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The Influence of Mites and a Photosynthetic
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Mark Anthony Hubbard

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Ph.D degree in Horticulture

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**THE INFLUENCE OF MITES AND A PHOTOSYNTHETIC
INHIBITOR (TERBACIL) ON PHOTOSYNTHESIS AND YIELD OF
SOUR CHERRY (*PRUNUS CERASUS* L. 'MONTMORENCY')**

By

Mark Anthony Hubbard

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

1995

ABSTRACT

THE INFLUENCE OF MITES AND A PHOTOSYNTHETIC INHIBITOR (TERBACIL) ON PHOTOSYNTHESIS, CARBON ACCUMULATION AND YIELD OF SOUR CHERRY (*PRUNUS CERASUS* L. 'MONTMORENCY')

By

Mark Anthony Hubbard

Orchard and container-grown sour cherry (*Prunus cerasus* L. 'Montmorency') trees were infested with two-spotted spider mites (*Tetranychus urticae*) to study mite-feeding effects on photosynthetic parameters, cold hardiness, starch accumulation and reproduction effort (flowering and yield). In the field, mite populations reached 1764 average cumulative mite•days (CMD) for mite-infested trees (MIT) and 1093 CMD for controls. Chlorophyll was reduced in MIT compared to controls late in the growing season; however, there was no reduction during the season in measured photosynthetic parameters, stem cambium cold hardiness or shoot starch levels. Additionally, miticides used commercially in Michigan tart cherry orchards were determined to have no effect on photosynthetic parameters. There was an increase in spring root starch levels of MIT the spring following infestation, but no effect on return fruit set or return yield. In the container grown trees, average CMD reached 917 for MIT and 90 for controls. Reductions were observed in single leaf A, whole tree A and chlorophyll fluorescence. This suggests that mite populations sustained in the orchard in 1993 were not high enough to reduce subsequent year yield, and therefore, control measures were of no economic benefit. The field trees demonstrated an ability to buffer the effects of mite feeding and maintain

reproductive capacity. Reduction in photosynthesis does occur, as in the container study, and that reduction in photosynthesis could ultimately affect yield and hardiness. In order to study photosynthetic reduction thresholds that affect tree hardiness and yield, trees were treated with 50-63 ppm terbacil to limit photosynthesis for specific periods of time.

Terbacil was applied to orchard trees at 4 physiological stages: fruit growth stages I and II, fruit harvest, and prior to leaf fall; at each date, A was reduced up to 60% within 24 h and showed 100% recovery within 7-10 days. Reductions were also observed in whole tree A and chlorophyll fluorescence, but not in chlorophyll, shoot cold hardiness, fruit yield or fruit quality. Root and shoot starch levels did vary slightly dependent on the timing of terbacil application but there was no effect on return yield.

DEDICATION

This dissertation is dedicated to Joanna for her sacrifice, love and encouragement. I was told years ago that the spouse of the Ph.D candidate does as much work for the degree than the candidate, and I believe it. This is also dedicated to Sarah who is a blessing from God and is due a lot of time from her father who spent too long in the lab and field during her first years.

ACKNOWLEDGMENTS

I am forever indebted to Jim Flore for his guidance, patience, constant support and his great help in completing this work. Thanks to Jim I have learned much more than could ever be written down or told concerning pomology, horticulture and science in general. Jim has also taught me many "cultural" things as well.

I am also grateful to my committee members for their encouragement and support. Dr. Ken Poff has always been available for discussion as well as keeping things in perspective with a sharp wit. Dr. Don Dickmann has provided much wisdom and encouragement and I am grateful to him for all his advice. Dr. Stan Ries has taught me many things and has provided much encouragement and advice. Also, I am thankful to Dr. M. John Bukovac for his friendship and his ability to inspire, as well as his support.

This work would never have been started had it not been for Lynne Sage and it would never have been finished without Sarah Breitzkreitz. I am grateful to both!!

There have been a host of people at Michigan State who have in one way or another contributed to my education and this work in particular. I am thankful to Des, Tom, Mark, Edgardo, Moreno, Ricardo, Claudio, Maurizio I, Maurizio II, Brent, John, Rebecca, Beth, Dave, Jon and the personnel at Clarksville and the Hort Farm. I have no doubt left someone out.

Finally, I must acknowledge that my motivation and source of strength are in my friend and savior Jesus Christ! His constant love saw this work through!

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ABBREVIATIONS

A.....	carbon dioxide assimilation
CMD	cumulative mite•days
Conventional.....	conventional level of chemical input
EPA.....	Environmental Protection Agency
Fv/Fm.....	variable fluorescence to maximal fluorescence ratio
GC	gas chromatography
gm	mesophyll conductance
gs.....	stomatal conductance
E.....	transpiration
IPM	integrated pest management
Low	low level of chemical input
Moderate.....	moderate level of chemical input
MPL.....	mites per leaf
Mylar.....	polyvinylidene coated polyethylene terephthalate
Omite....	propargite [2-(p-tert-butylphenoxy) cyclohexyl 2-propynyl sulfite]
Vendex...	fenbutin-oxide[Hexakis(2-methyl-2-phenylpropyl)-distannoxane]
WUE.....	water use efficiency
X-77	alkylaryl polyoxyethylene, glycol, fatty acids and isopropanol mix

Guidance Committee:

The journal–article format was adopted for this dissertation in accordance with departmental and university requirements. The manuscript was prepared for publication in the *Journal of the American Society for Horticultural Science*. The manuscript in Appendix H was prepared for publication in *HortScience*.

Introduction

Insects, mites and diseases present on leaves of fruit crop plants can have a number of deleterious effects on both vegetative growth and fruit development. These pests damage the photosynthetic machinery and reduce the potential of carbohydrates produced by those leaves, and a threshold is ultimately reached at which vegetative and reproductive growth is reduced. Mites (Acari: Tetranychidae) and other insects feeding on apple (*Malus* sp.) leaves reduce photosynthesis (Ferree, *et al.*, 1986; Hall and Ferree, 1975; Proctor *et al.*, 1982), growth, yield and return fruiting (Hull and Beers, 1990; Beers and Hull, 1987; Bailey, 1979). Reductions in CO₂ assimilation as a result of mite-feeding have also been demonstrated in peach, *Prunus persica* (Anderson and Mizell, 1987; Mobley and Marini, 1990), almond, *Prunus amygdalus* (Youngman, *et al.*, 1986), and grape, *Vitis* (Candolfi, *et al.*, 1992). However, only at very high population levels do mites affect photosynthesis, vegetative growth and yield of apple (Hull and Beers, 1990; Beers and Hull, 1987), and there was little effect on yield, fruit quality and vegetative growth of peach (McClerman and Marini, 1986). Photosynthesis was not affected by feeding of European red mite (*Panonychus ulmi*) on grape, although it was reduced by two-spotted spider mite (*Tetranychus urticae*) (Candolfi, *et al.*, 1993).

Specific low levels of pest populations that do not affect total yield and fruit quality can presumably be tolerated in an orchard. The reduction of photosynthesis and the resulting effects on cropping have not been studied on sour cherry, and therefore, biological, action and economic thresholds have not been established. Relatively low numbers of insects or mites feeding on the leaves does not result in lower yield or fruit quality (Mobley and Marini, 1990). The mechanism of tolerance may be that the tree either

alters carbon assimilation primarily toward fruit production at the expense of vegetative growth and storage or leaves compensate for lost photosynthetic tissues by increasing photosynthesis in the remaining tissues (Layne and Flore, 1992).

It is not clear how reduced vegetative growth and carbohydrate storage from mite or insect feeding affects physiological processes such as cold acclimation and hardiness of tissues and subsequent-year flower initiation and fruit development. These factors are particularly important with regard to sour cherry as insect and mite populations are often highest after sour cherry harvest when flower bud development and carbohydrate storage are occurring. Howell and Stackhouse (1973) have demonstrated that severe defoliation caused by cherry leaf spot can reduce subsequent years yield. Therefore, establishing insect and mite feeding thresholds that do not adversely affect these vegetative and reproductive processes for sour cherry orchards would be an important tool for orchard pest management. Also, as carbohydrate demand is lower after sour cherry harvest (Flore, 1994), thresholds for pest damage could be considerably higher than during the period prior to harvest, and considerably higher than thresholds established for apples. Additionally, it would be of value to growers to develop a system that could be applied in the field for early detection of photosynthetic injury and ultimately a photosynthetic threshold for management of phytophagous pests.

The application of chemical pesticides can be costly and may act on beneficial organisms as well as the targeted pest. It has also been demonstrated that many insecticides can reduce photosynthesis (Ayers and Barden, 1975; Andersen, *et al.*, 1986). Therefore, the use of pesticides can act to inhibit photosynthesis, reduce populations of natural predators and increase the levels of residues in the fruit. This calls into question the necessity of some pesticide applications, particularly for mites on sour cherry, in light of the lack of information on the effects of mite feeding on sour cherry trees.

The objectives of this research were to characterize the effects of mite-feeding on photosynthetic parameters, starch accumulation, cold hardiness and cropping; study mite

population thresholds that have no adverse effect on tree growth; determine the effects of miticide application on photosynthesis; and characterize the effects of photosynthetic reduction on these parameters and establish photosynthetic reduction thresholds that have no adverse effect on tree growth.

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**THE INFLUENCE OF MITES AND A PHOTOSYNTHETIC INHIBITOR
(TERBACIL) ON PHOTOSYNTHESIS AND YIELD OF SOUR CHERRY
(*PRUNUS CERASUS* L. 'MONTMORENCY')**

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Received for publication

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Cellular and Whole Plant Physiology

The Influence of Mites and a Photosynthetic Inhibitor (Terbacil) on Photosynthesis, Carbon Accumulation and Yield of Sour Cherry (*Prunus cerasus* L. 'Montmorency')

Additional index words. IPM, pest management, miticides, chlorophyll fluorescence.

Abbreviations: Carbon dioxide assimilation, A; transpiration, E; stomatal conductance, g_s ; mesophyll conductance, g_m ; water use efficiency, WUE; variable fluorescence to maximal fluorescence ratio, Fv/Fm; cumulative mite•days, CMD; mites per leaf, MPL; mite–infested trees, MIT; Omite: Propargite [2-(p-tert-butylphenoxy) cyclohexyl 2-propynyl sulfite]; Mylar: polyvinylidene coated polyethylene terephthalate; Vendex, Fenbutin-oxide[Hexakis(2-methyl-2-phenylpropyl)-distannoxane]; Omite, Propargite [2-(p-tert-butylphenoxy) cyclohexyl 2-propynyl sulfite]; X-77, alkylaryl polyoxyethylene, glycol, free fatty acids, isopropanol proprietary mix (Valent).

Abstract. Orchard and container–grown sour cherry (*Prunus cerasus* L. 'Montmorency') trees were infested with two–spotted spider mites (*Tetranychus urticae*) to study mite–feeding effects on photosynthetic parameters, cold hardiness, starch accumulation and reproduction effort (flowering and yield). In the field, mite populations reached 1764 average cumulative mite•days (CMD) for mite–infested trees (MIT) and 1093 CMD for controls. Chlorophyll was reduced in MIT compared to controls late in the growing season, yet there was no reduction during the season in measured photosynthetic parameters, stem cambium cold hardiness or shoot starch levels. Additionally, miticides used commercially in Michigan tart cherry orchards were determined to have no effect on photosynthetic parameters. There was an increase in spring root starch levels of MIT the spring following infestation, but no effect on return fruit set or return yield. In the

container grown trees, average CMD reached 917 for MIT and 90 for controls. Reductions were observed in single leaf A, whole tree A and chlorophyll fluorescence. This suggests that mite populations sustained in the orchard in 1993 were not high enough to reduce subsequent year yield, and therefore, control measures were of no economic benefit. The field trees demonstrated an ability to buffer the effects of mite feeding and maintain reproductive capacity. Reduction in photosynthesis does occur, as in the container study, and that reduction in photosynthesis could ultimately affect yield and hardiness. In order to study photosynthetic reduction thresholds that affect tree hardiness and yield, trees were treated with 50-63 ppm terbacil to limit photosynthesis for specific periods of time. Terbacil was applied to orchard trees at 4 physiological stages: fruit growth stages I and II, fruit harvest, and prior to leaf fall; at each date, A was reduced up to 60% within 24 h and showed 100% recovery within 7-10 days. Reductions were also observed in whole tree A and chlorophyll fluorescence, but not in chlorophyll, shoot cold hardiness, fruit yield or fruit quality. Root and shoot starch levels did vary slightly dependent on the timing of terbacil application but there was no effect on return yield.

The infestation of fruit orchards with mite species has increased significantly in recent years with the increased use of pyrethroid insecticides to control insect pests as those insecticides significantly reduce the populations of predatory mites that feed on phytophagous mites in the orchard. The result has been an increase in damage to the orchard trees as a result of higher populations of two-spotted mite and European red mite (*Panonychus ulmi*). Growers are currently using a number of pesticide applications aimed specifically at phytophagous mites. We estimated that one miticide application to 80% of Michigan sour cherries could cost growers between \$2.4 and 3.0 million and result in the use of 18,000 to 36,000 pounds of active ingredient, depending on the compound chosen.

The effects of phytophagous mites on fruit trees such as apple and peach have been well documented (Avery and Briggs, 1968; Bailey, 1979; Beers and Hull, 1987; Lienk, *et al.*, 1956; Mobley and Marini, 1990; Zwick, *et al.*, 1976) while research on the effects of phytophagous mite feeding on sour cherry has been limited. Jones (1990a) conducted a study on predator : prey relationships in sour cherry and later considered effects of pesticide applications on mite activity (Jones, 1990b). There have been no reports on the effects of phytophagous mite feeding on photosynthetic parameters and yield components of sour cherry. As damage to sour cherry leaves is visibly similar and proximate in time to apple leaf damage, the abundant research conducted on mite-feeding and apple tree photosynthesis has been extrapolated to management of sour cherries. Unlike apple and peach, sour cherry orchards experience high mite populations and damage primarily after the current season's crop has been harvested. The current season's yield of peach and apple can be adversely affected by mid- and late-summer mite populations (Ames, *et al.*, 1984; Bailey, 1979; McClellan and Marini, 1986) as can pear (Westgard, *et al.*, 1986) and grape (Welter, *et al.*, 1989). Additionally, this late season mite feeding has been shown to reduce apple flowering and yield in subsequent years (Beers and Hull, 1987; Beers and Hull, 1990). Utilizing these factors, thresholds have been developed for mite populations in apple and grape production in particular (Hull and Beers, 1990; Candolfi, *et al.*, 1993).

Several distinct thresholds for management purposes can be developed. The biological threshold is that population of mites that causes a biological response, such as a reduction in A, and is therefore reached at relatively low population of mites. The action threshold is the population level that dictates an action on part of the orchard manager to prevent adverse effects on the total crop. It presumably would occur at mite populations at least as high as those for the biological threshold. Ultimately, the economic threshold is reached, which is the mite population that results in an economic loss to the orchard

production system. Ideally, the biological threshold can be tolerated but is used as a guide to predict the action threshold which is then used to avoid reaching the economic threshold.

The question arises as to whether sour cherry can be affected by late season mite-feeding to the extent that flowering and yield are reduced in the subsequent year as can occur with apple (Beers and Hull, 1990). If yield is adversely affected, are the similarities with apple trees sufficient to warrant using apple threshold levels? Sour cherry growers are currently applying miticides at a substantial cost, and as no research has been conducted on thresholds for subsequent-year fruiting of sour cherry, the assumption is that sour cherries are damaged by late season mite-feeding to the same extent as apples.

In addition to damage caused by the pests themselves, some pesticides have been shown to limit photosynthesis of sour cherry (Murphy, 1937) as well as apple and peach (Ayers and Barden, 1975; Andersen, *et al.*, 1986). Repeated applications of pesticides can also reduce photosynthesis (Ferree and Hall, 1978). It is unclear if miticide chemicals have an adverse effect on tree photosynthesis and growth apart from any beneficial effect on pest populations.

Photosynthetic reduction in the orchard can occur as a result of any number of abiotic or biotic stresses apart from mite damage. Plants can compensate for moderate degrees of photosynthetic loss or for partial defoliation by an increase in photosynthetic rate of the undamaged portions of the leaves. Layne and Flore (1992) suggested that a loss of up to 20% of sour cherry leaf area resulted in no net reduction of photosynthesis. Similar results have been shown with other plants (Bassman and Dickmann, 1982; Proctor, *et al.*, 1982; Satoh, *et al.*, 1977). Increased damage, particularly from insects or mites, will ultimately reduce photosynthesis to a point that the leaf or tree can no longer compensate and net photosynthesis will decline. Reduced photosynthesis can have a variety of effects on the tree including reduced growth and yields (Bailey, 1979; Hull and Beers, 1990; Beers and Hull, 1987), and reduced carbohydrate levels and cold hardiness (Flore, *et al.*, 1983). Tissue hardiness has been correlated with starch concentration in peach (Flore *et*

al., 1987; Lasheen *et al.*, 1970), but low starch concentration does not necessarily result in lower hardness. The same factors that promote starch concentrations may also promote cold hardness (Flore and Howell, 1987). Of obvious importance is a reduction in yield, which can also occur in years subsequent to the season in which the photosynthetic reduction actually occurred. No studies have considered the amount of photosynthetic reduction that can occur in sour cherry before yield or tree hardness is affected, or how the timing of any photosynthetic reduction could effect yield and hardness.

Terbacil is the active ingredient of a commercial herbicide that inhibits photosynthesis by blocking the flow of electrons in photosystem II (Gardiner, 1981). At the labeled concentration of 2-4 kg•ha⁻¹ of concentrated spray volume (7676-15353 ppm terbacil), terbacil is an effective herbicide used in production of many fruit crops, but has been used to limit photosynthesis in apples for the purpose of fruit thinning (Byers *et al.* 1990). We hypothesized that at these significantly reduced concentrations, 50-200 ppm, terbacil would inhibit photosynthesis of sour cherry for a limited time after which the trees would recover to their full photosynthetic capacity. This temporary inhibition could mimic the effect of mite, insect or disease damage on plant photosynthesis, after which, control measures relieve the pest-induced inhibition and the tree typically recovers to full photosynthetic capacity.

A series of experiments were undertaken to address those unknowns and obtain information useful in sour cherry orchard management. A study was initiated to determine sour cherry leaf mite populations that reduced chlorophyll levels, photosynthesis and chlorophyll fluorescence. As any reduction in these parameters would not affect the current season's crop, consideration was to be given to the effects on hardness and cropping in the following season. Therefore, measurements were made on the effect of mite populations on stem cambium cold hardness, tissue starch levels, return fruiting characteristics and return yield. Of additional interest was the use of chlorophyll fluorescence measurements as an indicator of plant stress due to mite damage. Chlorophyll fluorescence, the Fv/Fm

ratio in particular, is a sensitive indicator of the physiological status of the photosynthetic apparatus (Krause and Weis, 1991). As continuous mite-feeding increasingly damages the photosynthetic apparatus of the leaf (Avery and Briggs, 1968), the fluorescence parameters should exhibit corresponding alterations. It was hypothesized that periodic chlorophyll fluorescence measurements would detect the mite damage to the leaf in advance of other measurable parameters. If such were the case, this technique would allow growers a method of early detection of mite injury – an early biological threshold – and become a valuable tool in orchard management in predicting action thresholds. A final objective was to reduce photosynthesis in a sour cherry orchard via application of a dilute terbacil concentration to study how the resulting photosynthetic reductions affect yield and tree hardiness.

Materials and Methods

Plant Materials. A sour cherry orchard established in 1982 at Clarksville Michigan was utilized for the mite-feeding and terbacil application experiments. The container studies utilized one-year-old sour cherry trees obtained from Newark Nurseries (Hartford, MI) that were potted in 12 L pots with a field soil mix, consisting of 7:1:1, soil:sand:organic matter and the trees pruned to two buds on a single stem. The mite study trees were grown outdoors under natural conditions in March–August 1994 for 45-60 days by which time there were ten or more fully expanded leaves before infesting with mites. The plants used for miticide studies were grown in a greenhouse on the Michigan State University campus with day temperatures of 25–30°C and night temperatures of 17–21°C under natural light conditions in March through May 1994. All trees were watered as needed, and fertilized biweekly with soluble 20–20–20 fertilizer at the rate of 60 g•L⁻¹.

Photosynthesis. All single leaf photosynthesis was measured from the 2 most recently expanded leaves using portable infrared gas analyzer units (ADC, Hoddesdon,

UK, model LCA-2, and P.P. Systems, Haverhill, Mass., model CIRAS) and Parkinson leaf chambers. The units measured and recorded net change in chamber CO₂, leaf and air temperatures, ambient and leaf chamber humidities, humidity increase due to transpiration, and incident light radiation. Net A; transpiration, E; stomatal conductance, g_s; mesophyll conductance, g_m; and water use efficiency, WUE were calculated using a BASIC computer program developed by Moon and Flore (1986), or when using the PP Systems unit, these parameters were calculated automatically by the unit.

Whole tree photosynthesis was measured with whole tree gas exchange chambers (Figure 1) that utilized a fan-forced air inlet; CO₂ concentrations were monitored at the inlet and the outlet using portable infrared gas analyzer units described above. The 44.9 m³ chambers were transparent Mylar (DuPont) and the seams were sealed with transparent tape (3M Corp.). Industrial type fans were utilized that introduced flow rates ranging from 2400 to 3600 L•min⁻¹. This flow rate was sufficiently high to minimize temperature increase inside the chamber and maximize CO₂ differential, approximately 1 complete exchange of. Flow rate was continuously monitored at the inlet using an anemometer (Cole-Parmer, Tri-sense model 37000-00) and taking the average of measurements across the inlet duct. The chambers were comparable to that described by Corelli-Grappadelli and Magnanini (1993). Smaller chambers were used for whole tree photosynthesis measurements of container trees, and are described in detail elsewhere (Miller, *et al.*, in press). Flow rates for the container tree chambers ranged from 36 to 70 L/min. Assimilation of CO₂ was calculated from the differential CO₂ concentrations and the air flow through the chamber based on the calculations of Moon and Flore (1986). Assimilation was determined on a leaf area basis using an estimation of the whole tree leaf area from data collected by Teichman and Flore (unpublished) for orchard trees and by actual leaf area measurements for container trees. Additionally, A was calculated on a trunk cross sectional area basis.

Chlorophyll Fluorescence. Chlorophyll a fluorescence was measured on the two most recently expanded leaves using one of two fluorescence measurement systems (P.K. Morgan Instruments, Inc., Andover, MA, model CF-1000; Opti-Sciences, Inc., Tewksbury, MA, model OS-500). Dark adaptation cuvettes were attached to leaves on trees of each treatment 20-30 min. prior to measurement. The optical cable of the fluorescence unit was then attached to the cuvette and the leaf irradiated with a pulse of $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 680 nm actinic light for 60 s. The following fluorescence parameters were measured and recorded: initial fluorescence, F_0 ; maximal fluorescence, F_m ; variable fluorescence, $F_v = F_m - F_0$; terminal fluorescence, F_t ; time to one-half fluorescence quenching, $t_{1/2}$; time to fluorescence quenching, t ; and calculation of the ratio F_v/F_m . The F_v/F_m ratio is significant in that it is closely correlated with photochemical efficiency (Krause and Weis, 1991). The Opti-Sciences unit also measured "photosynthetic yield," which is an indicator of quantum yield of photosynthesis, via pulse modulation and measurement of secondary maximal and variable fluorescences (Opti-Sciences, Inc., 1994).

Chlorophyll. Chlorophyll determinations were made by collecting 4 disc samples from the interveinal lamina proximate to the base of 4 different leaves on comparable canopy positions from each tree. The discs were collected using a paper hole punch yielding leaf discs with an area of 0.32 cm^2 and immediately placed in 20 ml glass scintillation vials containing 7 ml of N,N-dimethylformamide and placed on ice. The samples were stored at $+5^\circ\text{C}$ for 48 hours and then the absorbance of the N,N-dimethylformamide solution measured at 623, 647 and 664 nm relative to a N,N-dimethylformamide control. The levels of chlorophyll a, chlorophyll b, protochlorophyll and total chlorophyll were then calculated using the formulas from Moran (1982). The chlorophyll levels were converted to $\mu\text{g}\cdot\text{cm}^{-2}$ of leaf tissue.

Carbohydrate Status. Tissue samples for carbohydrate determinations were collected from the 1993 growing season's shoot and root tissue on November 10, 1993

and April 14, 1994. Current season shoots of 50-80 cm and 10g of freshly dug root tissue were collected from orchard trees and wrapped in polyethylene bags and transported on ice to the laboratory and then 1 cm sections placed in envelopes and frozen. Samples were frozen at -80°C and held for 3-4 weeks and then lyophilized and ground (40 mesh). To 100 mg samples, 3.5 ml of 80% ethanol was added, vortexed and allowed to stand for 30 min. The samples were then centrifuged for 5 min at $1879\times g$ and the supernatant collected for carbohydrate extraction and analysis. The pellet was extracted twice as above, suspended in 80% ethanol, vortexed, and centrifuged. The pellets from the samples were then frozen and dried (Speedvac SC200). Two ml of neutralized acetic acid (0.1 M, pH 5.0) was added to the dried pellet, vortexed gently and incubated at 100°C for 1 h. Samples were cooled to room temperature for 10 min and then 100 μl of amyloglucosidase solution ($0.0166\text{ g}\cdot\text{ml}^{-1}$) was added, the samples gently mixed and incubated at 55°C for 16 h. The samples were then centrifuged for 5 min at $1500\times g$ and 20 μl removed from each sample and combined with 1 ml double distilled H_2O . 250 μl of these sample solutions were combined with 2.5 ml of Sigma color reagent solution and incubated to develop color for 40 min. Absorbance at 440 nm was then measured and the percent starch calculated according to standards of known concentrations.

Cold Hardiness. Cold hardiness of stem cambium tissues was determined on eight dates from October to May based on a method described by Flore *et al.* (1983). On each collection date, six 50-80 cm stems of the most recent seasons growth were cut from the top of the canopy of each tree. Within 3 hours, the stems were prepared for cold hardiness tested as described below. The basal and apical thirds of each stem were discarded, and the middle third, 20-25 cm, was cut into 1.5-2.0 cm sections. These stem sections were taped together, wrapped in wetted gauze, and then rolled inside aluminum foil. Each roll of aluminum contained one stem section from each treatment, and a 26-gauge copper-constantan thermocouple inserted into one sample of each roll to monitor tissue temperature. Sufficient samples were prepared to allow freezing tests at 6 temperatures and

a control held continuously at 5°C. Three replicates for each temperature were prepared. The samples were placed inside a Beckmann freezer modified for precise temperature regulation and cold hardiness studies. The temperature was maintained at -1°C for 8-12 h and then the temperature lowered at the rate of 3°C per hour. Stem temperatures were monitored and 3 replicates removed when predesignated temperatures were achieved. The samples were thawed slowly at 5°C for 20-24 h and then the stem sections removed from the aluminum foil and gauze. The stem samples were then placed in plastic containers and air bubbled through a small volume of water to maximize humidity for 7-10 days. Each of the samples was examined and the cambium visually assessed as alive or dead. Calculations were then made to determine the T_{50} of the cambium for each treatment using the Spearman–Kärber method (Bittenbender and Howell, 1974). The T_{50} was calculated as

$$T_{50} = T_{100} - 0.5d + \frac{d \sum b_i}{n}$$

where

T_{50} = temperature at which 50% of the stems are killed,

T_{100} = temperature at which 100% of the stems are killed,

d = temperature interval between treatments

b_i = number of dead stems in i^{th} temperature

n = number of replicates sampled at each treatment temperature.

Cumulative cold hardiness was calculated as the average T_{50} value over the dormant period for each treatment.

Mite–Feeding Studies. Treatments consisted of control trees to which a miticide was routinely applied and mite-infested trees to which no miticides were applied. The treatments were assigned in the orchard utilizing a randomized complete block design with 4 blocks. Each plot consisted of four trees in a row with the two center trees used as data trees. Bean plants infested with two-spotted spider mites were attached to the trees on July 21, 22 and 23. Naturally occurring populations of European red mite were also present

and counted. Fifty leaves from each tree were collected biweekly and brushed to determine mite populations. Mite•days were used in an effort to express the duration of the mite population levels and calculated as the average number of mites for two consecutive counting dates multiplied by the number of days between the counting dates (Sances, *et al.*, 1981).

Container trees were infested with two-spotted spider mites by attaching infested bean leaves to the stems of the trees. The number of MPL on three representative leaves from each tree was counted weekly. An additional application of infested bean leaves was required after it was determined that there were 0 mites in the trees on 13 July. Trees were blocked by height and a randomized complete block design was used with 11 replications.

Vegetative and Reproductive Growth. In the orchard study, 5 one-year-old shoots were randomly selected from each treatment tree and initial measurements of stem diameter, stem length, number of leaves, fruit diameter, fruit weight and fruit number made on June 3, 1993. The fruit weights were determined by harvesting 30 developing fruit. Fruit diameter, weight and number were measured again on July 22 just prior to harvest by collecting 30 fruit. Stem diameter, stem length and number of leaves were measured again on September 29, 1993.

Miticide Study. After 8 weeks of growth, container trees were blocked according to shoot length and treatments were randomly assigned. The initial greenhouse experiment utilized 6 repetitions and a subsequent experiment utilized 4 repetitions. The treatments, applied by dipping leaves into 1L of solution, were a water control containing 1 ppm proprietary surfactant: alkylaryl polyoxyethylene, glycol, free fatty acids and isopropanol (X-77), Omite applied at the labeled rate of 6 pounds per 400 gallons ($1.8 \text{ g} \cdot \text{L}^{-1}$) with 1 ppm X-77, and Vendex applied at the labeled rate of 8 fluid ounces per 100 gallons ($0.6 \text{ ml} \cdot \text{L}^{-1}$) with 1 ppm X-77. All experiments were designed and analyzed as randomized complete blocks. Treatment differences were considered significant at $P \geq 0.05$ using analysis of variance. Photosynthetic parameters were measured at 0,1,3,5,7 and 10 days

after treatment. Shoot length was measured on the day prior to treatment and 30 days after treatment. Chlorophyll fluorescence parameters were measured at 0,1,3,5,7 and 14 days after treatment. Chlorophyll levels were measured 15 days after treatments were applied.

Terbacil Studies. A preliminary experiment was undertaken to determine how different rates of terbacil herbicide reduced photosynthesis in sour cherry. A sour cherry (*Prunus cerasus* L. 'Montmorency') orchard established in 1982 in East Lansing Michigan was utilized for the concentration study. The treatments were randomly assigned to a single leaves of orchard trees in a randomized complete block design with four repetitions. The experimental design for the greenhouse concentration study was 4 repetitions of each treatment. In both the orchard and greenhouse experiments, single leaves were dipped in 1 L solutions of 0, 25, 50, 100, 200, 400 and 800 ppm terbacil each containing 1 ppm X-77. Chlorophyll fluorescence and A were monitored at days 0, 1, 2, 3, 4, 6, 7 and 14 after treatment.

Four different times of terbacil application and a control were randomly assigned in the orchard in a randomized complete block design utilizing 4 blocks. Each experimental unit consisted of a four tree plot row, with the two center trees used as a data trees. Sixty-three ppm terbacil with 1 ppm X-77 was sprayed to drip, approximately 20L per tree, on the designated plots on the following dates in 1993: June 1 coinciding with stage I of cherry fruit development, June 15 coinciding with pit hardening or stage II, August 4 at harvest, and September 17, 6 weeks prior to leaf fall. The terbacil was applied using a handgun sprayer at mid-morning of each date.

Vegetative and Reproductive Growth. In the orchard study, 5 one-year-old shoots were randomly selected from each treatment tree and initial measurements made on June 3, 1993 of stem diameter, stem length, number of leaves, fruit diameter, fruit weight and fruit number. The fruit weights were determined by harvesting 10 developing fruit. Fruit diameter, weight and number were measured again on July 22 just prior to harvest. Stem diameter, stem length and number of leaves were measured again on September 29, 1993.

Statistical Protocol. All experiments were a randomized complete block design and statistical significance was determined using analysis of variance and F test. Treatment effects were considered significant at the 5% level or the 10% level where early detection of thresholds were sought.

Results

Mite-Feeding Studies. Orchard Study. Mite Populations. The average CMD in the orchard trees through the 1993 season are shown in Figure 2A. The CMD averages were similar in the two treatments until a Carzol miticide spray was applied to controls on 2 August which resulted in higher mite populations in MIT relative to controls. Mite numbers then fluctuated slightly with the treatment trees consistently having more mites than the miticide-treated controls. By the end of the 1993 season the average CMD was 1764 for the treatment trees and 1093 for controls, an increase of 61%.

Gas Exchange and Fluorescence. Single leaf A for 4 dates during the 1993 growing season along with corresponding CMD are given in Table 1. The treatment trees had increasing mite populations over these dates, but no change in single leaf A was observed. E and g_s were also not affected by mite-feeding: on 19 August, E was $2.38 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for control trees and $2.28 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for MIT, and g_s was $148 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for control trees and $140 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for MIT. Likewise, chlorophyll fluorescence was not altered by the treatments on 5 dates, specifically the Fv/Fm ratios (Table 2). The Fv/Fm ratio declined slightly over the growing season but this can be attributed to the aging of the leaves. Measurements of whole tree A taken on 17 August, $8.5 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for controls and $7.4 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for MIT, were not different among the two treatments. It was hypothesized that a regression of A or chlorophyll fluorescence against the mite population levels would give a better indication of mite-feeding effects. There was no

significant correlation for either tree or leaf A or fluorescence when compared to MPL or CMD (Appendix A).

Chlorophyll. Total chlorophyll levels in the MIT were not significantly different relative to control trees with increasing mite•days. On 8 October total chlorophyll for MIT was 59.8 and 64.0 $\mu\text{g}\cdot\text{cm}^{-2}$ for controls. The MIT did have reduced levels of chlorophyll a on 23 September, 46.8 $\mu\text{g}\cdot\text{cm}^{-2}$ for MIT and 50.6 $\mu\text{g}\cdot\text{cm}^{-2}$ for controls, but on 8 October the MIT chlorophyll a had recovered to the level of controls. On 8 October, the level of chlorophyll b was reduced in the MIT, 14.3 $\mu\text{g}\cdot\text{cm}^{-2}$ relative to 15.3 $\mu\text{g}\cdot\text{cm}^{-2}$ for controls. There were no differences on any date for the ratio of chlorophyll a to chlorophyll b.

Vegetative and Reproductive Growth. There were no treatment differences for stem diameter, stem length, and leaf number for the 1993 growing season, nor in subsequent year (1994) flowering, fruit set and yield. Stem length averaged 22.3 cm for controls and 22.2 for MIT. Return yield was 0.174 $\text{kg}\cdot\text{cm}^{-2}$ of trunk cross section for controls and 0.149 $\text{kg}\cdot\text{cm}^{-2}$ for MIT. Additionally, there were no treatment differences in the subsequent year for fruit diameter or fruit weight: fruit diameter averaged 20 mm for controls and 19 mm for mites, whereas fruit weight averaged 4.1g for both controls and MIT.

Carbohydrate Status. Starch levels measured from shoot tissues collected on 1 November 1993 and on 14 April 1994 showed no effects of the mite treatment. MIT shoots had 7.8% and 13.8% starch on 1 November and 14 April, respectively, and controls had 7.6% and 10.1% starch on 1 November and 14 April, respectively. Starch levels in root tissues collected on 14 April 1994 were slightly higher (significant at $p\geq 6\%$) in MIT, 7.0% compared to 5.8% for controls.

Cold Hardiness. Measurements of one-year old stem cambium cold hardiness of over the dormant period subsequent to the mite–feeding are shown in Appendix B. Actual cold hardiness depends on the environmental conditions the trees are exposed to in the orchard and the T_{50} values determined on each date were a reflection of actual minimum

temperatures recorded at the orchard. The mite populations had no effect the T_{50} values relative to control.

Container Study. Mite Populations. Mite populations were markedly different in the 2 treatments due to greater control of MPL on container grown trees. After initial establishment of a high number of MPL on the treatment trees, there was a decline to 0 MPL after which a second establishment was required and mite numbers then remained stable. The number of CMD in the mite treatment increased gradually over the season to a final maximum of 917 (Figure 2B), 10 times the number for control trees .

Gas Exchange and Fluorescence. Single leaf A for 4 dates over the season are shown in Table 3 along with the corresponding CMD. As CMD increased, there was no difference in A until 2 August when the mite treatment trees had accumulated 900 mite•days. All photosynthetic data measured on 2 August is given in Table 4. Whole tree A, single leaf A, photosynthetic yield from chlorophyll fluorescence, and WUE all reflect an effect of mite-feeding on the trees. However, Fv/Fm ratio from chlorophyll fluorescence, E and g_s do not reflect any effects of mite feeding.

Effects of Miticides. The application of the two miticides had no measurable effect on any of the photosynthetic parameters measured when compared to control or to each other. Assimilation levels remained constant over the period of measurement and there was no effect of the miticides on A, g_s , E, or Fv/Fm ratios (Appendix C) compared to the water-treated controls. As Fv/Fm ratio is an indicator of the physiological status of the photosynthetic apparatus, there is no indication of any adverse effects of the miticide applications.

There was no reduction in shoot growth, 10.9 cm for controls, 11.8 cm for Omite and 11.8 cm for Vendex, or total chlorophyll levels, $10.9 \mu\text{g}\cdot\text{cm}^{-2}$ for controls, $11.8 \mu\text{g}\cdot\text{cm}^{-2}$ for Omite and $11.8 \mu\text{g}\cdot\text{cm}^{-2}$ for Vendex, as a result of miticide applications.

The absence of alteration in shoot growth could be an indication that miticide-treated plants were not being affected by the chemicals. However, as photosynthetic

compensation in response to injury has been demonstrated in cherry (Layne and Flore, 1992), apple (Flore and Irwin, 1983), and other plants (Hodgkinson, 1974; Poston, *et al.*, 1976), shoot growth could remain unchanged as the tree compensates for any possible damage from the miticide. The miticide application resulted in no decrease in A and, therefore, no apparent photosynthetic damage. Also, there was no increase in A, and thus no photosynthetic compensation. The observation that there was no effect of the miticide chemicals on photosynthesis or the photosynthetic apparatus is confirmed by the chlorophyll fluorescence measurements. The Fv/Fm ratios remained constant and there was no change relative to control treatment. The E and g_s levels varied with the exact environmental conditions of the greenhouse at the time of measurement, but there was no increase or decrease relative to control. The miticide study was carried out under greenhouse conditions in which the leaf surface tends to have less waxes and therefore would be more susceptible to injurious chemical applications, whereas in the orchard the leaf has a much thicker cuticle. Thus, the effect of Omite and Vendex under orchard conditions would be expected to be less than any effects observed under the greenhouse conditions. As mite infestation and miticide applications occur late in the growing season, there would be no effect on the current season's fruit growth and yield, and presumably no effect on fruit growth and yield of the subsequent season.

Terbacil Studies. The results of the preliminary experiment to determine the effect of different terbacil concentrations on tart cherry photosynthesis are given in Appendix D. The Fv/Fm ratio from chlorophyll fluorescence was the best indicator of photosynthetic injury among the chlorophyll fluorescence parameters in this study. A of the terbacil-treated trees was initially reduced more than Fv/Fm, but A recovered more quickly than the Fv/Fm ratio. The 50 ppm terbacil-treated trees were reduced to 32% A and 43% Fv/Fm of controls one day after treatment, and both parameters returned to the level of the controls by 7 days after treatment. The 100 ppm terbacil treated trees were reduced to 5% A and 34% Fv/Fm ratio, but by day 7 the A was 85% of controls while Fv/Fm was only 72%. Higher

terbacil concentrations exhibited increasing reductions and longer periods of recovery. The 400 and 800 ppm terbacil-treated trees exhibited visual symptoms of phytotoxicity and did not recover full photosynthetic capacity.

The A and Fv/Fm ratios from the orchard trees for each of the spray dates is given in Figure 3. Again, A was initially reduced more than the Fv/Fm ratio but recovered quicker. Notably, the Fv/Fm for the spray on 23 September (Figure 3D) did not completely recover to the level of control trees. Whole tree photosynthesis for trees sprayed at stage II of fruit development is given in Figure 4. The reduction in whole tree A and recovery essentially mirrored single leaf A (Figure 3B).

Yield and fruit quality were not adversely affected from the photosynthetic reduction caused by the terbacil treatment (Table 2). Similarly, terbacil application and the resulting photosynthetic reduction did not reduce yield in 1994, the season following treatment. Fruit set was increased as a result of terbacil application at stage II of fruit development, but this increased fruit set did not lead to any increase in yield.

There was no effect of the terbacil applications on shoot extension or total chlorophyll levels (appendix E). Chlorophyll a and b levels fluctuated slightly over the season but this was not considered significant.

Percent starch levels were altered by application of terbacil at the different stages (Table 5). Starch samples from shoot tissue collected on 1 November 1993 showed reduced levels for terbacil application at stage I of fruit development only, Terbacil #1. The following spring, shoot starch levels were no different in Terbacil #1 than the control, yet starch levels were increased for the other terbacil application dates. Conversely, root starch levels were decreased for Terbacil #2 (stage II) and Terbacil #3 (harvest) compared to controls, but not affected for Terbacil #1 and Terbacil #4.

Shoot cambium cold hardiness was not affected at any time during the dormant season by terbacil application at any of the spray dates (Appendix F). Also, the average cold hardiness for the entire dormant season was also not affected by terbacil application.

Discussion

Mite-Feeding Studies. As there was no reduction in any photosynthetic parameters, but there was a reduction in chlorophyll levels, one may conclude that the orchard tree leaves increased their photosynthetic production of remaining chlorophyll to compensate for the loss of chlorophyll. This photosynthetic compensation for leaf damage has been described in sour cherry (Layne and Flore, 1992) as well as other tree species (Bassman and Dickmann, 1982; Proctor, *et al.*, 1982; Satoh, *et al.*, 1977). We were unable to detect a measurable increase. The chlorophyll loss was slight and therefore any compensation would have been small and difficult to detect even with careful and precise measurements. The trees were able to maintain full photosynthetic capacity even under the stress of continually increasing mite damage. Another possibility may be that the high mite populations in the control trees reduced A photosynthesis in those trees comparable to the MIT.

The higher mite populations and reduction in chlorophyll levels in MIT had no effect on fall starch levels (Table 6) in the shoots and no effect on shoot cold hardiness (Table 7). This suggests that either any photosynthetic compensation was sufficient, or mite damage was insufficient, to allow MIT shoots to maintain starch levels comparable to controls levels sufficient to maintain shoot cold hardiness. In the spring of the subsequent year, shoot starch levels were again not affected in MIT relative to controls, however, root starch levels were increased over controls. This increase could be due to a number of factors. The MIT could be maintaining high root starch levels at the expense of carbohydrate transport. That was not the case as there was no alteration in the shoot starch levels. The photosynthetic compensation of MIT over the season could be high enough to result in increased starch levels in the plant resulting in high levels transported and stored in

the roots. In effect, an over-compensation in response to the mite-feeding and subsequent reduction in chlorophyll. If this were the case, one would expect a measurable increase in photosynthesis, but no such increase was observed. Layne and Flore (1992) have suggested that photosynthetic compensation is most likely due to enhancement of both carboxylation efficiency and ribulose, 1-5-bisphosphate regeneration. This enhancement may account for sufficient starch being stored in the roots over the winter and present in the spring.

In the container study, mite populations were more easily managed due to the smaller tree size and the smaller number of total mites involved. The mite-free control trees were maintained consistently at less than 5 MPL (Figure 4) and often they had 0 MPL. Additionally, mites populations could be maintained in MIT by addition of mites added as needed. Consequently, MIT accumulated ten-fold the number of mites as the controls.

The large difference in CMD at the last measurement date in the container study coincided with significant reductions in several photosynthetic parameters. Reductions were observed in single leaf A, whole tree A, photosynthetic yield from chlorophyll fluorescence, and WUE (Table 4) when MIT had 900+ CMD. No reductions were evident 14 days earlier when there was again a ten-fold difference in CMD between the two treatments, but MIT had only 700 CMD. Thus, during the time period when mite populations went from 700 to 915 CMD, MIT reached the photosynthetic threshold for mite-feeding and damage. Effective control of mite populations below that photosynthetic threshold might have resulted in no decrease in any of the parameters. As these trees were relatively small and grown in containers, it is not known how that reduction might effect yield, or the yield threshold for mite damage. That photosynthetic threshold occurred after the 1994 harvest date and any effects on yield would not be observed until the following year. As the orchard trees tolerated mite damage with no effect on photosynthesis, similarly, trees could tolerate some postharvest photosynthetic inhibition with no effect on yield or tree hardiness.

In the orchard study, there was no decrease in photosynthesis, tree hardiness, and more importantly subsequent year's yield as a result of high mite populations, which suggests that control measures were unwarranted in 1993 at this orchard. There could be an effect of the measured mite population on photosynthetic parameters of controls, which accumulated 1093 CMD. If one were able to compare the controls to truly mite-free trees, there may indeed be an effect on photosynthesis as the controls in our study may have been above a biological threshold. Since completely mite-free trees cannot be achieved, and when comparing mite control with the absence of mite control, there was no benefit of the miticide applications. Much of the high CMD in controls was due to high numbers of MPL prior to any miticide application, suggesting that an earlier application of miticide would further reduce mite populations. Many miticides are not labeled for preharvest applications, and as demonstrated in this study, the miticide applications that were made did not reduce mite populations enough to enhance tree productivity. The container study involved trees that were much closer to being mite free and a photosynthetic threshold was reached at only 900 CMD, much lower than the 1093 CMD in the orchard control trees. That difference is most likely due to differences in tree size, age, and vigor that create an ability in the orchard trees to buffer stresses such as the mite feeding.

The 1764 CMD seen in the field MIT fall short of a photosynthetic threshold (biological threshold) as measured in this study. The photosynthetic threshold, and other biological thresholds are important for determining an action threshold and ultimately the economic threshold. As we detected no reduction in photosynthesis and no reduction in yield in either 1993 or 1994, it is unclear where the economic threshold lies, and thus where the action threshold should be. Of particular interest to growers is where the economic threshold lies with respect to the photosynthetic or other measurable biological thresholds. One possibility is that a particular biological threshold coincides with the action economic thresholds, which would require the grower to employ pest controls actions as soon as the biological threshold is reached. Another possibility, and more likely, is that the

action and economic thresholds come at a point beyond the biological threshold and therefore are difficult to determine. This study has demonstrated that the economic threshold is not reached prior to measurable losses in photosynthetic activity. Yield reduction likely occurs only after photosynthesis has been reduced by a specific level or for a specific period of time. The grower must then identify the period between the photosynthetic threshold and the economic threshold and utilize it for control measures. With respect to current season's yield, the mite damage in the year of this study occurred after harvest. The only effect possible on current seasons yield would be if mite populations were high enough early in the season to reduce the leaf to fruit ratio prior to harvest or reduce A significantly during the final stage of fruit growth. This is rarely the case in Michigan sour cherry orchards. Howell, *et al.* (1973) showed that significant levels of defoliation and photosynthetic reductions are required to result in fruiting losses in the subsequent year and thus reach the economic threshold. Also, the detrimental effect of mite feeding may take a number of years before it results in an measurable effect, particularly with regard to yield and hardness. As we did not reach a biological threshold in the field mite studies, we attempted to identify an economic threshold using a herbicide for photosynthetic reduction.

Direct comparisons of the effects of CMD between the orchard and the container studies are difficult. While the rate of increase in cumulative mite days over time was comparable for the orchard and the container studies, there are numerous, obvious physiological and environmental differences that prevent direct comparisons. Of particular importance is that the orchard trees are older and more vigorous than the container trees and therefore have an increased buffering capacity to mite damage. Therefore, the orchard trees may have a larger overall photosynthetic compensation effect, another mechanism of increased tolerance to mite damage. Mobley and Marini (1990) have also demonstrated a greater tolerance of orchard peach trees to mite-feeding compared to greenhouse-grown container trees. Additionally, the tolerance of trees to mite-feeding can be different

depending on the exact timing of mite damage (Candolfi, *et al.*, 1992), and thus CMD numbers can result in different thresholds depending on the time of injury.

An additional objective of this study was to determine if chlorophyll fluorescence, relatively easy to measure, could be used as an early indicator of mite damage to the photosynthetic apparatus, prior to any visual damage. There were no reductions in fluorescence in the orchard study even though mite damage was detected via chlorophyll levels and damage was evident visually. Quantum yield of photosynthesis as determined with the modulating fluorescence system was reduced in the container study but coincided with detected reductions in A. Other fluorescence parameters, in particular the Fv/Fm ratio, did not detect mite damage in the container study even though A had been reduced (Table 9). It is surprising that photosynthesis was reduced without affecting the Fv/Fm ratio which has been shown to be a sensitive indicator of the photosynthetic apparatus. The senior author noted while conducting the container study that initially Fv/Fm values may have actually increased with increasing mite damage, however, there was no such increase statistically. Visual damage was the best indicator of mite damage as determined from this study.

Sour cherries exhibit a considerably higher tolerance to mite-feeding than either apple or peach. This tolerance is in part due to the fact that mite damage occurred late in the growing season when photosynthetic demand in sour cherry is relatively low. Also, sour cherries are able to compensate for photosynthetic losses due to mite-feeding to the point that cold hardiness and return yield are not affected. This ability of tart cherries to withstand mite-feeding late in the season means that routine miticide applications can be reconsidered in Michigan orchards. Mite control measures that significantly reduce mite populations relative to the uncontrolled alternative had no significance on tree hardiness and yield in this study.

Effects of Miticides. The miticides used for sour cherry production in Michigan can be used with no concern that the chemicals themselves will have any adverse effects on

tree productivity. Repeated applications or applications of other chemicals may have an adverse effect on the crop (Ayers and Barden, 1975; Andersen *et al.*, 1986).

Terbacil Studies. By selecting the exact concentration of terbacil, photosynthesis, particularly A and Fv/Fm ratio, could be reduced by a predetermined amount for a predetermined amount of time. The degree and time of recovery of both A and Fv/Fm were concentration dependent. We purposefully chose a concentration that would reduce A by approximately 50% for 7–10 days. Presumably, naturally occurring stresses in the orchard would be detected and control measures employed that would allow the trees to recover in this time period.

A was initially reduced more than Fv/Fm ratio but recovered more quickly. The slow recovery of the Fv/Fm ratio suggested to us that that parameter was detecting photosynthetic damage that the A measurement was not. As mentioned previously, the Fv/Fm ratio was not effective as an early indicator of mite damage, but may still be an early indicator of other types of stresses. Photosynthetic recovery to the application of terbacil was similar on all applications dates except on spray date 4. Full recovery of Fv/Fm was not achieved prior to leaf fall, and full recovery of A took 14 days, twice the time for recovery for the other applications dates. Leaf senescence had been initiated at this point and the effect of terbacil was compounded by leaf senescence.

The effects of terbacil spray on whole tree A and recovery were not significantly different than single leaf measurements of A. It might be assumed that tree as a whole would be quicker to recover net photosynthesis due to photosynthetic compensation as more and more leaf area recovers. No compensation was observed, and the inhibition was likely not enough to induce measurable photosynthetic compensation in such a short time period.

The lack of any adverse effects of the terbacil treatments on fruit yield or quality indicates that sour cherry trees can tolerate substantial reductions in photosynthesis – during critical periods of photosynthate demand – with no effect on yield. Byers *et al.*

(1990) have shown an effect of terbacil on yield of peach and used photosynthetic inhibitors as fruit thinners. It is important to note that the leaf to fruit ratios in the orchard used in this study were well above the threshold for fruit production and the trees therefore had significant buffering capacity to the effects of the terbacil. Additionally, sour cherry trees appear to have significantly greater abilities to withstand both mite damage and photosynthetic reduction. Inhibition of photosynthesis can occur at one of several times during the growing season, including postharvest and still have no effect on yield or quality. Additionally, multiple reductions in photosynthesis that require up to 14 days for 100% recovery had no adverse effects on current season's yield or quality (Appendix G)

As the mode of action of terbacil is on electron transport of photosystem II (Gardiner, 1981), such low concentrations as used in this study should not have affected chlorophyll levels and did not. Yellowing of leaves due to chlorophyll loss is a signal of terbacil toxicity and concentrations that cause such damage were purposefully avoided to allow 100% recovery of leaves.

The treatment of trees with terbacil which led to the reduction in photosynthesis did have an effect on other parameters in this study. Shoot extension was reduced in the trees sprayed with terbacil immediately prior to harvest. The inhibition of photosynthesis during the final days of fruit carbohydrate assimilation and growth resulted in reduced production of photosynthate, apparently at the expense of stem tissue growth. The effect may have been to slow shoot growth or to accelerate terminal bud set, the timing of which was not measured in this study. There was no measurable increase in A after recovery to compensate for the reduced photosynthesis at such a critical stage. The application of terbacil and subsequent photosynthetic reduction at other stages of development had no effect on shoot extension. At those stages of growth, the trees apparently have the ability to withstand photosynthetic reduction due either to reduced demand during these periods, or to undetected photosynthetic compensation. The period of time immediately before

harvest is critical for maintaining maximum photosynthesis for fruit development and shoot growth.

While shoot extension was affected by photosynthetic reduction at harvest, shoot starch levels at the end of the growing season were not affected by photosynthetic reduction at harvest. Reduced autumn starch levels of trees treated at stage 1 indicates that fruit development at this stage has priority over assimilate partitioning to the point that fruit development was unaffected but storage carbohydrates were reduced. However, by the spring of the following year, the shoots had recovered such that they had starch levels comparable to controls. There was sufficient time between that early June photosynthetic reduction and leaf fall to produce sufficient starch that could be transported to the shoots in the early spring. The spring starch levels of the other treatment dates were increased over controls, even for the treatment date of 17 September. This indicates that the time of application relative to leaf fall had little to do with the amount of starch stored in the shoots. The response of the trees to photosynthetic inhibition was to preferentially transport starch to the shoots in the spring but not in the fall. That preferential transport may have been at the expense of root starch as levels were reduced for treatment date 2. The effect may have been for the treated trees, presumably lower in overall starch levels, to transport starch to the shoots to maximize shoot cold hardiness, which was not affected. The starch levels were consequently reduced in the roots, significantly for treatment date 2. The terbacil-induced photosynthetic reductions at stages 1 and 2 had contrasting responses with respect to starch storage between the roots and shoots. Inhibition of photosynthesis during stage I of fruit growth, a period of rapid and prolific cell division, resulted in less starch being stored in the shoots but no effect on the roots. This indicates that root starches are possibly produced or transported later in the season. Inhibition 14 days later resulted in no change in shoot starch levels but considerably less in the roots. Later dates of inhibition had no effect on root starch levels. This indicates that the period of pit hardening, when fruit development pauses, may be a period during which starch is produced for or transported to

the roots. Assimilate is still transported to the fruit during this time, and if starch is destined for the roots during this stage, significant levels of photosynthate are required during this brief period. This would be in direct contrast to the partitioning that occurred only 14 days earlier during stage I. Starch storage at stage II is obviously markedly different than the storage at stage I particularly under conditions of reduced photosynthesis.

These effects on starch levels may have been inconsequential as there was no effect on shoot cambium cold hardiness or subsequent season's yield. Starch concentrations have been correlated with cold hardiness of peach (Lasheen *et al.*, 1970; Flore and Howell, 1987), but there was no effect in our study of reduced starch levels on cold hardiness of tart cherry. Greater photosynthetic reduction would have been required to alter cold hardiness (Flore, *et al.*, 1983) or subsequent season's yield (Howell and Stackhouse, 1973).

The results of this study indicate that sour cherry trees can tolerate substantial losses in photosynthesis without any adverse effects on overall orchard production. While the levels of photosynthetic reductions in this study did not lower total yields or fruit quality, other factors were altered dependent upon the time of photosynthetic reduction. Shoot extension and root and shoot starch levels were altered as a result of photosynthetic reductions. The lack of changes in the yield components as well as cold hardiness indicate that the sour cherry trees were able to compensate for lost photosynthate with no cost to reproductive growth or tissue hardiness. Therefore, photosynthetic reductions that occur in the natural life of the orchard, whether those reductions be due to mites, insects, diseases, drought or flooding stress, or otherwise, can have little effect on the economically important factors of yield and tree vigor. Granted there are many other substantial differences between the terbacil induced photosynthetic reduction of this study and typical environmental stresses an orchard will encounter. When considering the effect of photosynthate loss alone, which the terbacil treatment allowed, photosynthetic reductions of up to 10 days can have little detrimental effect.

This data can be incorporated into integrated pest management programs for sour cherry. The demonstrated tolerance of orchard sour cherry trees to mite feeding and photosynthetic reductions will be useful information for growers as they make decisions concerning when to utilize mite and other pest control measures. It is important to note that the long term effects of mite feeding are still uncertain.

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Figure 1. Whole plant photosynthetic CO₂ assimilation chamber.

Figure 2. (A): Calculated cumulative mite•days on control and mite–infested orchard sour cherry trees on specified dates during the 1993 season at Clarksville Michigan. Arrow indicates miticide application to control treatment trees. Each point is the mean of 8 trees. Vertical bar represents LSD at 5%. (B): Calculated cumulative mite•days on control and mite–infested container sour cherry trees on specified dates during the 1994 at East Lansing Michigan. Each point is the mean of 22 trees. Vertical bar represents LSD at 5%.

Figure 3. The effect of spraying 63 ppm terbacil on CO₂ assimilation and Fv/Fm ratio of orchard sour cherry leaves at Clarksville Michigan in 1993. Treatment dates were (A): 1 June, (B): 15 June, (C): 4 August and (D): 17 September. Each point is the mean of 4 trees. Vertical bar represents LSD at 5%.

Figure 4. The effect of spraying 63 ppm terbacil on whole tree CO₂ assimilation of orchard grown sour cherry trees at Clarksville Michigan in 1993. Each point is the mean of 2 trees. Vertical bar represents LSD at 5%.



Figure 1.

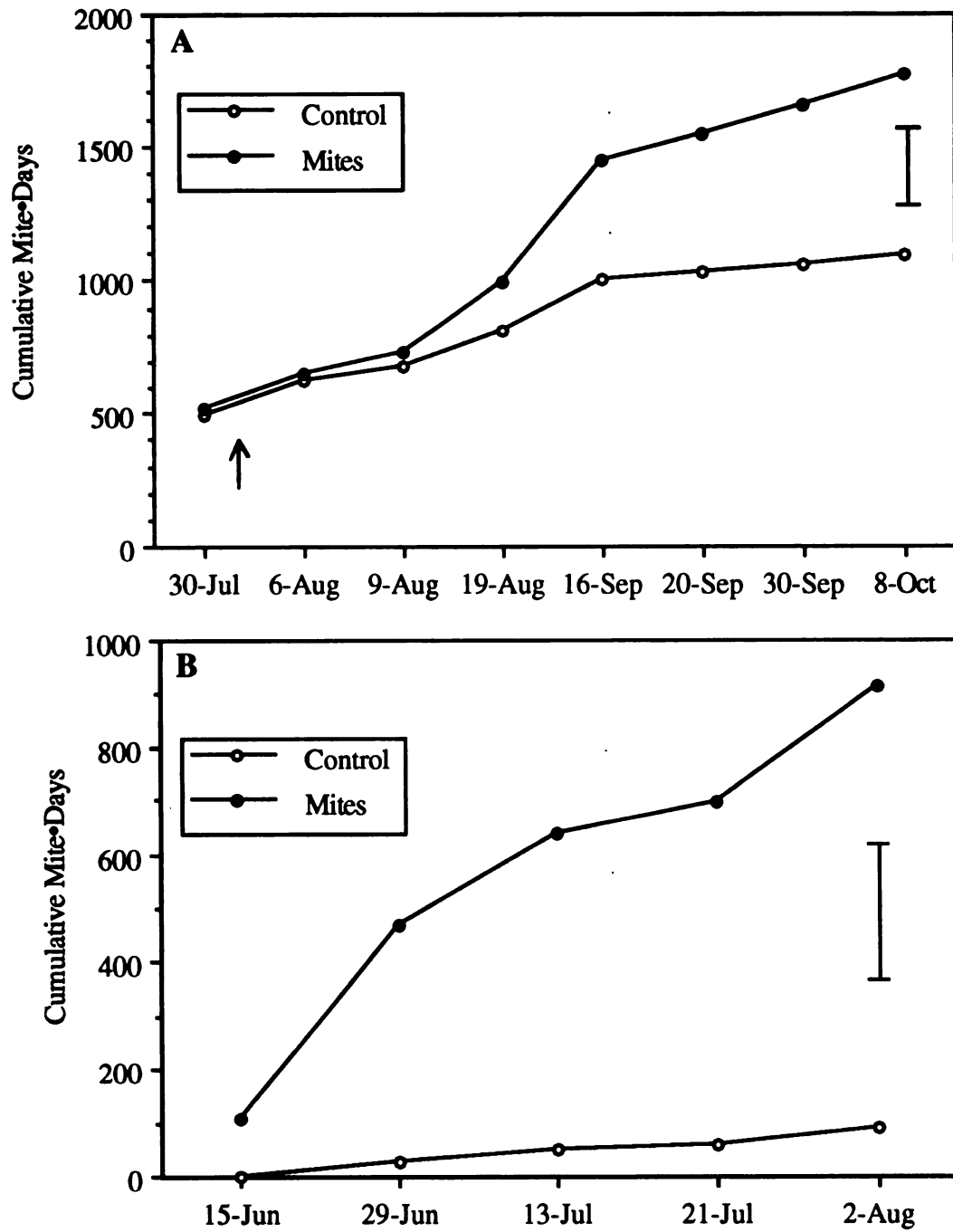


Figure 2.

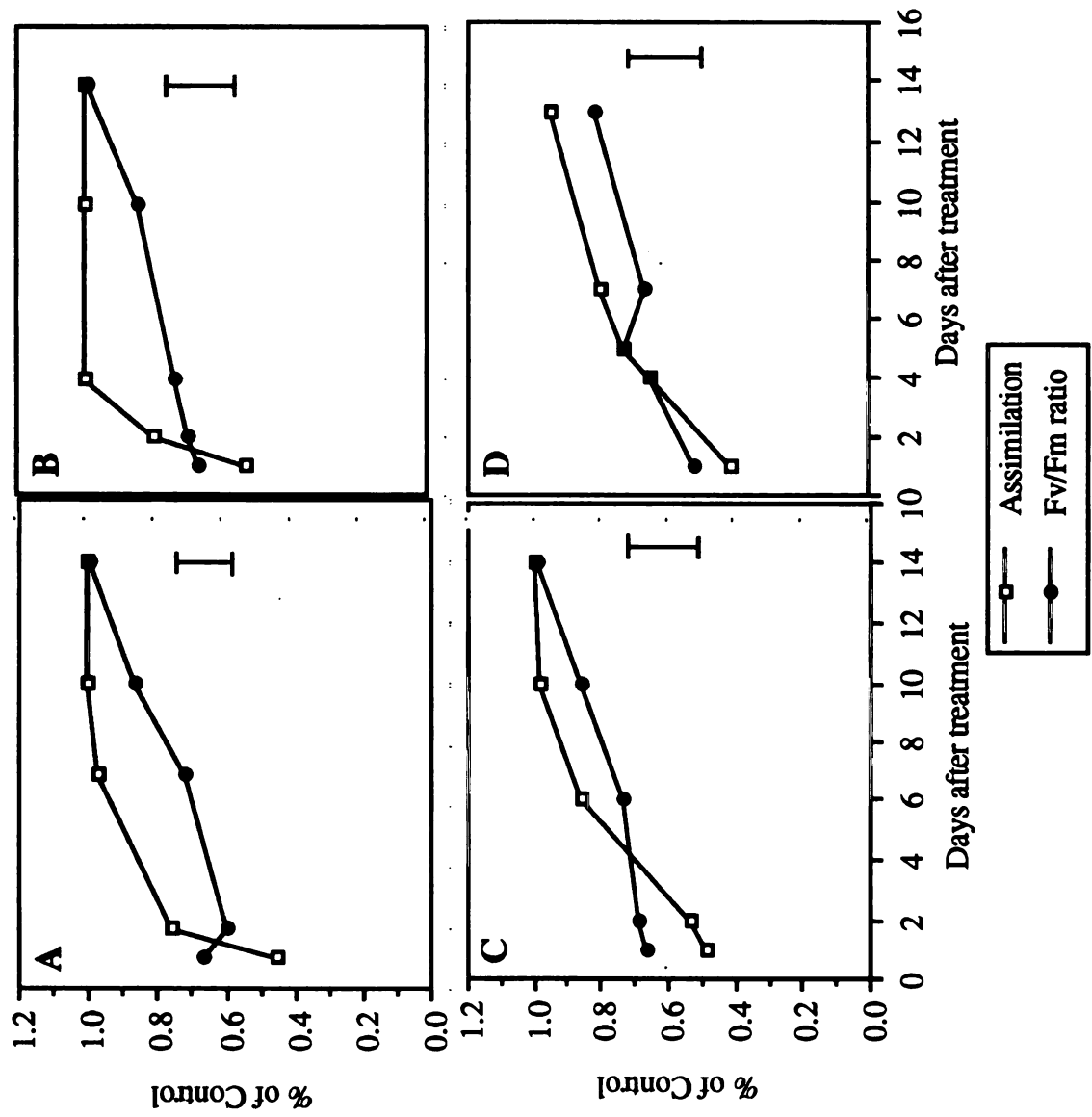


Figure 3.

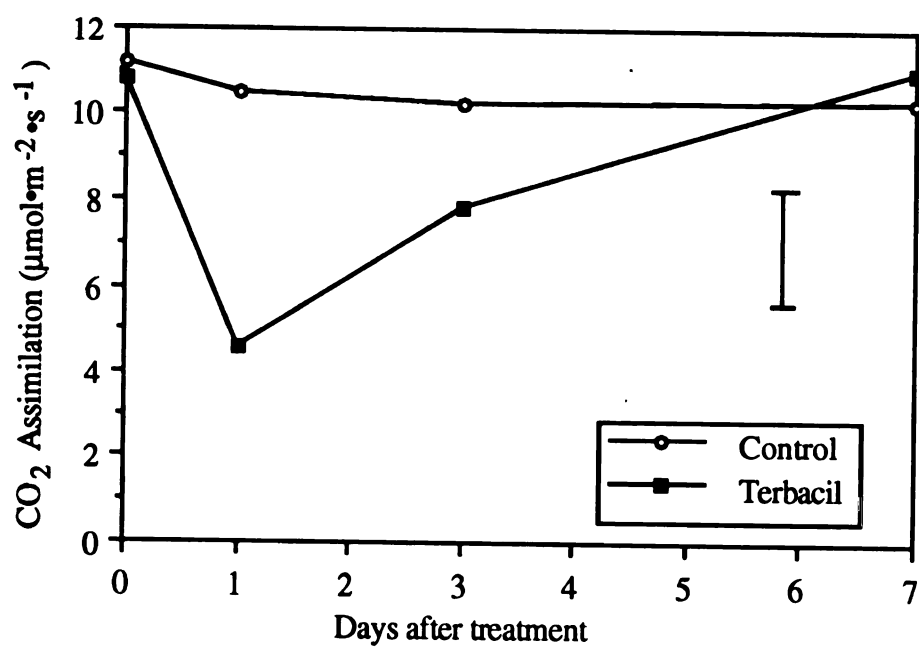


Figure 4.

Table 1. The effect of mite-infestation on single leaf CO₂ assimilation of sour cherry leaves from Clarksville Michigan orchard trees on specified dates in 1993.

Treatment	Assimilation ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			
	12 August	19 August	23 September	29 September
Control	4.13	4.38	4.80	6.12
Mites	4.46	4.30	4.63	6.24
	NS	NS	NS	NS

Treatment differences are not significant at 10% probability level, NS, or significant at the probability level indicated.

Table 2. The effect of mite infestation on Fv/Fm ratio, Fo, Fm and Fv from chlorophyll fluorescence of sour cherry leaves from orchard trees on selected dates in 1993.

		4 August		10 August		16 August		16 September		30 September	
Treatment	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fv/Fm	Fo	Fm	Fv
Control	0.649	0.683	0.659	0.644	0.556	898.2	2024.6	1126.2			
Mites	0.652	0.693	0.667	0.634	0.536	760.4	1659.2	898.8			
	NS	NS	NS	NS	NS	NS	NS	NS			

Treatment differences are not significant at 10% probability level, NS, or significant at the probability level indicated.

Table 3. The effect of mite infestation and cumulative mite•days (CMD) on CO₂ assimilation (A) and Fv/Fm ratio of fully expanded sour cherry leaves of container grown trees on specific dates in 1994.

		Date											
		7 July			13 July			21 July			2 August		
Treatment		CMD	A	Fv/Em	CMD	A	Fv/Em	CMD	A	Fv/Em	CMD	A	Fv/Em
Control	26	14.7		0.641	50	13.9	0.664	56	12.9	0.742	90	10.7	0.691
Mites	467	13.9		0.664	637	13.0	0.655	695	12.7	0.730	917	8.6	0.684

Treatment differences are not significant at 10% probability level, NS, or significant at the probability level indicated.

Table 4. The effect of mites and cumulative mite•days (CMD) on whole tree CO₂ assimilation, single leaf CO₂ assimilation, transpiration (E), stomatal conductance (gs), water use efficiency (WUE), photosynthetic yield, and Fv/Fm ratio of expanded sour cherry leaves of container–grown trees on 2 August 1994.

Treatment	Assimilation		E (mmolH ₂ O•m ⁻² •s ⁻¹)	gs (mmolCO ₂ •m ⁻² •s ⁻¹)	Photosynthetic		
	CMD	Tree Leaf (μmolCO ₂ •m ⁻² •s ⁻¹)			WUE* (mmolCO ₂ •m ⁻² •s ⁻¹)	Yield	Fv/Fm Ratio
Control	90	13.43	10.69	12.1	154	0.00090	0.720
Mites	917	9.53	8.64	12.1	140	0.00072	0.702

p<1% p<1% NS NS p<2% p<8% NS

Treatment differences are not significant at 10% probability level, NS, or significant at the probability level indicated.

*WUE: water use efficiency (mol CO₂ / mol H₂O)

Table 5. The effect of application of 63 ppm terbacil on shoot starch levels on 1 November 1993 and shoot and root starch levels on 14 April 1994 of orchard sour cherry trees. Terbacil #1 sprayed on 1 June, Terbacil #2 sprayed on 15 June, Terbacil #3 sprayed on 4 August and Terbacil #4 sprayed on 17 September.

	<u>1 November 1993</u>		<u>14 April 1994</u>	
<u>Treatment</u>	<u>Shoot</u>		<u>Shoot</u>	<u>Root</u>
Control	8.38% b		8.11% b	5.77% ab
Terbacil #1	6.13% a		11.49% ab	6.98% a
Terbacil #2	7.73% b		12.13% a	2.17% c
Terbacil #3	8.99% b		12.23% a	4.58% bc
Terbacil #4	8.46% b		12.64% a	5.26% abc
	p<5%		p<8%	p<10%

Treatment differences are not significant, NS, or significant at the probability level indicated.

SUMMARY AND CONCLUSIONS

It was demonstrated that moderate levels of mite populations that occur in the absence of chemical miticide applications can result in no significant decrease in tree yield or hardiness compared to typical control measures taken. Compared to an absolutely mite-free tree, the effects may be very different. In this study, 1764 CMD did not reduce photosynthesis, yield or hardiness compared to 1080 CMD in the orchard study. In the container study, 900 CMD reduced a number of photosynthetic parameters when compared to only 90 CMD of controls. Therefore, while orchard trees not treated with miticides were shown not to be adversely affected, there may have been adverse affects if they had been compared to truly mite-free trees. The latter consideration may be inconsequential since absolutely mite-free orchards are not attainable. Additionally, the orchard trees may have a significantly greater capacity to withstand the effects of mite feeding. It may be possible that during certain years sufficient control measures can be taken that would reduce explosive mite populations enough to achieve a benefit, although this was not the case in our orchard in 1993.

The question of whether the miticide sprays themselves have a detrimental effect on photosynthesis and therefore tree yield and hardiness was addressed also as part of this study. The two major miticides labeled for sour cherries and used in Michigan demonstrated no inhibitory effects on photosynthesis.

As the mite populations in the orchard did not affect photosynthesis and the mite populations in the container study did reduce photosynthesis, it is unclear as to how much photosynthetic reduction the tree and the orchard can tolerate before yield and hardiness are affected. It was demonstrated that reductions in photosynthesis that required 10 or more days to fully recover had no effect on current season or subsequent season yields. These were photosynthetic reductions of 50% or more that gradually recovered to 100% in 5-14

days. Therefore, plant stresses that reduce photosynthesis to these levels for similar time periods may be insignificant in regard to sour cherry yield and vigor.

All of these data indicate that in some seasons, applications of miticides may be unnecessary even when reductions in photosynthesis and growth can be detected. Mite and insect population thresholds for sour cherry may be much higher than originally believed, particularly with regard to thresholds established for apple and peach. Additionally, economic and action thresholds could be pushed back as well to reflect the tolerance to photosynthetic reduction.

While reduction of chemical inputs into the orchard can be of benefit to the grower, the loss of these management tools must be replaced by other pest control measures. However, it was demonstrated that for our orchard in 1993, a mite population threshold was not reached that reduced photosynthesis, yield or hardiness. Likewise, a photosynthetic reduction threshold was not reached that reduced yield or hardiness. Therefore, mite control measures were not necessary in that year. Additionally, measures to alleviate other stresses to photosynthesis may not be as necessary as well in similar situations. Thus the tart cherry grower could effectively produce a crop with reduced chemical inputs.

APPENDIX A

Regression Figures of 1993 Orchard Single Leaf CO₂ Assimilation, Whole Tree CO₂ Assimilation, and Chlorophyll Fluorescence Against Mites Per Leaf and Cumulative Mite•Days.

Figure 5. Single leaf CO₂ assimilation ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for orchard sour cherry trees in 1993 at Clarksville Michigan plotted against (A) mites per leaf ($r^2 = 0.03$, $n=44$), and (B) cumulative mite•days ($r^2 = 0.12$, $n=44$).

Figure 6. Whole tree CO₂ assimilation ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for orchard sour cherry trees in 1993 at Clarksville Michigan plotted against (A) mites per leaf ($r^2 = 0.12$, $n=8$), and (B) cumulative mite•days ($r^2 = 0.0001$, $n=8$).

Figure 7. Fv/Fm ratio from chlorophyll fluorescence for orchard sour cherry trees in 1993 at Clarksville Michigan plotted against (A) mites per leaf ($r^2 = 0.13$, $n=74$), and (B) cumulative mite•days ($r^2 = 0.33$, $n=74$).

Figure 8. Effect of cumulative mite day populations for orchard sour cherry trees in 1993 at Clarksville Michigan on (A) 1994 yield (kg per tree) ($r^2 = 0.01$, $n=16$), and (B) cold hardiness (T₅₀) of 1993-94 dormant season ($r^2 = 0.004$, $n=16$).

Figure 9. Effect of cumulative mite day populations for orchard sour cherry trees in 1993 at Clarksville Michigan on (A) 1993 fall shoot starch concentrations (percent by weight) ($r^2 = 0.004$, $n=16$), (B) 1994 spring shoot starch concentrations (percent by weight) ($r^2 = 0.27$, $n=16$), and (C) 1994 spring root starch concentrations (percent by weight) ($r^2 = 0.26$, $n=16$).

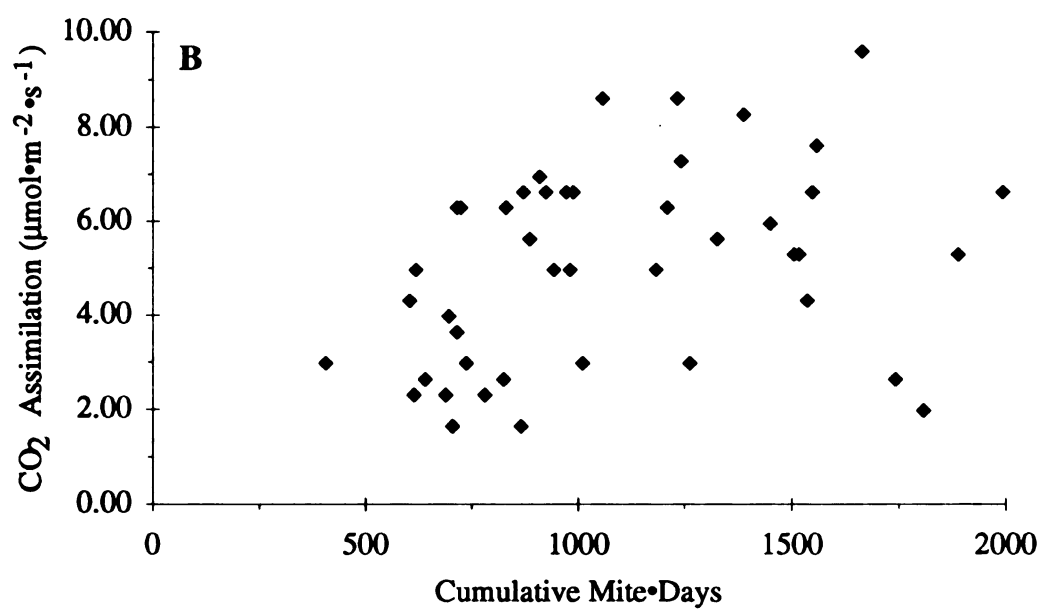
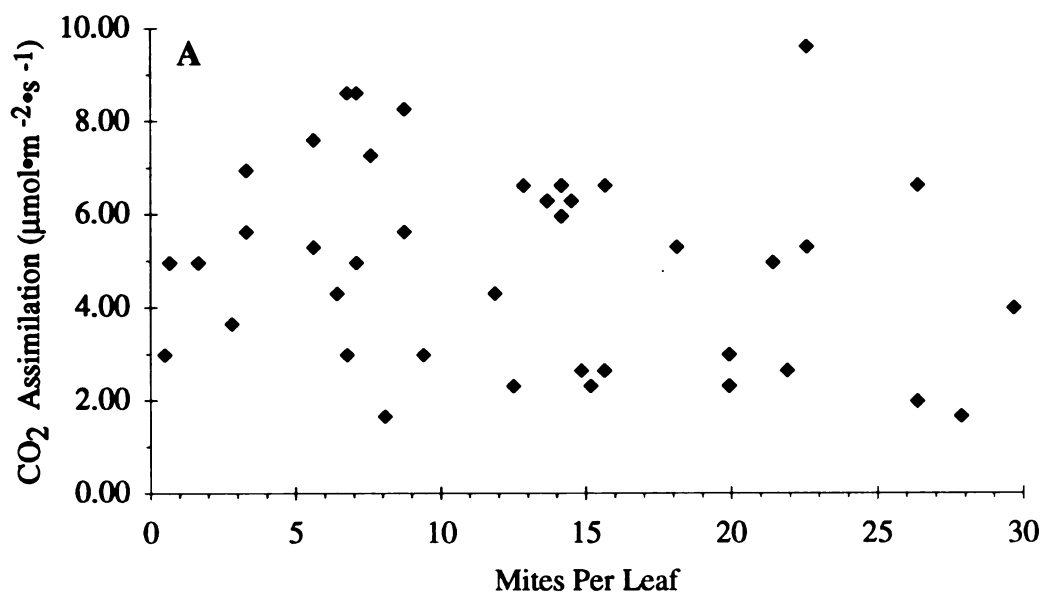


Figure 5

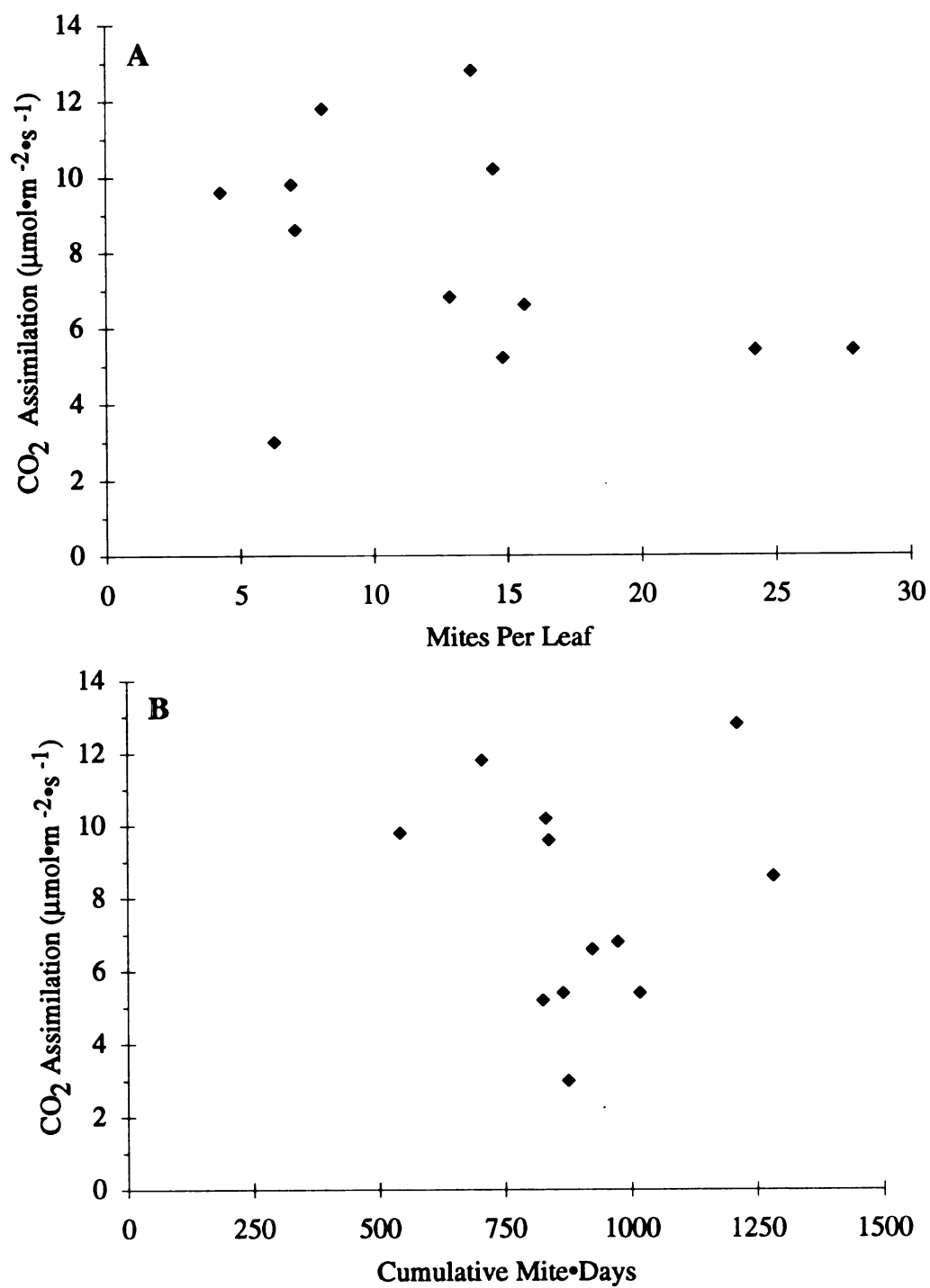


Figure 6

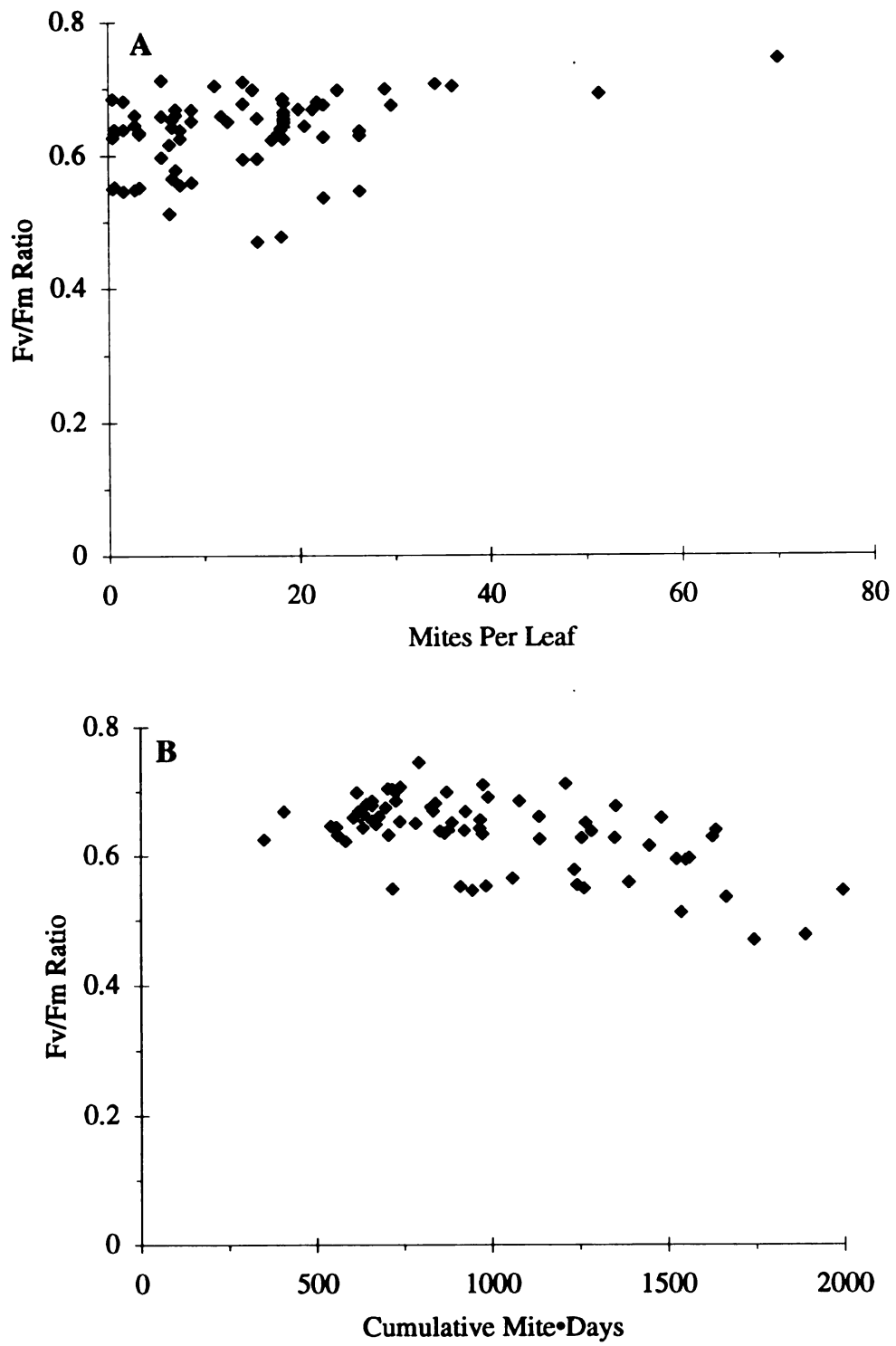


Figure 7

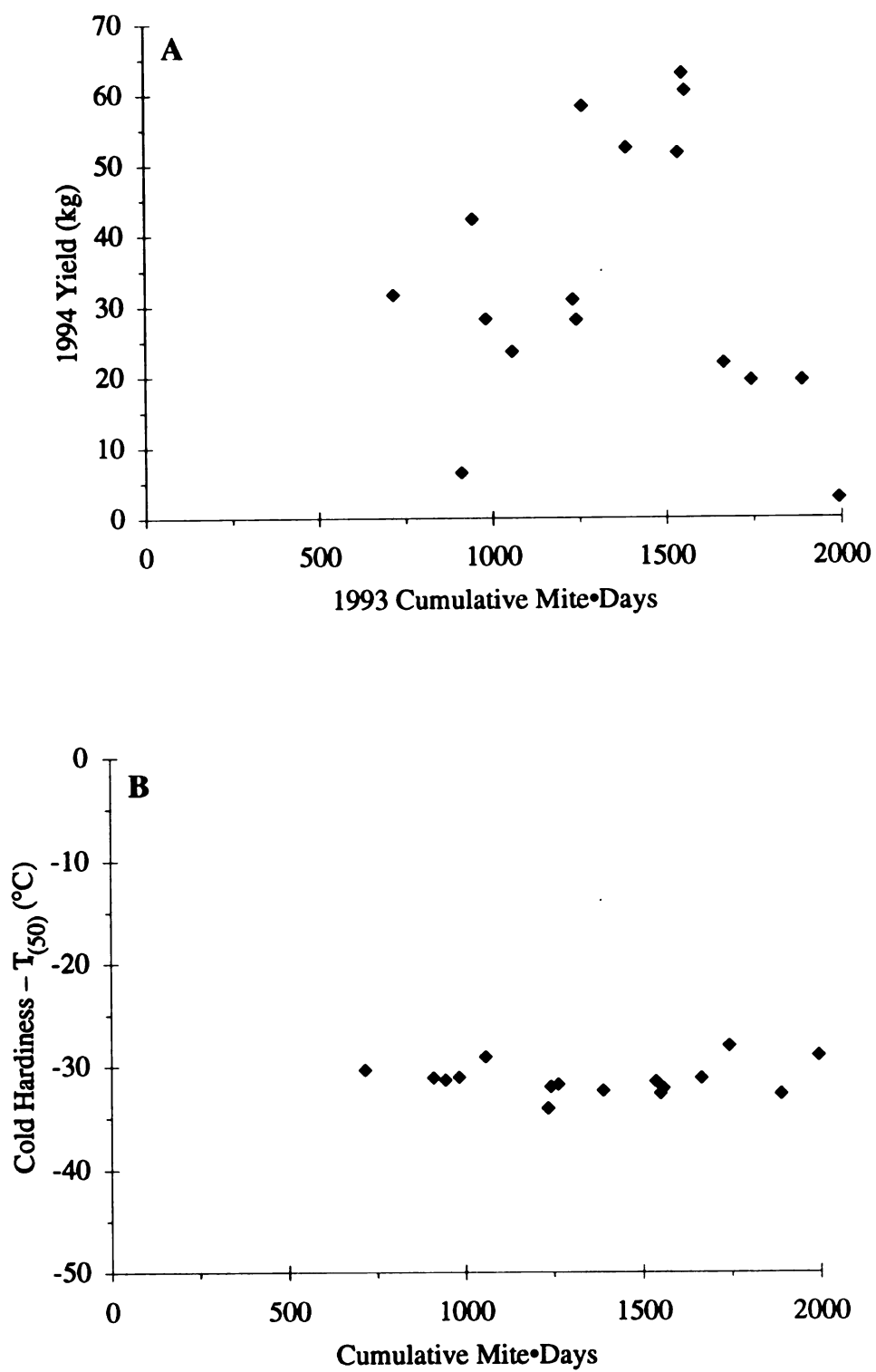


Figure 8

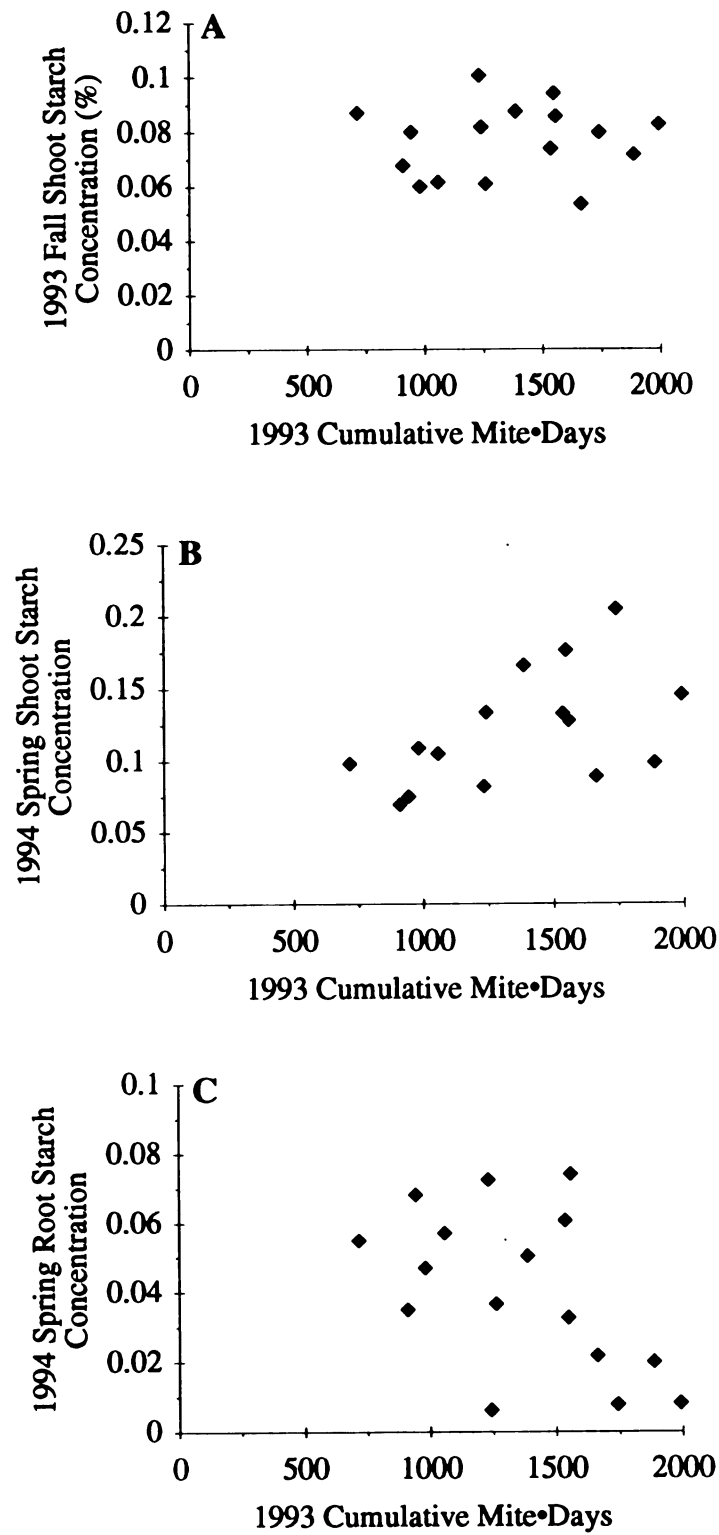


Figure 9

APPENDIX B

Cold Hardiness of Orchard Sour Cherry Trees Infested with Mites in 1993.

Figure 10. Stem tissue cold hardiness as determined by T(50) values for controls and mites-infested trees on the specified dates in the 1993-94 dormant season at Clarksville Michigan (each bar is the average of 8 trees). Treatment means were not significantly different analyzed using ANOVA and F test at 5%.

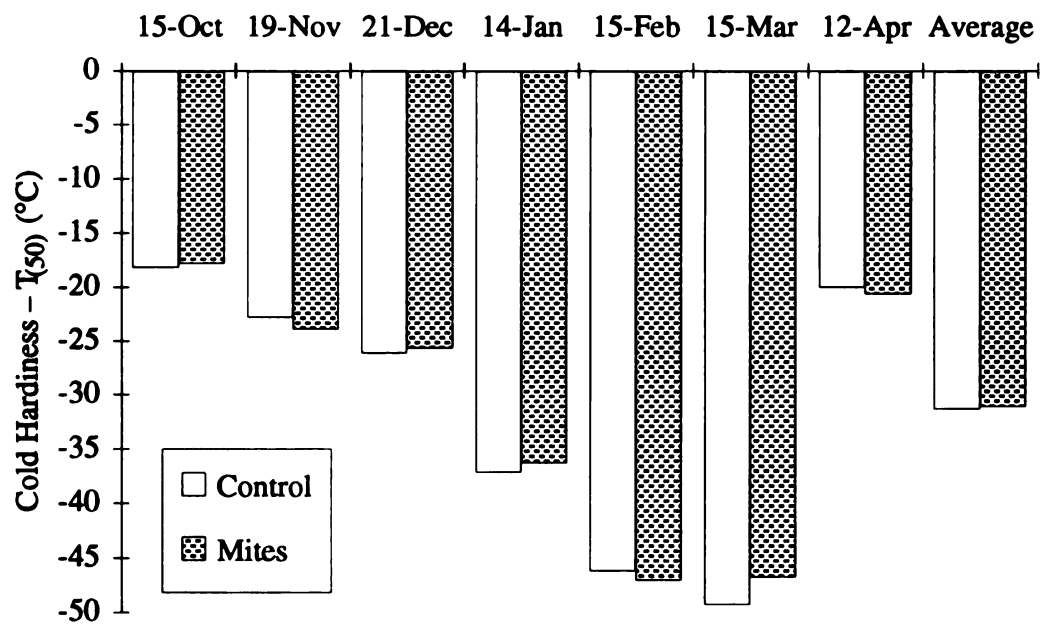


Figure 10

APPENDIX C

**Effects of Miticides on CO₂ Assimilation, Stomatal Conductance, Transpiration and
Chlorophyll Fluorescence of Greenhouse Grown Sour Cherry Trees.**

Figure 11. The effect of miticide application on (A): CO₂ assimilation (A), (B): stomatal conductance (g_s), (C): transpiration (E), and (D): Fv/Fm of expanded sour cherry leaves in East Lansing Michigan greenhouse in 1994. Means \pm SE (n=10).

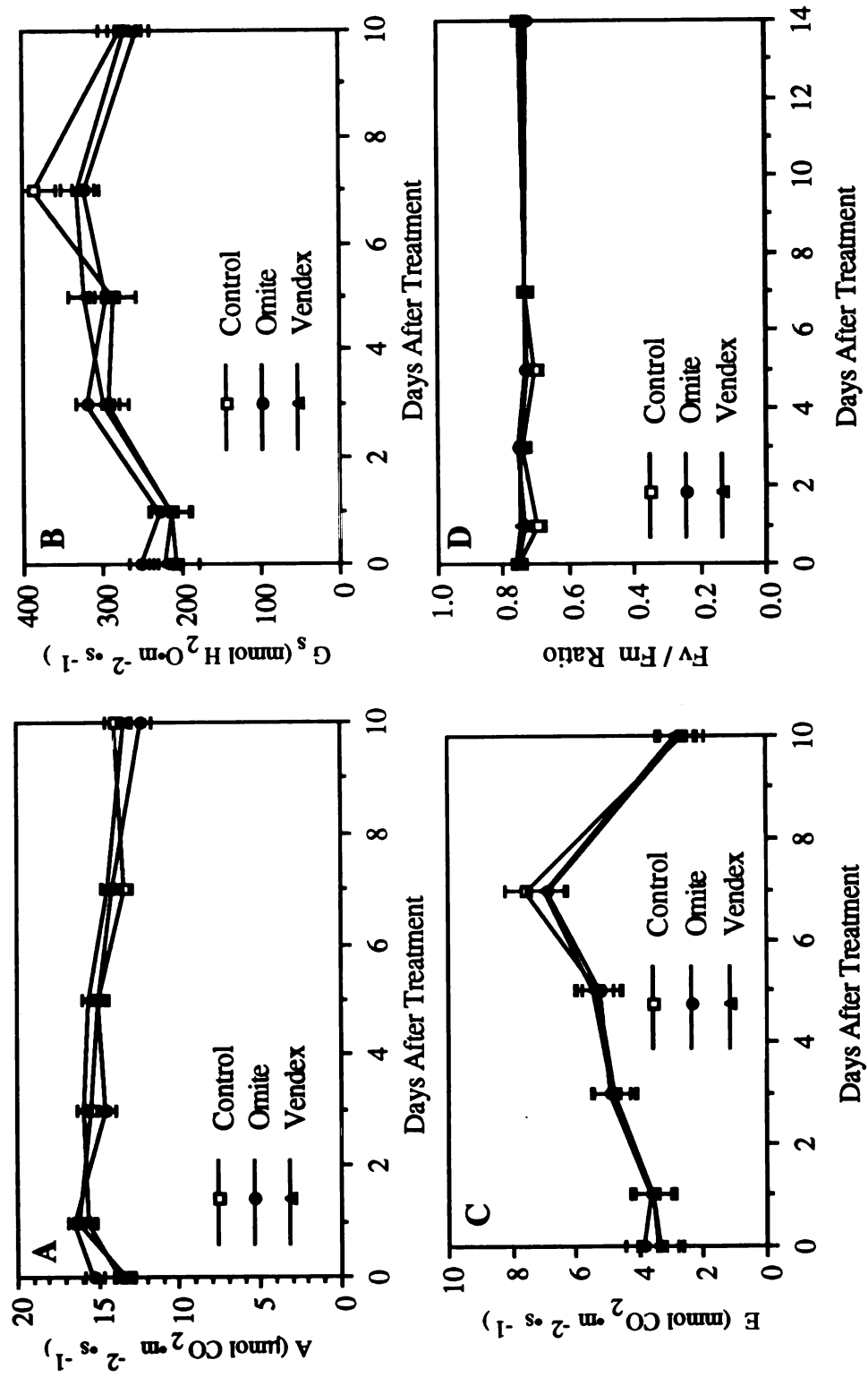


Figure 11

Table 6. The effect of miticide application on shoot length increase and chlorophyll of greenhouse grown sour cherry trees after application.

Treatment	Length (cm)	Total Chlorophyll ($\mu\text{g}\cdot\text{cm}^{-2}$)
Control	10.92	92.4
Omite	11.75	92.3
Vendex	11.75	95.2
	NS	NS

Treatment differences are not significant at 5% probability level, NS, or significant at the probability level indicated.

APPENDIX D

**Effects of Differing Concentrations of Terbacil on CO₂ Assimilation and Chlorophyll
Fluorescence of Sour Cherry Trees.**

Figure 12. The effect of terbacil concentration on (A): CO₂ assimilation (A) and (B): Fv/Fm ratio of expanded leaves of container grown sour cherry trees. Means \pm SE (n=6).

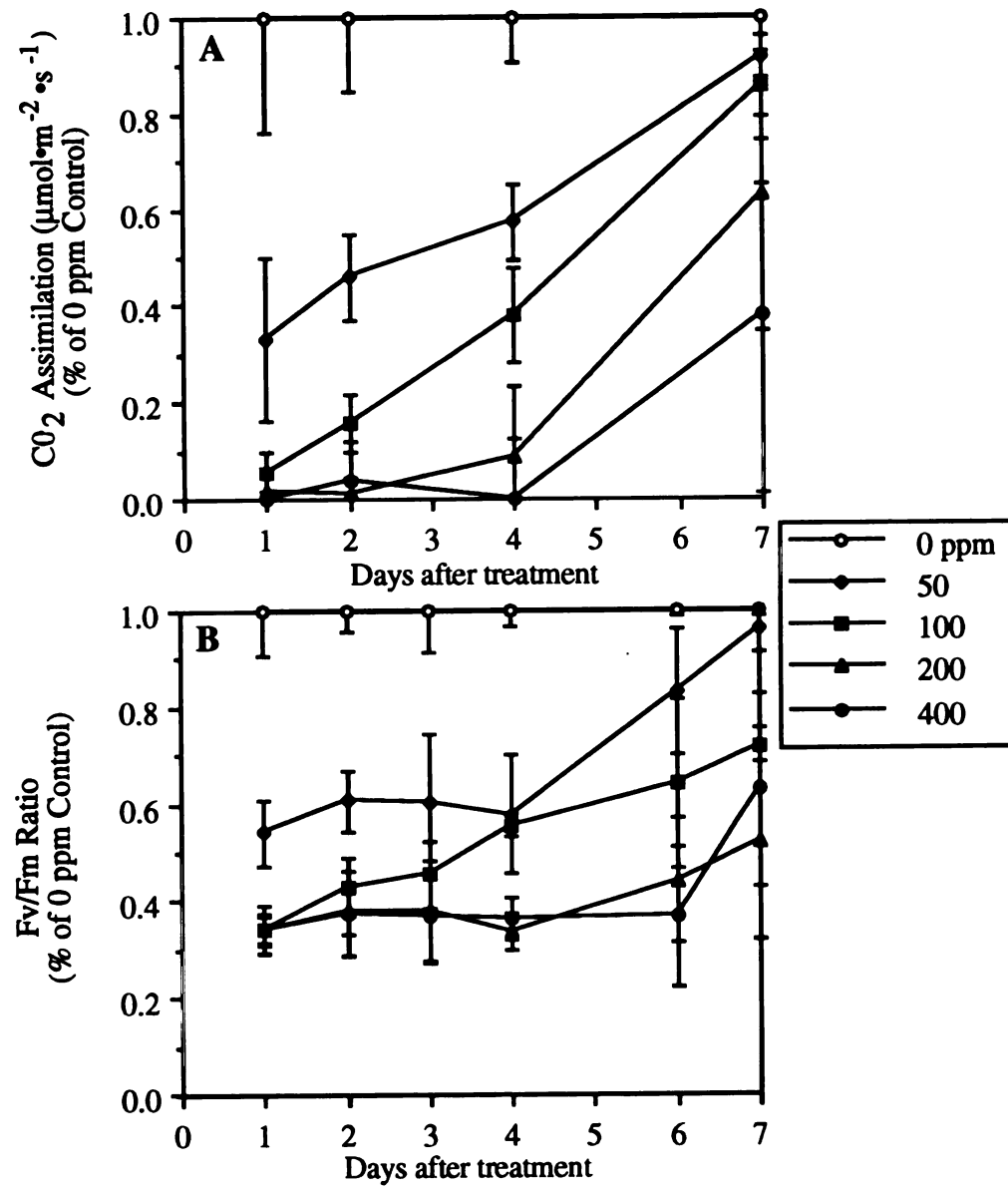


Figure 12

APPENDIX E

**Shoot Extension and Chlorophyll Levels of Orchard Sour Cherry Trees Treated with 63
ppm Terbacil on Specified Dates in 1993.**

Table 7. The effect of application of 63 ppm terbacil on shoot extension, and total chlorophyll on selected date of orchard sour cherry trees in 1993. Terbacil #1 sprayed on 1 June, Terbacil #2 sprayed on 15 June, Terbacil #3 sprayed on 4 August and Terbacil #4 sprayed on 17 September.

Treatment	Shoot Extension (cm)	Total Chlorophyll ($\mu\text{g}/\text{cm}^2$)		
		24 August	20 September	20 October
Control	22.85 a	83.70	69.64	63.56
Terbacil #1	22.75 a	83.76	76.33	64.78
Terbacil #2	21.50 a	83.81	71.19	60.03
Terbacil #3	18.45 b		68.25	61.98
Terbacil #4	22.82 a			61.78
p<8%		NS	NS	NS
Treatment differences are not significant, NS, or significant at the probability level indicated.				

APPENDIX F

**Cold Hardiness of Orchard Sour Cherry Trees Treated with 63 ppm Terbacil on Specified
Dates in 1993.**

Figure 13. Stem tissue cold hardiness as determined by T(50) values for controls and trees treated with 63 ppm terbacil on the specified dates in the 1993-94 dormant season at Clarksville Michigan. Cont: unsprayed controls, Terb1: sprayed on June 1, Terb2: sprayed on June 15, Terb3: sprayed on Aug 4, Terb4: sprayed on Sept 23 (each bar is the average of 4 trees). Treatment means were not significantly different analyzed using ANOVA and F test at 5%

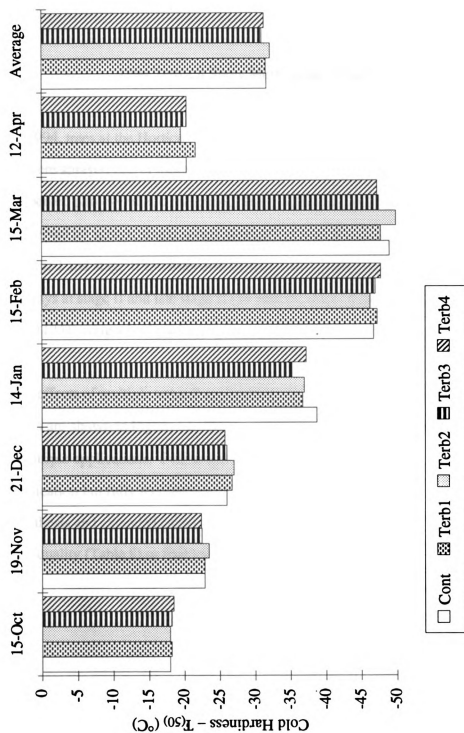


Figure 13

APPENDIX G

1994 Terbacil Spray Methods and Results

In 1994, trees at the Horticultural Research and Teaching Center at East Lansing Michigan were sprayed one or more times with 63 ppm terbacil with 1 ppm X-77, as a study of repeated photosynthetic reduction. Trees were sprayed as one of 5 treatments: controls received no sprays; single sprays at stage I of fruit development (30 May); multiple sprays at stage I and stage II (30 May and 14 June); single spray at stage II (14 June); or multiple sprays at stage II and late stage II (14 and 27 June). Spray applications were made prior to 9:00 EDT using a backpack sprayer with foliage sprayed to drip, approximately 8 L/tree.

The effects of multiple terbacil applications during fruit development on A and Fv/Fm ratios are shown in Figure 14. Figure 14A shows that A recovered within 7 days even for multiple applications. Fv/Fm ratios (Figure 14B) took longer to recover after the second application but fully recovered by 14 days. Significantly, this additional reduction in photosynthesis and extended reduction in photochemical efficiency (Fv/Fm) did not reduce fruit quality (Table 8) or fruit yield per limb cross sectional area.

Figure 14 . The effect of single and multiple spray applications of 63 ppm terbacil on (A): CO₂ assimilation and (B): Fv/Fm ratio of expanded sour cherry leaves from orchard trees in 1994. Terbacil #1 was treated on 30 May, Terbacil #1 x 2 was treated on 30 May and 14 June, Terbacil #2 was treated on 14 June and Terbacil #2 X 2 was treated on 14 June and 27 June. Arrows indicate dates of terbacil application. Means \pm SE (n=4).

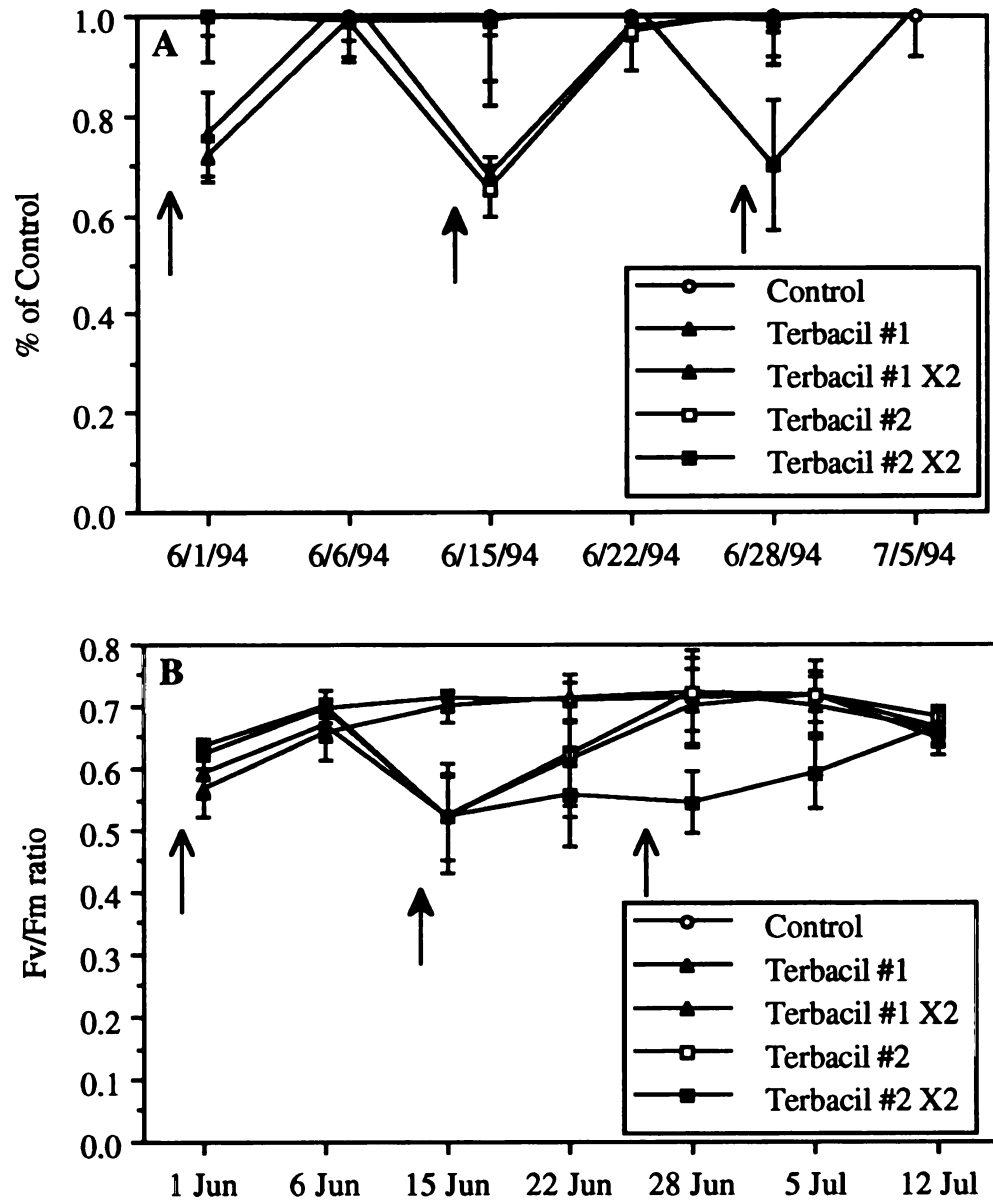


Figure 14

Table 8. The effect of application of 63 ppm terbacil on fruit weight, soluble solids and removal force for orchard sour cherry trees in 1994. Terbacil #1 sprayed on 30 May, Terbacil #1 X 2 sprayed on 30 May and 14 June, Terbacil #2 sprayed on 14 June and Terbacil #2 X 2 sprayed on 14 June and 27 June.

Treatment	Fruit Weight	Soluble Solids	Fruit Removal Force
	(g)	(%)	(g)
Control	4.50	11.50	376
Terbacil #1	4.21	12.13	311
Terbacil #1 X 2	4.08	11.67	354
Terbacil #2	4.31	11.58	430
Terbacil #2 X 2	4.75	10.92	387
	NS	NS	NS

Treatment differences are not significant, NS, or significant at the probability level indicated.

APPENDIX H

Pesticide Residue Study

**PESTICIDE RESIDUES IN PEACH (*PRUNUS PERSICA* L.
'NEWHAVEN') FRUIT GROWN UNDER IPM AND CONVENTIONAL
PEST CONTROL**

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Received for publication .

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Cellular and Whole Plant Physiology

Pesticide Residues In Peach (*Prunus persica* L. 'Newhaven') Fruit Grown Under IPM And Conventional Pest Control

Additional index words. IPM

Abbreviations: EPA, Environmental Protection Agency; GC, gas chromatography; IPM, integrated pest management; CONV, conventional level of chemical input; MOD, moderate level of chemical input; LOW, low level of chemical input

Abstract. Six peach (*Prunus persica* L.) orchards were established in 1990 and three distinct management strategies were employed that compared different levels of synthetic chemical input: a conventional system (CONV) that utilized standard chemical control measures, a moderate level of chemical input (MOD) that monitored pest populations for pesticide use and integration of nonchemical controls, and a low level of chemical input (LOW) that further reduced chemical inputs by employing additional IPM strategies and applying pesticides only when absolutely necessary as determined by entomologist. Selected pesticide residues in the fruit and soil were determined for 1992 and 1993. For fruit harvested in 1992, there were no significant differences between the pesticide residues from the different orchards, although there had been a distinct difference in the number of pesticide sprays each orchard received. In 1993, there was again a distinct difference in the pesticide sprays for each orchard with only minor difference in residues. The CONV orchards did have higher residues of the fungicide Captan than the MOD or the LOW orchards. Also, the conventional orchards had residues of chlorpyrifos of 0.08 ppm which is above the Environmental Protection Agency (EPA) tolerance of 0.05 ppm. The chlorpyrifos residues in the MOD and LOW orchards were below the EPA tolerance, 0.03

ppm and 0.04 ppm respectively. For the two management years of this study, few differences were found in the pesticide residues among the different treatments, however, CONV orchards did have chlorpyrifos residues above EPA tolerances in 1993.

There is growing concern about the use of synthetic pesticides in the production of food crops. This has sparked considerable interest and study in the use of management practices that reduce chemical control measures and utilization of nonchemical strategies. Integrated pest management (IPM) incorporates the technologies of horticulture, entomology, plant pathology and other fields to maintain quality crop production using innovative pest management strategies to minimize environmental impact to the site as well as the crop itself.

Peach growers are encountering pressure to produce quality fresh fruit and at the same time meet the increasing consumer demand for fruit produced with a minimum of chemical input to the orchard environment. The orchard manager must control a number of persistent insects, mites, and diseases to produce a crop and maintain crop quality. With the advent of IPM strategies and new biological controls, there exist the possibility of producing the quality and quantity of fruit with reduced use of synthetic chemicals. However, these techniques require greater skill in orchard management and the production results of exclusively using these strategies on a commercial scale is unknown. Also, the effects of reduced synthetic inputs on pesticide residues in the fruit is also uncertain. Residues have become a major health concern, but not without controversy. The debate ranges from dramatic press headlines (Blume, 1987) to scientific review (CAST, 1990). The EPA has established pesticide residue tolerances for all synthetic chemicals registered for use in the U.S. (Code of Federal Regulations, 1994). The methodology by which the EPA establishes health risks has also been called into question (Gold *et al.* 1992; Ames and Gold, 1990).

This study was initiated to compare three levels of chemical input and IPM strategies to determine if reducing synthetic chemical input into the orchards will reduce the detectable pesticide residues in the fruit and soil.

Materials and Methods

Orchard Management Strategies. The establishment and management strategies of the orchards has been previously discussed in detail (Flore, *et al.* 1994) and will be briefly described here. Six 'Newhaven' peach orchards were established in 1990, and the treatments applied were conventional chemical input, moderate level of chemical input and low level of chemical input. The conventional treatment consisted of production practices typical of those used by peach producers in southwest Michigan: preplant fumigation, clean cultivation of the soil, broadcast application of fertilizer, scheduled insecticide sprays and herbicide sprays of paraquat and simazine, and dormant pruning. Pesticide sprays were utilized according to spray guidelines issued by Michigan State University Cooperative Extension Service. The moderate level of chemical input included a fescue ground cover, fertilizer application through drip irrigation lines, insect scouting for spray scheduling, application of sulfur in place of synthetic fungicide sprays, conventional herbicide sprays of simazine and paraquat, and dormant pruning. Scouting included monitoring the presence of Oriental fruit moth, tarnished plant bug, and peach tree borers with sticky boards and traps. Once treatment thresholds were exceeded a spray was made to control populations. The low level of chemical input included an endophytic rye ground cover for tarnished plant bug control, utilization of novel insect controls, insect scouting for spray scheduling, control of nematodes with *Pseudomonas* predation, nitrogen fertilizer applied in the form of horse manure, application of sulfur in place of synthetic fungicide sprays, straw mulch in tree rows for weed control, no synthetic fertilizer application, and

summer pruning. Insect controls included pheromone disruption for control of Oriental fruit moth by placing pheromone ties in the trees to effectively saturate the orchard environment and thus prevent mating. The amount of horse manure applied gave the equivalent amount of actual nitrogen per ha as the other fertilization methods.

Pesticide Extraction and Analysis. Fifty kg of fruit were randomly collected from each orchard and the samples for each orchard combined. Twenty fruit samples were then cut into 4-5 g sections, retaining the epidermis but removing the pit, and stored at -30° until extracted. For extraction, fruit was thawed for 10 min at room temperature to allow for additional sectioning and blending. Soil samples were collected from 4 predesignated locations on the perimeter as well as from the center of each orchard and combined and the 200 g samples stored at -30° until extraction. All pesticides were extracted according to procedures obtained from M. Zabik of the Pesticide Research Center at Michigan State University and were in accordance with EPA methodology: simazine was extracted from the soil using the procedure of Smith (1981) and polyclonal antibody kits obtained from Millipore Corp. (Bedford, MA); chlorpyrifos and azinphos methyl were extracted from a procedure originally obtained from Shell (Modesto, CA); fenvalerate was extracted based on a procedure originally obtained from DuPont (Wilmington, DL); iprodione, chlorothalonil and captan were extracted based upon the procedure of Liao, *et al.* (1991); elemental sulfur was extracted using a procedure originally obtained from the EPA (Washington, DC). Triplicate samples were extracted from the 1992 fruit and duplicate samples extracted from the 1993 fruit. Samples spiked with each pesticide were extracted every six samples.

Extracted samples were analyzed on a Hewlett Packard gas chromatograph (model 5890 series II) with a 25m capillary DB-5 column and an electron capture detector. Injection was via a Hewlett Packard automatic injector (model 7673 series II). GC parameters were as follows: split injection mode, inlet temperature 240°; helium carrier gas with a column head pressure of 12.9 psi, nitrogen makeup gas, initial oven temperature of

180° for 20 min and then increasing at 10° per min to 275° and holding at 275° for 5 min; and detector temperature was 300°. Duplicates were run of each sample along with the spiked samples of pesticide standards obtained from Chem Service (Chester, PA). A standard was delivered no less than every six samples. Standards that were run included the first degradation products of chlorpyrifos, chlorothalonil and iprodione as these compounds are included in the EPA tolerance levels (Code of Federal Regulations, 1994). Retention times for the samples were compared to retention times for the standards and the amount of pesticide in each sample calculated from the area of the detector response as measured by integration (Hewlett Packard model 3396 series II Integrator). Recoveries of the extraction procedures were calculated based on the amount of pesticide found in the spiked samples.

Statistical Procedure. Treatment means were analyzed for differences using ANOVA and F test. Differences were considered significant at the 5% level. Residues were statistically analyzed separately for 1992 and 1993 as pest control treatments were different for each year and residue values were markedly different each year.

Results

Orchard Management Strategies. The number of pesticide spray applications to each treatment orchard are reported in Table 9 for 1992 and Table 10 for 1993. In 1992, the CONV orchards received a total of 14 synthetic chemical sprays compared to 9 for the MOD and 2 for the LOW. In addition the LOW orchards received 3 pesticide sprays that were applied only to the perimeter of the orchard, thus minimizing the amount of pesticide applied to harvested fruit. Also, the MOD orchard was treated with 6 sprays of elemental sulfur and the LOW was treated with 2 sulfur sprays. In 1993, the CONV orchard received 16 spray applications of synthetic chemicals, the MOD 6 spray applications and

the LOW 2 applications. Again, the MOD orchard received 6 sprays of elemental sulfur while the LOW received 2.

Pesticide Extraction and Analysis. The results from the pesticide extraction and analysis of the fruit are given in Tables 11 and 12. In 1992, Iprodione was detected in the CONV but not in the MOD or the LOW as it was not applied to these orchards. No iprodione was detected in any of the 1993 fruit samples. Chlorothalonil, applied only to the CONV was not detected in any of the samples except for detection of trace levels in 1993 in the CONV orchards. No captan was detected in 1992, but was detected in the two orchards to which it was applied to in 1993. Chlorpyrifos was not detected in 1992, but was detected in fruit from each treatment orchard in 1993,. In the case of the CONV, chlorpyrifos exceeded the EPA tolerance of 0.05 ppm. No azinphos methyl was detected in any of the samples analyzed for either year. Fenvalerate was detected in equal amounts in each treatment for both 1992 and 1993. Sulfur residues were highest in the MOD as those orchards received the most sulfur spray applications.

Simazine residues in the soil were not statistically different between treatments in 1992, the CONV had 8.0 ppb, the MOD 22.3 ppb and the LOW 12.2 ppb.

Recoveries for the extraction and analysis procedures ranged from 80-160% as determined from analysis of the spiked samples.

Discussion

Distinctly different levels of pesticides were applied to each of the different treatment orchards as a result of the distinct management strategies employed for each. The MOD orchards utilized a number of IPM techniques that eliminated the need for several of the synthetic chemical sprays. In particular, the use of sulfur as a fungicide markedly reduced the chemical input into those orchards. The fruit quantity and quality was comparable to the CONV (Flore *et al.*, 1994) indicating that IPM strategies can effectively

control pest populations and reduce chemical input into the orchard. The LOW orchards utilized additional IPM strategies and used synthetic chemicals only if absolutely necessary to prevent damage to the entire crop. However, the LOW orchards had a lower level of fruit free of insect and disease damage, 79% compared to the 95% for the CONV (Flore *et al.*, 1994). Thus, a significant crop can be produced by drastically reducing chemical inputs, but at present there is a tradeoff in quality and quantity.

There are obvious benefits to the producer and the consumer from producing fruit crops with reduced levels of synthetic chemicals. The producer may realize a net cost savings as a result of lower pesticide expenses, and the cumulative effect of pesticide application to the orchard site is reduced. The consumer benefits from knowing that fewer pesticides have been added to their food and the environment as a whole.

There is considerable controversy over the health effects of reduced pesticide input in fruit production. It is assumed that lower chemical inputs will mean more beneficial fruit as a result of lower pesticide residues. This study demonstrates that all chemicals inputs are not clearly reflected in the fruit residues, but reducing late season chemical inputs can reduce pesticide residues. The levels of fungicides applied were most noticeably different each year, but only in 1992 were any residue differences detected. The CONV orchards received the most fungicide applications and consequently had the higher iprodione residue in 1992. In 1993, captan was detected at 6.3 ppm in the CONV, yet there was no significant difference due to the high variability in the captan residues in the CONV orchards. Additionally, while fruit from the LOW and MOD orchards were free from synthetic fungicide residues, sulfur residues from those orchards was highest, although not statistically different. The insecticide fenvalerate was detected in each orchard each year, but only at higher levels in the 1993 in the CONV and MOD orchard fruit. In 1993, the level of chlorpyrifos in the CONV orchards was higher than the EPA tolerance for peach fruit, and the residues detected in the other treatment orchards were below the tolerance limit and different from the CONV residue level. As seen in this study, conventional pest

management can lead to fruit with excessive levels of pesticide residues as can poorly timed applications of pesticides in an IPM scheme. Implementation of IPM strategies is becoming a necessary pest management tool due to increased restriction on pesticide use, producer concern for the orchard environment and consumer preference. Reduced synthetic chemical pesticide input does not necessarily mean lower residues in the fruit. The manager must take into consideration the timing of chemical application with respect to harvest as well as alternative pest control measures.

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Table 9. Pesticide applications made to peach orchards in 1992. Treatments are Conventional, conventional level of chemical pest management; Moderate, moderate level of chemical pest management; and Low, low level of chemical pest management.

Pesticide (Rate·ha ⁻¹)*							
Treatment	Iprodione (2.2 kg)	Chlorothalonil (4.6 L)	Captan (1.1 kg)	Chlorpyrifos (3.27 kg)	AzinphosMethyl (3.27 kg)	Fenvalerate (0.55 kg)	Sulfur (16.3 kg)
Conventional	1	2	4	3	2	2	0
Moderate	0	0	0	2	4	1	6
Low	0	0	0	2**	2**	1**	2

* The Moderate level orchards received 2 benomyl applications.

** One of the chlorpyrifos sprays, the azinphosmethyl sprays and the fenvalerate spray were applied only to the perimeter trees of the Low level orchards.

Table 10. Pesticide applications made to peach orchards in 1993. Treatments are Conventional, conventional level of chemical pest management; Moderate, moderate level of chemical pest management; and Low, low level of chemical pest amangement.

Treatment	Pesticide (Rate·ha ⁻¹)*					
	Iprodione (2.2 kg)	Chlorothalonil (4.6 L)	Captan (2.2 kg)**	Chlorpyrifos (3.3 kg)	AzinphosMethyl (3.3 kg)	Fenvalerate (0.55 kg)
Conventional	1	3	4	4	2	2
Moderate	0	0	1	2	1	2
Low	0	0	0	1	1	3

* The Moderate level orchards received 6 sulfur and 2 benomyl applications and the Low level orchards received 2 sulfur applications.

** The Moderate level orchards were sprayed at a rate of 1 lb•acre⁻¹ of captan.

Table 11. The effect of different levels of chemical pesticide input on pesticide residues of peach fruit in 1992. Treatments were Conventional, conventional chemical pesticide input level; Moderate, moderate level of chemical pesticide input; Low, low level of chemical pesticide input. Values are the mean of triplicate extraction samples from two orchards of each treatment.

Treatment	Pesticide Residues (ppm)					
	Iprodione	Chlorothalonil	Captan	Chlorpyrifos	Azinphosmethyl	Fenvalerate
Conventional	0.29 a	0.00	0.0	0.00	0.0	1.22
Moderate	0.00 b	0.00	0.0	0.00	0.0	1.09
Low	0.00 b	0.00	0.0	0.00	0.0	0.93
5%		NS	NS	NS	NS	NS
EPA tolerance	20.0	0.50	50.0	0.05	2.0	10.0
MDL*	0.01	0.50	0.4	0.01	0.19	0.90
						5.0

*MDL, minimum detectable limit.

Treatment differences are not significant, NS, or significant at the probability level indicated.

Table 12. The effect of different levels of chemical pesticide input on pesticide residues of peach fruit in 1993. Treatments were Conventional, conventional chemical pesticide input level; Moderate, moderate level of chemical pesticide input; Low, low level of chemical pesticide input. Values are the mean of triplicate extraction samples from two orchards of each treatment.

Treatment	Pesticide Residues (ppm)				
	Iprodione	Chlorothalonil	Captan	Chlorpyrifos	Azinphosmethyl Fenvalerate
Conventional	0.00	trace	6.3	0.08 a	0.00 0.02 a
Moderate	0.00	0.00	trace	0.03 b	0.00 0.03 a
Low	0.00	0.00	0.0	0.04 b	0.00 trace b
<hr/>					
	NS	NS	NS	5%	5%
EPA tolerance	20.0	0.50	50.0	0.05	2.0 10.0
MDL*	0.09	0.04	1.3	0.01	0.38 0.02

*MDL, minimum detectable limit.

Treatment differences are not significant, NS, or significant at the probability level indicated.

APPENDIX I

Pesticide Residues Extraction Protocols

Protocol for azinphos methyl and chlorpyrifos:

1. Add 65 mL acetone to 20.0g of peach fruit and blend for 3 min. at medium speed.
2. Filter through Whatmann #4 paper using suction; rinse the blending apparatus thoroughly and also filter.
3. Extract with equal volume of methylene chloride in separatory funnel by shaking for 1 min. and allowing the phases to separate for 3+ min.
4. Percolate the lower, acetone–methylene chloride layer through a 2x5cm plug of anhydrous sodium sulfate.
5. Extract the upper aqueous phase with 25 mL methylene chloride via separatory funnel , shaking 30 sec. and allowing 3+ min. separation, percolate lower phase through anhydrous sodium sulfate.
6. Evaporate the acetone–methylene chloride eluate to dryness with a jet of air and 50°C water bath.
7. Dissolve residue in 50 mL hexane and transfer to 250 mL separatory funnel, using additional 50 mL of hexane to aid transfer.
8. Partition the hexane with three 25 mL portions of acetonitrile shaking 1 min. each time.
Combine acetonitrile extracts and discard hexane layer.
9. Evaporate acetonitrile extracts to dryness with a jet of air and 50°C water bath, dissolve residue in 2 mL acetone.

Protocol for fenvalerate:

1. Add 200 mL of hexane:isopropanol (3:1) to 20.0g of peach fruit and blend for 3 min at medium speed.
2. Filter using vacuum into a 250 mL separatory funnel.
3. Add 100 mL of water, shake carefully for 1 min. and discard lower, aqueous phase.
4. Wash hexane with 2 additional 100 mL volumes of water to remove all isopropanol, measure and record volume of hexane.
5. Column preparation: cover 6.0g of activated Florisil (overnight heating at 145°C) with hexane, and add to a glass column using hexane to complete transfer. Tap column to settle Florisil and add a 1cm layer of anhydrous sodium sulfate to the top of the column and drain excess hexane keeping anhydrous sodium sulfate covered in hexane.
6. Add 2.0 mL of the hexane extract to the column and drain, adding 5 mL additional hexane to wash.
7. Add 50 mL of hexane to column and discard hexane fraction.
8. Add 50 mL of 5% ethyl acetate in hexane and save this fraction for analysis, evaporate to <5 mL.

Protocol for chlorothalonil, iprodione and captan:

1. Add 50 mL of acetonitrile to 20.0g of peach fruit and blend for 5 min at medium speed.
2. Filter using vacuum, add 10g of NaCl and shake vigorously for 1 min.
3. Allow phases to separate; if no separation occurs, centrifuge at low g for 3 min.
4. Aliquot 40mL of upper, acetonitrile phase to a flask (recording exact volume of aliquot).
5. Add 0.5 mL of toluene and percolate through a column containing 5g of anhydrous sodium sulfate.
6. Rotoevaporate to 0.5-1.0 mL – DO NOT DRY COMPLETELY!
7. Filter into a vial and rinse filter twice with 0.5 mL acetone to complete transfer.

Protocol for benomyl:

1. Add 25 mL of methanol:ethyl acetate (1:1) to 20.0g of peach fruit and blend for 1 min at low speed.
2. Filter using vacuum and wash the cake with additional 25 mL of methanol:ethyl acetate.
3. Transfer the filtrate to a 1L flask containing 1.0 mL of 0.5N HCl.
4. Extract with 10 mL of hexane and discard the upper hexane layer.
5. Adjust this solution to pH 7.5–8.0 by addition of 1N NaOH.
6. Extract twice with 100 mL portions of ethyl acetate and save the ethyl acetate extract.
7. Dry the ethyl acetate extracts by percolating through anhydrous sodium sulfate and then wash the sodium sulfate with 50 mL of additional ethyl acetate.
8. Rotoevaporate at 35°C to 4–5mL.

Protocol for sulfur:

1. Prepare a dry, clean beaker and weigh accurately!
2. Add 10 mL of carbon disulfide to 20.0g of blended peach fruit in a filter and mix gently, catching the filtrate in the weighed beaker..
3. Wash with 3 additional 5 mL portions of carbon disulfide.
4. Evaporate the carbon disulfide in an exhaust hood at room temperature.
5. When carbon disulfide is completely evaporated, heat the beaker and residue for 15-20 min. at 100-105°C and weigh.
6. Subtract to determine the weight of elemental sulfur.

Protocol for simazine:

1. Weigh 20.0g wet soil into a 33x94mm Soxhlet extraction thimble and cover with glass wool. Make a moisture content determination of a separate soil sample.
2. Place thimble of soil in Soxhlet extractor with 150mL of 10% water in methanol and reflux for 23 hours and then collect water:methanol into 250 mL flask and rotoevaporate to dryness.
3. Add 50 mL methylene chloride to dissolve residues and transfer to a 250 mL separatory funnel.
4. Rinse flask with 10-15 mL of methylene chloride and 50 mL of water and add to separatory funnel. Rinse a second time with 25 mL methylene chloride but keep second rinse separate.
5. Shake separatory funnel vigorously for 1 min., allow phases to separate completely and drain methylene chloride into a 250 mL flask. Add second rinse to separatory funnel, shake for 30 sec., collect and combine methylene chloride fractions and rotoevaporate to dryness.
6. Redissolve in 2 mL isooctane and transfer through a glass funnel containing isooctane-saturated glass wool to a preweighed 13x100mm glass, teflon-lined screw cap culture tube. Repeat twice bringing total volume to 6-7 mL.

APPENDIX J

Data for 1993 Mite Population Counts

Table 13. Data for Mite Population Counts at Clarksville Michigan in 1993.

plot	8/9/93			8/19			9/16			9/20			9/30			10/8		
	MPL	MD	CMD	MPL	MD	CMD	MPL	MD	CMD	CMD			MPL	MD	CMD	CMD		
I-C	11.2	44	703	14.5	129	832	7.1	302	1134	1183			7.1	99	1233	1290		
I-M1	24.1	64	725	15.7	199	924	8.7	342	1266	1327			8.7	122	1388	1458		
I-M2	34.3	79	738	12.9	236	973	14.2	378	1352	1451			14.2	198	1550	1663		
I-M3	70.1	133	791	13.7	419	1210	5.6	270	1480	1519			5.6	79	1558	1603		
II-C	11.9	49	605	8.1	100	705	3.3	159	864	887			3.3	46	910	936		
II-M1	15.2	50	614	9.4	123	737	6.8	226	964	1011			6.8	95	1058	1112		
II-M2	21.9	59	641	14.8	184	825	22.6	524	1349	1507			22.6	316	1665	1845		
II-M3	21.4	59	619	27.9	247	865	26.4	760	1625	1810			26.4	369	1994	2205		
III-C	19.9	57	407	6.9	134	541	2.8	136	677	697			2.8	39	716	739		
III-M1	12.5	46	781	6.3	94	875	0.7	97	972	977			0.7	9	981	987		
III-M2	19.9	57	689	12.7	163	852	7.6	284	1136	1189			7.6	106	1242	1303		
III-M3	29.7	72	696	24.3	270	966	15.7	559	1524	1634			15.7	219	1743	1868		
IV-C	18.3	55	725	4.3	113	838	1.7	83	921	932			1.7	23	944	957		
IV-M1	29.0	71	871	12.2	206	1077	0.5	178	1255	1258			0.5	7	1262	1266		
IV-M2	51.5	105	989	7.1	293	1282	18.1	353	1635	1762			18.1	254	1889	2034		
IV-M3	36.1	82	716	24.3	302	1018	6.4	430	1447	1492			6.4	90	1537	1589		

APPENDIX K

Data for 1993 Chlorophyll Fluorescence Measurements of Field Study on Mites

Table 14. Data for chlorophyll fluorescence measurements for control and mite infested trees at Clarksville Michigna in 1993.

	8/10/93				9/16/93				9/30/93			
	Fo	Fm	Fv	Fv/Fm	Fo	Fm	Fv	Fv/Fm	Fo	Fm	Fv	Fv/Fm
1C	668	2263	1595	0.705	704	2081	1377	0.662	902	2135	1234	0.578
1M1	719	2375	1656	0.697	772	2215	1443	0.652	842	1908	1066	0.559
1M2	638	2178	1541	0.707	649	2013	1365	0.678	659	1625	966	0.595
1M3	564	2209	1645	0.745	765	2248	1484	0.660	728	1807	1079	0.597
2C	857	2518	1661	0.660	867	2368	1501	0.634	971	2167	1196	0.552
2M1	733	2433	1700	0.699	799	2241	1442	0.643	969	2224	1255	0.564
2M2	820	2567	1747	0.680	864	2323	1459	0.628	913	1975	1062	0.538
2M3	812	2454	1643	0.669	889	2404	1516	0.630	898	1980	1083	0.547
3C	754	2279	1525	0.669	783	2309	1526	0.661	890	1975	1085	0.549
3M1	801	2298	1497	0.651	833	2280	1447	0.635	945	2113	1168	0.553
3M2	799	2426	1627	0.671	914	2445	1532	0.626	908	2043	1135	0.555
3M3	764	2352	1589	0.675	903	2229	1326	0.595	609	1162	553	0.476
4C	697	2205	1509	0.684	748	2071	1324	0.639	879	1925	1046	0.543
4M1	680	2264	1584	0.700	750	2010	1260	0.627	789	1751	962	0.550
4M2	696	2259	1563	0.692	821	2288	1467	0.641	1020	1965	945	0.481
4M3	679	2291	1613	0.704	791	2062	1271	0.616	349	719	370	0.515

APPENDIX L

Effect of Mites on Shoot Growth and Flowering

Table 15. The effect of mites on 1993 shoot growth and 1994 flowering and fruit set for orchard trees infested in 1993.

	Shoot Growth	Flowers	Fruit Set
Treatment	(cm)	(#)	(%)
Control	22.85	62.0	16.0
Mites	22.12	60.1	17.3
	NS	NS	NS

Treatment differences are not significant at 10% probability level, NS, or significant at the probability level indicated.

APPENDIX M

Effect of terbacil on fruit yield and quality in 1993 and 1994

Table 16. The effect of application of 63 ppm terbacil on 1993 fruit yield, diameter, weight, soluble solids and removal force; and 1994 return fruit set and yield of orchard sour cherry trees at Clarksville Michigan. Terbacil #1 sprayed on 1 June, Terbacil #2 sprayed on 15 June, Terbacil #3 sprayed on 4 August and Terbacil #4 sprayed on 17 September.

Treatment	1993					1994	
	Total Yield (kg/cm ² trunk)	Fruit Diameter (mm)	Fruit Weight (g)	Soluble Solids (%)	Removal Force (g)	Fruit Set (%)	Yield (kg/cm ² trunk)
Control	0.172	20.5	5.32	9.8	450	16.0 a	0.152
Terbacil #1	0.167	20.6	5.40	10.4	430	17.3 a	0.145
Terbacil #2	0.186	20.3	5.20	10.0	435	24.1 b	0.152
Terbacil #3						21.5 ab	0.144
Terbacil #4						21.8 ab	0.149

NS NS NS NS NS p<5% NS

Treatment differences are not significant, NS, or significant at the probability level indicated.

APPENDIX N

Chlorophyll Data Collected in 1993 on Mite Treated Trees

Table 17. Chlorophyll data from control and mite infested trees at Clarksville Michigan in 1993.

trmt	8/24/93					9/20/93					10/8/93				
	chl a	chl b	chl a/b	P chl	total chl	chl a	chl b	chl a/b	P chl	total chl	chl a	chl b	chl a/b	P chl	total chl
I-C	55.0	16.2	3.4	1.6	72.8	53.8	17.0	3.2	4.9	75.6	45.0	15.4	2.9	5.2	65.7
I-M1	55.3	18.9	2.9	4.5	78.7	50.9	15.4	3.3	5.0	71.3	43.1	14.4	3.0	4.7	62.2
I-M2	51.9	16.1	3.2	3.2	71.2	45.8	14.1	3.3	5.2	65.1	46.0	15.0	3.1	4.8	65.8
I-M3	55.9	17.4	3.2	1.8	75.1	56.2	16.1	3.5	4.9	77.3	45.1	15.2	3.0	6.4	66.8
II-C	46.9	17.3	2.7	5.4	69.6	47.8	16.3	2.9	4.6	68.6	44.8	16.2	2.8	4.7	65.7
II-M1	53.0	20.0	2.6	4.5	77.5	50.0	17.9	2.8	4.9	72.8	46.4	16.7	2.8	5.1	68.2
II-M2	50.3	17.5	2.9	4.5	72.3	43.8	15.4	2.8	4.5	63.8	39.8	15.1	2.6	4.9	59.8
II-M3	48.4	16.1	3.0	1.3	65.7	49.1	17.0	2.9	4.7	70.8	42.0	15.3	2.7	4.6	61.9
III-C	47.8	16.0	3.0	1.3	65.1	43.0	14.7	2.9	4.7	62.3	36.7	13.2	2.8	4.6	54.4
III-M1	43.5	15.1	2.9	3.0	61.6	54.6	23.6	2.3	13.7	91.9	45.3	15.7	2.9	4.8	65.8
III-M2	53.7	17.8	3.0	1.4	72.8	45.9	15.9	2.9	4.6	66.3	41.4	14.5	2.9	4.8	60.7
III-M3	46.9	15.0	3.1	1.3	63.2	37.2	13.2	2.8	4.4	54.8	26.9	10.8	2.5	4.5	42.2
IV-C	52.8	17.4	3.0	2.1	72.3	50.9	16.2	3.1	4.9	72.0	47.5	16.2	2.9	4.8	68.5
IV-M1	53.6	17.0	3.2	2.0	72.6	53.9	17.8	3.0	4.9	76.6	42.0	14.6	2.9	4.8	61.4
IV-M2	45.7	15.0	3.0	1.4	62.0	50.6	17.3	2.9	4.5	72.4	44.1	15.4	2.9	4.8	64.3
IV-M3	46.8	14.9	3.1	1.3	63.0	45.3	14.5	3.1	4.7	64.5	38.8	13.3	2.9	4.6	56.7

APPENDIX O

Yield Data Collected in 1993 and 1994

Table 18. Yield Data collected in 1993 and 1994 from orchard sour cherry trees.

1993 Yield

	I	II	III	Average
Control	84.43	50.91	101.72	79.02
Terb1	114.48	30.37	90.31	78.38
Terb2	87.65	72.34	76.62	78.87

1994 Yield

	I	II	III	IV	Average
Control	41.65	15.03	29.93	50.34	34.24
Mites	61.72	12.38	23.79	35.55	33.36
Control	30.94	6.48	31.64	42.32	27.85
Terb1	43.10	16.98	45.04	24.66	32.45
Terb2		14.94	21.02	62.96	32.97
Terb3	42.58	8.72	24.18	46.64	30.53
Terb4	55.18	17.74	18.92	28.08	29.98

APPENDIX P

Starch Data Collected in 1993 and 1994

Table 19. Starch data collected from orchard sour cherry trees in 1993 and 1994.

Fall Shoot Starch			Spring Shoot Starch			Spring Shoot Starch		
<u>Rep</u>	<u>Treatment</u>	<u>% Starch</u>	<u>Rep</u>	<u>Treatment</u>	<u>% Starch</u>	<u>Rep</u>	<u>Treatment</u>	<u>% Starch</u>
2	T3	9.02%	1	C	8.20%	1	C	7.26%
2	T1	3.73%	2	C	6.95%	2	C	3.51%
4	T3	8.95%	3	C	9.79%	3	C	5.49%
1	M1	8.73%	4	C	7.50%	4	C	6.81%
4	M1	6.09%	1	M1	16.57%	1	M1	5.04%
1	T1	6.98%	2	M1	10.50%	2	M1	5.71%
2	M2	5.33%	3	M1	10.89%	3	M1	4.71%
3	T4	8.22%	4	M1	9.68%	4	M1	3.67%
3	T3	8.02%	1	M2	17.62%	1	M2	3.28%
2	C	6.76%	2	M2	8.92%	2	M2	2.18%
1	T4	9.33%	3	M2	13.34%	3	M2	0.63%
3	M3	7.96%	4	M2	9.83%	4	M2	2.01%
3	M1	6.00%	1	M3	12.81%	1	M3	7.41%
1	M2	9.40%	2	M3	14.56%	2	M3	0.82%
3	T1	7.10%	3	M3	20.46%	3	M3	0.79%
2	M1	6.16%	4	M3	13.26%	4	M3	6.07%
3	M2	8.17%	1	T1	16.39%	1	T1	11.02%
2	T4	9.35%	2	T1	7.59%	2	T1	5.11%
4	M2	7.14%	3	T1	10.07%	3	T1	7.40%
3	T2	9.29%	4	T1	11.91%	4	T1	4.40%
4	M3	7.37%	2	T2	13.68%	2	T2	1.27%
4	T1	6.69%	3	T2	7.46%	3	T2	1.51%
3	C	8.70%	4	T2	15.25%	4	T2	3.74%
4	T4	6.93%	1	T3	11.50%	1	T3	7.01%
2	T2	7.23%	2	T3	8.25%	2	T3	2.89%
1	M3	8.55%	3	T3	13.27%	3	T3	6.27%
4	T2	6.67%	4	T3	15.90%	4	T3	2.15%
4	C	8.00%	1	T4	15.37%	1	T4	10.85%
1	T3	9.99%	2	T4	10.81%	2	T4	3.31%
2	M3	8.26%	3	T4	14.67%	3	T4	3.22%
1	C	10.07%	4	T4	9.70%	4	T4	3.64%

APPENDIX Q

Data for Container Grown Mite Study

Table 20. Data collected from container grown control and mite trees in 1994.

REP	TRTMT	WGT	HGT	6/15/94		6/22/94	6/27/94	6/29/94	
		(kg)	(cm)	#MPL	CMD	Fv/Fm	Fv/Fm	#MPL	CMD
1	C	0.126	65	0.0	0	0.612	0.687	1.3	9
1	M1	0.176	58	17.0	68	0.630	0.671	15.0	292
1	M2	0.144	55	22.5	90	0.621	0.511	13.3	341
1	M3	0.215	56	30.0	120	0.639	0.665	26.7	517
2	C	0.123	67	0.0	0	0.658	0.721	1.0	7
2	M1	0.158	64	35.0	140	0.649	0.654	16.7	502
2	M2	0.201	64	30.0	120	0.670	0.674	28.3	528
2	M3	0.181	60	50.0	200	0.703	0.672	13.3	643
3	C	0.095	59	0.0	0	0.674	0.640	0.0	0
3	M1	0.144	62	12.5	50	0.643	0.683	13.3	231
3	M2	0.237	57	22.5	90	0.681	0.679	25.0	423
3	M3	0.126	60	17.5	70	0.650	0.688	9.7	260
4	C	0.108	66	0.0	0	0.629	0.647	0.0	0
4	M1	0.150	61	19.0	76	0.672	0.685	18.3	337
4	M2	0.241	54	30.0	120	0.686	0.662	30.0	540
4	M3	0.101	60	41.0	164	0.666	0.630	53.3	824
5	C	0.197	58	0.0	0	0.698	0.665	2.3	16
5	M1	0.189	66	19.5	78	0.698	0.656	26.7	401
5	M2	0.186	64	24.5	98	0.597	0.626	5.7	309
5	M3	0.110	65	81.5	326	0.691	0.653	41.7	1188
6	C	0.146	60	1.5	6	0.727	0.689	15.0	122
6	M1	0.176	54	10.0	40	0.717	0.666	16.7	227
6	M2	0.106	57	54.5	218	0.673	0.665	21.7	751
6	M3	0.153	56	34.5	138	0.697	0.646	36.7	636
7	C	0.171	62	0.0	0	0.697	0.662	5.3	37
7	M1	0.057	50	3.5	14	0.646	0.674	13.3	132
7	M2	0.091	61	15.0	60	0.622	0.624	21.7	317
7	M3	0.149	59	30.5	122	0.587	0.685	43.3	639
8	C	0.124	48	0.0	0	0.687	0.663	2.3	16
8	M1	0.115	51	11.5	46	0.657	0.687	6.7	173
8	M2	0.129	56	27.0	108	0.618	0.593	10.0	367
8	M3	0.130	54	49.0	196	0.702	0.633	33.3	772
9	C	0.076	47	0.0	0	0.691	0.691	8.0	56
9	M1	0.158	44	16.5	66	0.687	0.663	53.3	555
9	M2	0.068	50	11.0	44	0.696	0.669	15.0	226
9	M3	0.179	55	37.5	150	0.714	0.665	13.3	506
10	C	0.157	53	0.0	0	0.509	0.601	1.0	7
10	M1	0.149	56	11.5	46	0.613	0.697	13.3	220
10	M2	0.106	44	36.5	146	0.650	0.621	30.0	612
10	M3	0.262	53	35.5	142	0.640	0.649	26.7	577
11	C	0.103	57	0.3	1	0.656	0.626	1.5	14
11	M1	0.088	52	8.3	33	0.658	0.700	25.0	266
11	M2	0.076	54	18.0	72	0.579	0.662	44.5	510
11	M3	0.154	50	29.0	116	0.681	0.566	39.3	594

Table 20 (cont'd)

REP	TRTMT	7/7/94		7/11/93	7/13/94	7/13/94	7/13/94	
		A	Fv/Fm	Fv/Fm	A	Chlor	#MPL	CMD
1	C		0.622	0.649	13.4	75.71	0.0	19
1	M1		0.664	0.687	9.5	75.12	0.0	397
1	M2		0.688	0.706	11.9	72.83	0.0	434
1	M3		0.639	0.698	12.1	76.34	0.0	703
2	C		0.689	0.683	9.3	75.81	0.0	14
2	M1		0.708	0.705	12.8	59.54	0.0	618
2	M2		0.736	0.665	12.2	78.35	0.0	727
2	M3		0.685	0.690	13.6	71.51	0.0	737
3	C		0.683	0.691	12.0	64.22	0.0	0
3	M1		0.619	0.601	14.0	70.76	0.0	324
3	M2	9.8	0.681	0.658	10.2	75.95	0.0	598
3	M3		0.678	0.577	12.9	62.91	0.0	328
4	C		0.633	0.637	8.7	78.93	0.0	0
4	M1		0.646	0.669	10.6	74.89	0.0	466
4	M2		0.661	0.621	13.4	71.47	0.0	750
4	M3		0.661	0.575	13.0	66.49	0.0	1198
5	C	11.9	0.629	0.667	11.9	74.28	0.0	33
5	M1		0.680	0.693	14.5	72.05	0.0	588
5	M2	12.5	0.621	0.631	12.9	73.30	0.0	349
5	M3	12.2	0.665	0.637	13.0	77.49	0.0	1480
6	C	12.3	0.658	0.655	11.9	74.18	0.0	227
6	M1	14.7	0.659	0.700	11.5	71.07	0.0	343
6	M2	14.6	0.688	0.697	11.7	78.86	0.0	903
6	M3	14.5	0.650	0.699	14.1	77.84	0.0	893
7	C	15.7	0.646	0.658	11.4		0.0	75
7	M1	14.2	0.601	0.680	13.6		0.0	225
7	M2	12.7	0.674	0.686	11.0		0.0	468
7	M3	16.5	0.702	0.605	13.7		0.0	942
8	C	15.7	0.598	0.689	14.5		0.0	33
8	M1	16.0	0.694	0.650	13.8		0.0	220
8	M2	15.6	0.630	0.641	16.1		0.0	437
8	M3	14.1	0.648	0.632	14.0		0.0	1006
9	C	14.8	0.663	0.669	23.9		0.0	112
9	M1	14.9	0.625	0.620	10.6		0.0	928
9	M2	13.8	0.710	0.694	12.0		0.0	331
9	M3		0.680	0.608	14.6		0.0	599
10	C	15.0	0.671	0.646	16.8		0.0	14
10	M1	12.5	0.675	0.634	15.5		0.0	313
10	M2	15.0	0.661	0.557	13.1		0.0	822
10	M3	13.4	0.602	0.682	15.2		0.0	764
11	C	14.6	0.557	0.664	18.6		0.0	24
11	M1	11.7	0.667	0.711	13.9		0.0	441
11	M2	10.1	0.649	0.682	12.5		0.0	821
11	M3	12.3	0.653	0.617	14.6		0.0	870

Table 20 (cont'd)

REP	TRTMT	7/21/94		7/21/94		8/1/94		8/2/94	
		Fv/Fm	A	#MPL	CMD	Fv/Fm	Yield	#MPL	CMD
1	C	0.744	12.8	1.7	25	0.695	0.674	4.7	64
1	M1	0.721	11.2	5.3	418	0.670	0.691	8.3	500
1	M2	0.753	10.8	10.0	474	0.686	0.711	21.7	664
1	M3	0.741	11.5	20.0	783	0.690	0.678	23.3	1043
2	C	0.723	13.2	1.0	18	0.680	0.728	5.7	58
2	M1	0.699	12.6	16.7	685	0.690	0.707	36.7	1005
2	M2	0.709	15.3	11.7	773	0.687	0.702	20.0	963
2	M3	0.738	8.7	6.7	763	0.714	0.711	15.0	893
3	C	0.716	11.3	0.7	3	0.677	0.730	3.0	25
3	M1	0.750	14.3	5.0	344	0.706	0.744	3.3	394
3	M2	0.741	11.9	25.0	698	0.689	0.758	28.3	1017
3	M3	0.747	13.7	8.3	361	0.662	0.705	16.7	511
4	C	0.766	14.6	0.7	3	0.699	0.703	2.0	19
4	M1	0.760	14.3	13.3	519	0.721	0.649	33.3	799
4	M2	0.736	14.5	23.3	843	0.685	0.690	16.7	1084
4	M3	0.757	11.1	20.0	1278	0.687	0.755	15.0	1488
5	C	0.733	16.0	2.3	42	0.679	0.685	3.0	74
5	M1	0.738	15.7	10.0	628	0.694	0.761	11.7	758
5	M2	0.713	10.2	11.7	396	0.635	0.691	25.0	616
5	M3	0.753	12.5	10.0	1520	0.690	0.649	23.3	1720
6	C	0.765	15.1	1.3	232	0.690	0.750	3.0	258
6	M1	0.728	12.7	10.0	383	0.701	0.717	11.7	514
6	M2	0.747	14.9	20.0	983	0.671	0.758	18.3	1213
6	M3	0.759	14.0	15.0	953	0.675	0.760	18.3	1153
7	C	0.752	14.7	4.0	91	0.706	0.724	1.0	121
7	M1	0.755	12.5	23.3	319	0.675	0.693	20.0	579
7	M2	0.760	14.3	13.3	522	0.723	0.691	16.7	702
7	M3	0.736	11.0	10.0	982	0.684	0.732	33.3	1242
8	C	0.748	16.3	0.7	35	0.711	0.708	3.7	62
8	M1	0.748	13.9	5.7	243	0.635	0.657	31.7	467
8	M2	0.739	14.2	2.3	446	0.693	0.687	21.7	591
8	M3	0.714	14.9	21.7	1092	0.688	0.722	23.3	1362
9	C	0.747	4.1	0.0	112	0.702	0.771	7.7	158
9	M1	0.716	13.6	18.3	1002	0.677	0.652	15.0	1202
9	M2	0.708	13.0	10.0	371	0.685	0.696	23.3	571
9	M3	0.742	14.9	23.3	693	0.668	0.671	30.0	1013
10	C	0.734	9.9	2.3	23	0.688	0.729	5.7	72
10	M1	0.757	13.5	5.0	333	0.664	0.648	23.3	503
10	M2	0.764	5.6	10.0	862	0.673	0.662	25.0	1072
10	M3	0.752	14.4	27.3	873	0.659	0.708	25.0	1187
11	C	0.739	13.7	2.7	35	0.676	0.722	5.7	85
11	M1	0.738	1.0	23.3	535	0.716	0.700	53.3	994
11	M2	0.462	17.3	19.7	900	0.693	0.706	23.3	1157
11	M3	0.720	15.2	23.0	962	0.681	0.714	30.0	1280

Table 20 (cont'd)

		8/2/94			8/10/94
REP	TRTMT	A	E	Tree A	Chlor
1	C	6.5	9.69	29.29	71.40
1	M1	8.7	10.36	26.85	67.95
1	M2	8.1	10.62	26.85	69.27
1	M3	7.7	9.04	29.29	70.31
2	C	11.5	11.67	17.09	66.57
2	M1	9.5	10.67	17.09	69.61
2	M2	6.9	10.71	14.65	70.08
2	M3	6.9	11.15	12.21	67.69
3	C	13.8	11.22	12.21	63.19
3	M1	11.1	11.05	9.76	61.33
3	M2	8.3	11.28	9.76	64.05
3	M3	9.7	11.25	9.76	62.52
4	C	13.5	11.12	36.62	68.08
4	M1	8.5	13.20	14.65	60.43
4	M2	6.6	11.40	25.63	67.06
4	M3	7.6	12.00	14.65	68.34
5	C	8.9	13.88	32.96	74.18
5	M1	11.6	13.98	21.97	70.91
5	M2	7.5	14.36	21.97	68.11
5	M3	6.3	14.29	21.97	70.33
6	C	10.7	15.32	32.96	72.85
6	M1	10.7	15.07	21.97	64.69
6	M2	13.4	14.37	21.97	61.04
6	M3	10.4	14.92	21.97	64.56
7	C	14.4	14.77		
7	M1	6.2	14.62		
7	M2	9.9	14.51		
7	M3	8.5	14.92		
8	C	9.1	13.18		
8	M1	9.1	13.69		
8	M2	6.8	12.84		
8	M3	7.6	13.09		
9	C	10.9	12.13		
9	M1	10.1	11.92		
9	M2	9.9	11.71		
9	M3	6.5	11.86		
10	C	10.3	11.64		
10	M1	9.3	11.57		
10	M2	8.2	11.20		
10	M3	10.3	10.62		
11	C	8.0	8.10		
11	M1	6.9	8.35		
11	M2	8.7	9.95		
11	M3	7.5	9.77		

APPENDIX R

**Daily Minimum and Maximum Temperatures for 1993-1994 Dormant Period at Clarksville
Michigan**

**Figure 15. Daily Minimum and Maximum Temperatures for 1993-1994 Dormant Period at
Clarksville Michigan**

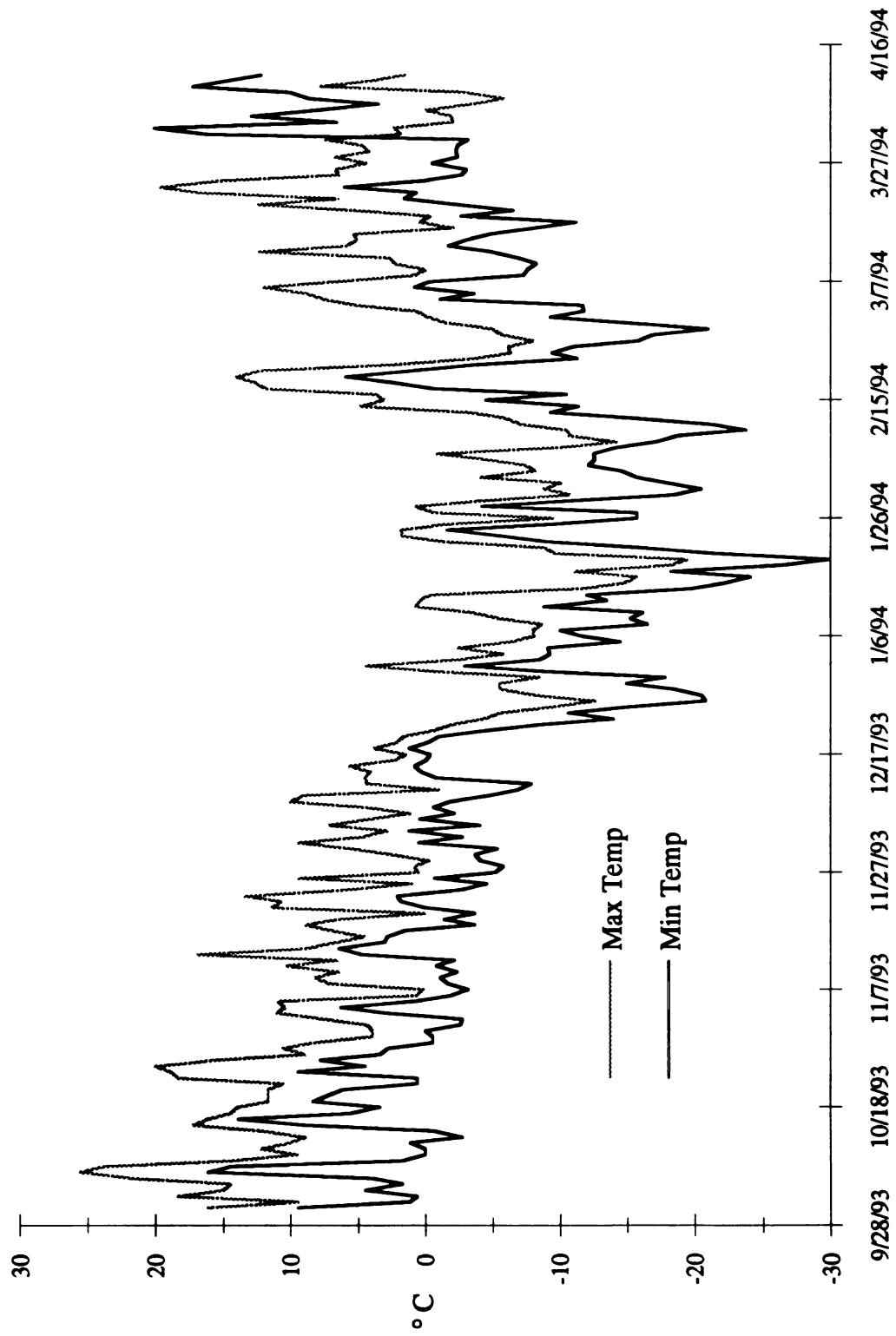


Figure 15

APPENDIX S

Air flow generating fan and inlet setup for whole plant photosynthetic CO₂ assimilation
chambers

Figure 16. Air flow generating fan and inlet setup for whole plant photosynthetic CO₂ assimilation chambers.



Figure 16

APPENDIX T

Schematic drawing of whole plant photosynthetic CO₂ assimilation chambers.

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