johnson, K.B. 1987. Defoliation, disease, and growth; A reply. Phytopathology 77:1495-1497.

Jones, H.G. 1985. Partitioning stomatal and non-stomatal limitation to photosynthesis. Plant Cell Environ. 8:95-104.

Krause, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis; The basics. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.

Lakso, A.M., C. Pratt, R.C. Pearson, R.M. Pool, and M.J. Welser. 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (powdery mildew). Phytopathology 72:232-236.

Layne, D.R. and J.A. Flore. 1992. Photosynthetic compensation to partial leaf area reduction in sour cherry. J. Amer. Soc. Hort. Sci. 117:279-286.

Layne, D.R. and J.A. Flore. 1993. Physiological responses of *Prunus cerasus* to whole-plant source manipulation. Leaf gas exchange, chlorophyll fluorescence, water relations and carbohydrate concentrations. Physiologia Plantarium 88:44-51.

Layne, D.R. and J.A. Flore. 1995. End-product inhibition of photosynthesis in *Prunus cerasus* L. in response to whole-plant source-sink manipulation. J. Amer. Soc. Hort. Sci. 120:583-599.

Magyarosy, A.C., P. Schürmann, and B.B. Buchanan. 1976. Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. Plant Physiol. 57:486-489.

Marquardt, D.W. 1963. An algorithm for least squares estimation of parameters. J. Soc. Ind. Appl. Math. 11:431-441.

Ough, C.S. and H.W. Berg. 1979. Powdery mildew sensory effect on wine. Am. J. Enol. Vitic. 30:321.

Pearson, R.C. and A.C. Goheen. 1988. Compendium of Grape Diseases, APS Press St. Paul, MN.

Petrie, P.R., M.C.T. Trought, and G.S. Howell. 2000. Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. Vitis 39:31-36.

Pool. R.M., R.C. Pearson, M.J. Welser, A.N. Lakso, and R.C. Seem. 1984. Influence of powdery mildew on yield and growth of Rosette grapevines. Plant Dis. 68:590-593.

Poston, F.L., L.P. Pedigo, R.B. Pearce, and R.B. Hammond. 1976. Effects of artificial and insect defoliation on soybean net photosynthesis. J. Econ. Entomol. 69:109-112.

Rabbinge, R., I.T.M. Jorritsma, and J. Schans. 1985. Damage components of powdery mildew in winter wheat. Neth. J. Plant Pathol. 91:235-247.

Shtienberg, D. 1992. Effects of foliar diseases on gas exchange processes: A comparative study. Phytopathology 82:760-765.

Stacey, D.L. 1983. The effect of artificial defoliation on the yield of tomato plants and its relevance to pest damage. J. Hort. Sci. 58:117-120.

Yarwood, C.E. 1967. Responses to parasites. Ann. Rev. Plant Physiol. 18:419-438.

APPENDIX

Table 1. Effects of powdery mildew of grape (GPM) infection on single leaf photosynthesis (Pn), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration (E) of potted Chardonnay grapevines at four phenophases in Year 1. Grapevines were either infected or not infected with GPM.

Time of inoculation	Most recent fully expanded leaf @bloom				Most recent fully expanded leaf @5mm berry size				Most recent fully expanded leaf @pre-veraison				Most recent fully expanded leaf post-veraison*			
	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E
Infected	15.1 b	278	222	6.56	9.9 b	246	243	3.67	8.9 b	117	188	1.56	2.5 b	76 b	275	1.29
Control	17.6 a	305	221	6.27	12.9 a	161	234	3.88	10.4 a	139	193	1.84	5.8 s	120 a	240	1.92
p-value	0.0004	0.1261	0.7067	0.0996	0.0044	0.5115	0.0577	0.4425	0.0437	0.2247	0.6940	0.2113	0.0064	0.0183	0.0593	0.0552

Time of inoculation	Most recent fully expanded leaf @bloom				Original most recent fully expanded leaf @5mm berry size				Original most recent fully expanded leaf @pre-veraison				Original most recent fully expanded leaf post-veraison*			
	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E
Infected	15.1 b	278	222	6.56	10.7	129	183	2.54	4.9	54	198	0.67	-0.1	26 a	363	0.55 a
Control	17.6 a	305	221	6.27	12.2	169	194	3.01	4.9	55	201	0.68	0.3	0.66 b	323	0.33 b
p-value	0.0004	0.1261	0.7067	0.0996	0.0209	0.0009	0.0933	0.0024	0.6245	0.8435	0.8868	0.9048	0.4247	0.0132	0.1234	0.0134

*High degree of senescence, especially on 'Early' and 'Late' treatments.

Table 2. Effects of powdery mildew of grape (GPM) infection on single leaf photosynthesis (Pn), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration (E) of potted Chardonnay grapevines at four phenophases in Year 2. Grapevines were either infected or not infected with GPM.

Time of inoculation*	Most recent fully expanded leaf @bloom				Most recent fully expanded leaf @5mm berry size				Most recent fully expanded leaf @midseason				Most recent fully expanded leaf @veraison				Most recent fully expanded leaf @harvest			
	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E
Infected	10.7	170	200	5.12 a	11.4	145	174	4.12	10.1	108	161	2.21	6.4 b	71 b	184 a	2.01 b	3.2 b	46 b	231 a	0.88 b
Control	12.4	167	180	4.03 b	11.4	150	177	4.23	11.0	114	153	2.37	11.9 a	136 a	171 b	3.34 a	10.7 a	113 a	170 b	1.86 a
p-value	0.0998	0.8927	0.0853	0.0145	0.9747	0.386	0.2965	0.3577	0.1153	0.4629	0.2112	0.4335	<0.0001	<0.0001	0.0017	<0.0001	<0.0001	<0.0001	0.0005	<0.0001

Time of inoculation	Most recent fully expanded leaf @bloom				Original most recent fully expanded leaf @5mm berry size				Original most recent fully expanded leaf @midseason				Original most recent fully expanded leaf @veraison				Original most recent fully expanded leaf @harvest			
	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E
Infected	10.7	170	200	5.12 a	9.9	132	180	3.91	8.4	86	152	1.9	3.1 b	39 b	222 a	1.24 b	1.1 b	41 b	273 a	0.8 b
Control	12.4	167	180	4.03 b	9.9	132	182	3.98	9.1	92	157	1.97	7.8 a	76 a	158 b	2.12 a	6.2 a	67 a	181 b	1.23 a
p-value	0.0998	0.8927	0.0853	0.0145	0.9824	0.9759	0.5652	0.7285	0.1017	0.412	0.5252	0.532	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0095	0.0054	0.0109

Whole Vine Pn

Time of inoculation	1200 DD	Veraison	Harvest
Infected	8.7 b	7.6	3.1 b
Control	11.8 a	9.9	7.4 a
p-value	0.0026	0.0642	0.0097

Table 3. Effects of powdery mildew of grape (GPM) infection on single leaf photosynthesis (Pn), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration (E) of potted Chardonnay grapevines at four phenophases in Season 1. Grapevines were inoculated with *Uncinula necator* prior to bloom (Early), after the 5mm berry stage (Late), or not inoculated (Control).

Time of inoculation	Most recent fully expanded leaf @bloom				Most recent fully expanded leaf @5mm berry size				Most recent fully expanded leaf @pre-veraison				Most recent fully expanded leaf post-veraison*			
	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E
Early	15.1 b	278	222	6.27	9.9 b	246	243	3.67	9.1 b	117	180	1.58	2.4 b	76.4 b	279 a	1.32 b
Late	16.4 a	311	224	6.56	13.0 a	256	231	3.79	8.7 b	118	196	1.55	2.7 b	74.7 b	269 a	1.27 b
Control	17.1 a	299	217	6.47	12.7 a	269	237	3.92	10.4 a	139	193	1.84	5.8 a	120 a	240 b	1.92 a
p-value	0.0011	0.0948	0.3573	0.2112	0.0087	0.6967	0.4004	0.6134	0.0183	0.2564	0.2623	0.2109	0.0009	0.0051	0.0445	0.0170

Time of inoculation	Most recent fully expanded leaf @bloom				Original most recent fully expanded leaf @5mm berry size				Original most recent fully expanded leaf @pre-veraison				Original most recent fully expanded leaf post-veraison*			
	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E	Pn	g_s	C_i	E
Early	15.1 b	278	222	6.27	10.6 b	128 b	183	2.54 b	4.5	48.7 b	197	0.6 b	0.2	24.4 ab	354	0.54
Late	16.4 a	311	224	6.56	12.2 a	166 a	195	3 a	5.4	59.5 a	199	0.72 a	-0.2	27.1 a	369	0.57
Control	17.1 a	299	217	6.47	12.2 a	172 a	195	3.03 a	4.9	54.7 ab	201	368 ab	0.3	15.6 b	323	0.33
p-value	0.0011	0.0948	0.3573	0.2112	0.0096	0.0006	0.1947	0.0005	0.3635	0.0429	0.9423	0.0496	0.5564	0.0444	0.2884	0.0513

*High degree of senescence, especially on 'Early' and 'Late' treatments.

Table 4. Effects of powdery mildew of grape (GPM) infection on fresh and dry weights of potted Chardonnay grapevines. Grapevines were inoculated with *Uncinula necator* prior to bloom (Early), after the 5mm berry stage (Late), or not inoculated (Control).

Season 1

Time of inoculatior	Fresh Weight (g)					Dry Weight (g)				
	Root	Trunk	Shoot	Leaf	Total	Root	Trunk	Shoot	Leaf	Total
Early	48.9 b	32.9	41 b	38.2	161 b	20.9 b	15.8	10 b	24.9	59.9 b
Late	62 a	34.4	50.4 a	39.8	187 a	26.1 a	18.3	13.8 a	26.3	73.7 a
Control	58.5 a	33.8	48.7 a	43.2	184 a	25.9 a	17.8	13.6 a	27.7	74.2 a
p-value	0.0096	0.7486	0.0038	0.4678	0.0086	0.0480	0.1211	0.0106	0.5483	0.0089

Season 2

	Fresh Weight (g)					Dry Weight (g)				
	Root	Trunk	Shoot	Leaf	Total	Root	Trunk	Shoot	Leaf	Total
Infected	90.6 b	38.1	20.5 b	17 b	162 b	48.2 b	21.3	9.9 b	5.2 b	80.3 b
Noninfected	124 a	40.7	23.3 a	41.2 a	230 b	63.1 a	21.6	11.9 a	13.1 a	102 a
p-value	<0.0001	0.1582	0.0212	<0.0001	<0.0001	<0.0001	0.6995	0.0012	<0.0001	0.0012

	Fresh Weight (g)					Dry Weight (g)				
	Root	Trunk	Shoot	Leaf	Total	Root	Trunk	Shoot	Leaf	Total
Early	92.9 b	38.7	21.5 ab	18.3 b	162 b	46.9 b	21.5	10.2 b	5.6 b	81.9 b
Late	88.4 b	37.6	19.6 b	15.6 b	161 b	44.7 b	21	9.5 b	4.8 b	78.7 b
Control	124 a	40.7	23.3 ab	41.2 a	230 a	63.1 a	21.6	11.9 a	13.1 a	102 a
p-value	<0.0001	0.2189	0.0104	<0.0001	<0.0001	<0.0001	0.8072	0.0007	<0.0001	<0.0001

Table 5. Effects of powdery mildew of grape (GPM) infection on fresh and dry weights of potted Chardonnay grapevines in Season 2. Grapevines were inoculated with *Uncinula necator* prior to bloom (Early(2°)), after the 5mm berry stage (Late(2°)), or not inoculated (Control(2°)) in Season 1, and were not infected with GPM in Season 2.

	Fresh Weight (g)						
Time of inoculation	Root	Trunk	Shoot	Leaf	Fruit	Total Plant (including fruit)	Total Plant (no fruit)
Early(2°)	160.9 b	50.2	40.7 c	97.2 b	356.9	702.8	349.1 b
Late(2°)	183.7 ab	52.3	47.1 b	105.6 ab	388.6	777.3	388.7 ab
Control(2°)	225.1 a	52.9	54.8 a	113.6 a	389.1	820.1	431 a
p-value	0.0057	0.2822	0.0016	0.0380	0.5868	0.0515	0.0124

	Dry Weight (g)						
Time of inoculation	Root	Trunk	Shoot	Leaf	Fruit	Total Plant (including fruit)	Total Plant (no fruit)
Early(2°)	72.9 b	32.6	18.4 c	35.1 b	81.4	240.3 c	159 b
Late(2°)	80.8 ab	33.8	21.3 b	38 ab	96	269.8 b	173.9 ab
Control(2°)	95.6 a	33.5	25.7 a	41.3 a	96.1	292.3 a	196.2 a
p-value	0.0072	0.4113	0.0002	0.0452	0.1993	0.0004	0.0045

	Shoot Lengths (mm)				
Time of inoculation	Bloom	5mm Berry Size	1200 DD	Veraison	Harvest
Early(2°)	240 c	250 b	255 b	252 b	238 b
Late(2°)	269 b	281 b	280 ab	273 b	275 ab
Control(2°)	297 a	308 a	311 a	307 a	292 a
p-value	0.003	0.0059	0.0119	0.0113	0.0138