This is to certify that the
dissertation entitled

DETERMINATION OF DAMAGE THRESHOLD LEVELS OF STRAWBERRIES (*Fragaria* × *ananassa*)

presented by

A. Zafer Makaraci

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Horticulture

James M. Flore
Major Professor's Signature

May 1, 2003
Date

DETERMINATION OF DAMAGE THRESHOLD LEVELS OF STRAWBERRIES (*Fragaria × ananassa*)

By

A. Zafer Makaraci

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

2003

ABSTRACT

DETERMINATION OF DAMAGE THRESHOLD LEVELS OF STRAWBERRIES

(*Fragaria* × *ananassa*)

by

A. Zafer Makaraci

Damage thresholds of strawberry plants (*Fragaria* × *ananassa*) were investigated by using two different methods. The first method was mechanical damage by punching holes in leaves such that a predetermined leaf area was removed from each leaf. The second method was terbacil application. Mechanical damage was applied such that 10%, 20% and 30% of the leaf area of a fully expanded single leaf was removed. Terbacil was applied to field-grown plants during 2001 and 2002. In 2001, terbacil was applied at concentrations of 12.5, 25, 50, 100, 200 and 400 ppm. In 2002, a previously untreated group of two year old strawberry plants were sprayed with terbacil at concentrations of 50, 100 and 200 ppm at two growth stages (during fruit set and after harvest stages).

Strawberry leaves that were mechanically damaged did not recover their photosynthetic capacity following the damage. Chlorophyll fluorescence (F_v/F_m) values were not affected by the mechanical damage. Increasing damage levels decreased the ability of the strawberry leaf to use light and carbon dioxide in photosynthesis. Difference in stomatal conductance and transpiration rates were

insignificant. Internal CO_2 (C_i) levels were higher in damaged plants compared to the control plants.

Strawberry plants that were treated with terbacil (12.5, 25, 50, 100 and 200 ppm) were able to recover at certain levels, except 400 ppm level during the first year experiment. Recovery occurred between 4 and 10 days after the terbacil treatment. Average fruit weight was adversely affected during the year following the 400 ppm terbacil treatment. Other concentrations of terbacil did not have any affect on fruit yield. Stage of development did not alter the response of the plants to terbacil. Difference in stomatal conductance and transpiration rates were insignificant. Internal CO_2 (C_i) levels were higher in plants that were treated with high terbacil concentrations. Chlorophyll a and total chlorophyll content decreased following the terbacil treatment. However, chlorophyll a and total chlorophyll increased 8 days after terbacil treatment. Plant dry matter and chl b values were not affected by the terbacil treatments.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Dr. James Flore, my major professor, for the support and advice that he has given during my doctoral program. He always encouraged me and provided motivation for completion of my doctoral program.

I also would like to thank to the members of my guidance committee, Dr. James Hancock, Dr. Stanley Howell, Dr. Kenneth Poff and Dr. Frank Telewski for their interest and suggestions with the dissertation projects.

I also thank to the Turkish government for providing scholarship for my Ph.D. education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATONS

A	net CO_2 assimilation ($\mu mol.m^{-2}.s^{-1}$)
C.E.	carboxylation efficiency
chl a	chlorophyll a
chl b	chlorophyll b
C_i	internal CO_2 concentration (ppm)
E	transpiration rate ($mmol.m^{-2}.s^{-1}$)
F_m	maximal fluorescence
F_0	instantaneous fluorescence
F_v	variable fluorescence
F_v/F_m	photochemical fluorescence
g_s	stomatal conductance rate ($\mu mol.m^{-2}.s^{-1}$)
I_s	relative stomatal limitation to A
P chl	protochlorophyll
PAR	photosynteticly active radiation ($\mu mol.m^{-2}.s^{-1}$)
R^2	regression coefficient
RuBP	ribulose-1,5-bisphosphate
Q.E.	quantum efficiency

INTRODUCTION

The damage threshold when used in a pest management context is defined as the level of pest damage above which there are negative effects on the growth or health of the plant. For a perennial crop like strawberry the effect could either be in the current year, or in the subsequent crop year.

Foliage damage threshold levels have been determined for several plants, but have not been determined for strawberry. Such thresholds have been observed in wheat and barley (Shaw, 1956), soybean (Wareing, 1968), lucerne (Hodgkinson, 1974), sour cherry (Layne, 1989), (Disegna, 1994), apple (Ferree, 1982), (Lakso 1996). Foliage damage threshold levels for other crop plants range from 5% - 20% depending on the crop, and the crop load (Disegna, 1994). Determination of such a value would be useful in IPM and pesticide application programs, the assessment of environmental impacts, and on economical studies.

Damage to strawberry foliage can be biotic (insect, disease or weeds) or abiotic (temperature, drought, anoxia, etc). Biotic damage is a major concern to growers. Growers need to intervene at different stages of growth and this intervention may differ depending on the level of damage (Gut, 2003). Plants may have different photosynthetic recovery levels at different growth stages, such development stage of the leaves. Thicker leaves are usually more resistant to damage from herbicides and pathogens. Kirwood (1983), Unrath (1981) and Bukovac (1979) found that cuticle of older leaves is less permeable and thicker. This decreases herbicide absorption.

CO_2 assimilation rates are similar to those of many other fruit crops (Flore, 1989). In *Fragaria* × *ananassa* Duch. Photosynthesis rate range from 15 to 25 $\mu mol.m^{-2}.s^{-1}$ (Hancock, 1989). High photosynthesis rates do not result in increase in strawberry fruit yield (Strick 1986). Strawberry plants are known to have active sinks. Roots, runners, fruits and leaves are the sinks for strawberry plants (Alpert, 1986). In order to have high yield high portion of the fixed carbon has to be allocated to fruit (Hancock, 1991).

Deblossoming causes new leaf formation and total photosynthetic rate increases on per plant basis in strawberry plants (Forney, 1985). Defoliation of in excess of 66% leaf area result in higher CO_2 assimilation rate per leaf area. However, photosynthesis of the whole plant is not compensated completely (Kerkhoff, 1988). Removal of the flowers during the first year caused increased vegetative growth in both years and increased yield during the second year (Daugaard, 1999).

Fruit load may also affect photosynthetic recovery, since fruits are one of the major sinks for carbohydrates. Fruit removal often result in decrease in CO_2 assimilation rates on a per leaf area basis for at least a few weeks in strawberry plants (Schaffer, 1986).

Leaf removal decreases the total dry weight of strawberry plants (Chandler, 1988).

Gucci (1990) found different responses in CO_2 assimilation rates in plum trees depending on the stage that fruits were removed from trees. Allocation of carbohydrates to fruit may also affect the recovery process. Perennial crops may

also have a carry over effect into the next season. This carry over effect may be in the form of a decrease in cold hardiness. It has been found that early leaf loss caused a decrease in cold hardiness of the sour cherry buds and this effect was carried over into the next season (Howell, 1973).

The amount of the leaf damage may also have an affect on photosynthetic recovery. Damage may occur by different organisms such as insects, diseases, nematodes and mammals. Some environmental factors can also damage the leaves such as low temperature, wind, hail and fire. Cultural practices may also cause damage such as mechanical harvest or herbicide toxicity. Different parts (organs) of the plant can be damaged and each plant part may have a different damage threshold. Root damage may occur by nematodes in strawberry plants and different insects and diseases cause leaf damage. Some major diseases for strawberry plants in Michigan as follows: Leaf spot, Leaf blight, Scorch, Stem end rot, Angular leaf spot, Red stele, Powdery mildew, Anthracnose, Gray mold, Leather rot. Major insects that cause damage in strawberry plants in Michigan as follows: Strawberry sap beetle, Mites, Tarnished plant bug, Spittlebug, Strawberry leafroller, Strawberry clipper, Slugs, Leafhoppers, Strawberry aphips, Grubs (Gut, 2003).

In woody plants trunk damage may occur by cold damage, small mammals or mechanical harvest. Trunk damage thresholds have been investigated in some trees (Layne, 1989).

The hypothesis tested in this research was "Leaf photosynthetic capacity will determine the damage threshold levels for strawberry productivity".

For this purpose two different methods were used to determine the threshold levels in strawberry. Simulation of insects damage (mechanical damage) to the leaves by a leaf punch and use of terbacil a photosytem II inhibitor as a tool to reduce photosyntesis.

Hole punching has been used as a method to simulate insect damage to leaves on other species (Kappel ,1986; Layne, 1989).

Terbacil is a uracil type herbicide that blocks both the Hill reaction and photosytem II in the photosynthetic pathway (Ashton , 1973). Terbacil was used on fruit trees as a method to limit photosyntesis and to cause thinning (DelValle, 1985). Others have used terbacil as tool to investigate the damage thresholds (Byers, 1990; Disegna, 1994). In this research, terbacil is used as a tool to investigate the photosynthetic threshold of strawberry plants and to investigate other effects which may be related to photosynthesis (e.g. fruit yield, dry weight of plant). It is commonly used to control the weeds in strawberry. It is usually applied before planting, in early season and after harvest renovation (Mahr et. al, 2002).

The objective of the first research (first chapter) was to determine the leaf damage threshold for strawberry (*Fragaria* × *ananassa* cv. Honeoye) by simulating leaf damage with hole punches (0.33 cm^2) . Because of the difficulty to calculate the dynamically changing canopy area of strawberry plants observations were conducted on single leaf.

The objective of second research (second chapter) was to determine the leaf damage threshold for strawberry (*Fragaria* × *ananassa* cv. Honeoye) on

whole plants under field conditions at different times during the growing season. Leaf damage was simulated by applying terbacil to the foliage at different concentrations and at different critical stages in crop development. The degree and duration of photosynthetic inhibition are dose dependant and crop-specific. It was hypothesized that different levels of P_n reduction could reduce the production and storage of carbohydrates needed for growth and that reduced carbohydrate production may affect yield and runner production negatively and the ability of the plant to resist environmental stress.

LITERATURE REVIEW

Photosynthetic compensation and damage thresholds

Photosynthetic compensation in response to leaf injury and leaf area loss has been reported in several species of plants. Such compensation has been observed in wheat and barley (Shaw, 1956), soybean (Wareing 1968) and lucerne (Hodgkinson, 1974), sour cherry (Layne, 1989), (Disegna, 1994), apple (Ferree, 1982), (Lakso 1996).

The effect of insect injury on whole plant productivity has been evaluated by simulation of the injury caused by an insect. To simulate the damage caused by the spotted tentiform leaf miner (*Phyllonorycter blancardella*), Kappel (1986) punched holes in leaves of apple trees. This treatment reduced trunk growth, rootstock growth, fruit number and fruit yield. Kappel (1986) also demonstrated that leaf injury reduced return bloom and fruit set in the following year. In poplar trees, removing 75% to 80% of the leaf area reduced the growth of young poplar trees by 20% in nursery conditions (Bassman, 1982). In tomato, removing 75% of the plant's leaf area reduced fruit yield by 40% Stacey, 1983). Fruit yield was reduced by 80% when all the spur leaves of Golden Delicious apple trees were removed (Ferree, 1982).

Layne (1989) demonstrated that, in non fruiting 'Montmorency' sour cherry trees, the removal of 20% of leaf area caused no significant reduction in net carbon dioxide assimilation and had no effect on tree dry weight. In these trees,

photosynthetic compensation following leaf injury was observed four days after the leaf damage had occurred.

Photosynthesis

CO_2 assimilation rates in strawberry are similar to other fruit crops (Flore and Lakso, 1989). Under field conditions, strawberry plants (*Fragaria* x *ananassa*) typically have CO_2 assimilation rates of 15-25 $\mu mol.m^{-2}.s^{-1}$ (Hancock, 1989).

The light saturation point for photosynthesis in field-grown strawberry plants (*Fragaria* x *ananassa*.) is between 800 and 1000 $\mu mol.\ m^{-2}.s^{-1}$ (Cameron, 1990).

Diseases

Viral, bacterial and fungal diseases that infect leaves can cause a decrease in CO_2 assimilation rates. When young peach leaves were infected with Peach rosette virus and decline disease, decreases in CO_2 assimilation rate and leaf growth were observed (Smith 1977).

Apple scab infection (*Ventura inaequalis*) decreases CO_2 assimilation rates of apple leaves within 28 days of inoculation (Spotts, 1979). However, the average decrease in CO_2 assimilation rate was smaller than the decrease in leaf area caused by the disease. This would indicate that the remaining healthy

leaves may have increased their CO_2 assimilation rate to compensate for the leaf loss (Spotts, 1979).

Apple powdery mildew (*Podospharea leucotricha*) reduced the CO_2 assimilation and transpiration rates of all leaves but had the greatest effect on CO_2 assimilation rate in young apple leaves (Ellis, 1981). Leaves that are infected during the early stages of growth, never regain their full photosynthetic capacity (Ellis, 1981).

Diseases reduce the amount of light penetrating the leaf (Smith 1977). Contrary, Lakso (1982) found that low levels of powdery mildew (*Unincula* necator) increased CO_2 assimilation rate in grape vines (Lakso 1982).

Damage thresholds for insect and mite infestations

Spider mites (*Tetranchus urticae* Koch) can reduce fruit yield in strawberries. Thirty cumulative mite days during any monthly period was found to be threshold level for strawberry plants. Higher values decreased the yield of strawberry plants. (Walsh, 1998).

Tarnished plant bug (*Lygus lineolaris*) damages the blossom clusters and reduces the yield of strawberries. Economic injury level, as indicated by strawberry weight, was approximately 0.95-0.99 tarnished plant bug nymphs per blossom cluster. Action threshold for the 'Redcoat' strawberry cultivar was estimated at 0.26 nymphs per blossom cluster (Mailloux, 1988).

Flower bud removal trials on 12 strawberry cultivars indicated that most could compensate for a significant amount of flower bud loss caused by the strawberry bud weevil (*Anthonomus signatus*), provided that the loss occurred during early development of the inflorescence (Pritts, 1999). In most of these 12 cultivars injury remained below the damage threshold (Pritts, 1999).

In 'Redchief' strawberry, the economic threshold for yield reduction by the nymphs of the cercopid *Philaenus spumarius* was found to be 20 nymphs per m^{-2} (Zajac, 1984).

The effect of two-spotted spider infestations was investigated on 'Franklin' apple trees. Mite infestation levels of 15, 30 and 60 mites per leaf reduced CO_2 assimilation by 26, 30 and 43 percent respectively, when compared to control plants (Hall, 1976).

Proctor (1982) investigated the effects of leaf injury caused by the spotted tentiform leaf miner (*Phyllonorycter blancardella*) on CO_2 assimilation rate. The lowest net CO_2 assimilation rate was measured in leaves that had 3 mines per leaf. Leaves injured by 20 mines per leaf suffered a 32.9% reduction in leaf area. However, the decrease in the photosynthetic rate of these leaves was only 23.2%, which indicated that photosynthetic compensation had occured (Proctor, 1982).

Effect of Fruit Load on Photosynthesis

It has been reported that fruiting and non-fruiting had no difference CO_2 assimilation rate on either seasonal or diurnal basis in sweet cherries. It has been suggested that CO_2 assimilation rates in sweet cherry were primarily affect by ontogeny and environment. Strength of the sink did not have influence the CO_2 assimilation rates (Roper, 1988).

Gucci (1990) investigated the effects time of the season that fruits were removed on CO_2 assimilation rate. Removing fruit at pit hardening stage decreased the CO_2 assimilation rate by 25% within 24 hours. However, removing mature fruits did not have any effect on CO_2 assimilation rate.

Photosynthetic inhibition

Among the most commonly used herbicides in agriculture are herbicides that act as photosynthetic inhibitors (Trebst, 1981), which includes ureas, triazines and bipyridiniums (Van Rensen, 1989). Fifty percent of commercially used herbicides are inhibitors of photosynthesis (Trebst, 1981). Terbacil, which is used to control weeds in strawberries, is classified as a uracil herbicide (Aston, 1977).

Terbacil controls many annual weeds and some perennial weeds. Terbacil is absorbed primarily by roots and translocated apoplastically to the leaves, but can also be taken up directly by the leaves with the aid of surfactants. Adjuvants

increase herbicidal activity by increasing retention, penetration, absorption and translocation of the herbicide (Kirkwood, 1983). A general symptom of terbacil toxicity is chlorosis, which is a consequence of the degeneration of chloroplasts in the leaves of susceptible plants (Izawa, 1965).

Photosynthetic inhibitors interfere with the Hill reaction, which occurs in chloroplasts. Hill reaction is defined as the evolution of oxygen by a suspension of isolated chloroplasts when illuminated in the presence of an artificial electron acceptor (Moreland, 1980). When the Hill reaction is interfered, ATP formation is also inhibited. Thus, energy production stops in the chloroplast. Tresbst (1981), indicated that most of the herbicides are inhibitors of electron flow at the functional site between the primary and secondary electron acceptors of photosystem II.

Van Rensen (1989) reported that the damage caused by many uracil herbicides was reversible. Izawa (1965) concluded that diuron binds weakly to the receptor molecule in the thylakoid membrane.

Designa (1994) found that damage caused by the terbacil was reversible in apple trees.

Degradation of terbacil also varies in plants. Terbacil was degraded more in beans which are susceptible to terbacil than citrus. Citrus is considered tolerant to terbacil (Herholdt, 1968). However, Barrentine (1970) found that terbacil was metabolized at a higher rate in tolerant peppermint plants than in susceptible sweet potato plants

Use of Chlorophyll Fluorescence to Determine the Herbicide inhibition

Measuring chlorophyll fluorescence has been used to determine photosynthetic activity and this method gives detailed information about photosystem integrity system (Krause, 1984). Chlorophyll fluorescence measurement at 685 nm indicates the energy state of the P 680 reaction centre of photosytem II and its associated pigments reflects the rate of electron transport from photo system II to chemical acceptors and the coupling between ATP and electron transport (Krause, 1984).

It has been reported an that there is an inverse relationship between assimilation and photosynthesis after the application of herbicide which limits electron transport (Panneels, 1987).

Leaf fluorescence changes were found from the inhibition of photosynthetic electron by using herbicide simazine and diuron (Miles, 1973).

Voss (1984) reported that when analyzing the chlorophyll fluorescence from the leaves of different species treated with photosynthesis inhibitors, the F_v/F_m provides a good estimate about the changes in the photosynthetic capacity of the leaves after the herbicide treatment.

Designa (1994) found that F_v/F_m values can be used to assess the photosynthetic inhibition cause by terbacil.

Terbacil Tolerance in Strawberries

The tolerance of strawberry to terbacil has been shown to be at least partially attributable to restricted translocation of root-absorbed herbicide to the site action in mesophyll chloroplasts. Uptake by the root did not appear to be a factor in tolerance to terbacil (Genez, 1983).

Honeoye, Guardian and Darrow strawberry cultivars are reported to susceptible to terbacil. Recommended rate is 138-419 g/ha . 559 g/ha are found to be toxic to the strawberry. Rate should be chosen depending on the soil type. Lower rate suggested on coarse type soils. (Mahr et.al. , 2002)

'Chambly' strawberry a hybrid of Sparkle X Honeoye, is reported to be tolerant to terbacil (Khanizadeh, 1990)

LITERATURE CITED

Alpert, P., H.A. Mooney. 1986 Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*. Oecologia 70:227-233.

Ashton, F.M. , O.T. De Villiers, R.K. Glenn, W.B. Duke, 1977. Localization of metabolic sites of action of herbicides. Pestic. Biochem. Physiol. 7:122-124.

Bassman, J., W. Myers, D, Dickman, L. Wilson. 1982. Effects of simulated insect injury on early growth of nursery grown hybrid poplars in Wisconsin. Can. J. For. Res. 12:1-9.

Byers, R.E., J.A. Barden, R.F. Polomski, R.W. Young, D.H. Carbaugh. 1990. Apple thinning by photosynthetic inhibition. J. Amer. Soc. Hort. Sci. 115:14-19.

Cameron, J.S., C.A. Hartley. 1990. Gas Exchange Characteristics of *Fragaria chiloensis* genotypes. Hort-Science 25(3): 327-329.

Chandler, C.K., D.D. Miller, D.C. Ferree D.C. 1988. Influence of leaf removal, root pruning, and soil addition on the growth of greenhouse-grown strawberry plants. J. Amer. So. Hort. Sci. 113:4, 529-532.

DelValle, T.B.G., J.A. Barden, R.E. Byers. 1985. Thinning of peaches by temporary inhibition of photosynthesis with terbacil. J. Amer. Soc. Hort. Sci. 110:804-807

Disegna, E.J. 1994. The use terbacil as a tool to establish a photosynthetic threshold in apples. Michigan State Univ., East Lansing, Msc thesis.

Daugaard, H. 1999. The effect of the flower removal on the yield and vegetative growth of A+ frigo plants of strawberry (*Fragaria* × *ananassa* Duch). Scientia-Horticulturae. 98:1-2, 153-157.

Ellis, M.A., D.C. Ferree, D.E. Spring. 1981. Photosynthesis, transpiration and carbohydrate content of apple leaves infected by *Podosphaera leucotricha*. Phyopahology 71:329-395.

Ferree, D.C. and J.W. Palmer. 1982. Effect of spur defoliation and ringing during bloom on fruiting, fruit mineral level and net photosynthesis of 'Golden Delicious' apple. J. Amer. So. Hort. Sci. 107(6): 1182-1186.

Flore, J.A., A. Lakso. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. Horticulture Reviews 11, 111-157.

Forney, C.F., P.J. Breen. 1985. Dry matter partitioning and assimilation in fruiting and deblossomed strawberry. J. Amer. Hort. Soc. 110:181-185.

Genez, A.L. , T.J. Monaco. 1983. Uptake and translocation of terbacil in strawberry (*Fragaria* × *ananassa*) and goldenrod (*Solidago fistulosa*). Weed Science. 31:56-62.

Gucci, R., J.A. Flore. 1990. Net Photosynthesis and non-structural carbohydrates in leaves of field-grown plum trees following fruit removal. In: 3rd International meeting on "Regulation of photosynthesis in fruit crops". Perigia. Italy. P.127.

Gut, L.J., R. Isaacs, J.C. Wise, A.L. Jones, A.M.C. Snider, B. Zandstra, E. Hanson. 2003. 2003 Michigan Fruit Management Gide. Mich. Agr. Expt. Sta. Extension Bulletin E-154.

Hall, F.R., and D.C. Ferree. 1976. Effects of two-spotted spider mite populations on photosynthesis of apple leaves. J. Econ. Ent. 68:517-520.

Hancock, J.F., J.A. Flore, G.J. Galletta, (1989). Gas exchange properties of strawberry species and their hybrids. Scientia Horticulturae 40, 139-144.

Hancock, J.F., (1991). Photosynthesis in strawberries and the possibility of genetic improvement. The strawberry into the 21st century. Timber Press Inc. Portland, Oregon.

Herhodt, J.A. 1968. The mode of action and metabolism of terbacil in bean (*Phaseolus vulgaris* L.) and citrus (*Citrus sinensis* L. Obbeck. And *Citrus jambhiri* Lush). Ph.D. Thesis, Univ. Calif. Riverside, 91 pp.

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

Howell, G.S., S.S. Stackhouse. 1973. The effect of defoliation time on acclimation and dehardining in tart cherry (*Prunus cerasus* L.) J. Amer. Soc. Hort. Sci. 98: 132-136.

Izawa, S., S.S. Stackhouse. 1965. The number of sites sensitive to 3 – (3,4 – dichlorophenly) – 1, 1 – dimethylurea, 3 (4 – chlorophenly) -1, 1-dimentylurea and 2 – chloro – 4 – (2 – propylamino) 6 – ethylamino-s-triazine in isolated chloroplasts. Acta 102:20-26.

Kappel, F. 1986. Influence of spotted tentiform leaf miner on apple leaf and tree physiology. Ph. D. Thesis. University of Guelph, Guelph, Ontario, Canada.

Kerkhoff, K.L., J.M. Williams, J.A. Barden. 1988. Net photosynthetic rates and growth of strawberry after partial defoliation. Hortscience 23:1086.

Khanizadeh, S. D. Buszard, M. Lareau, D. Bagnara. 1990. 'Chambly' strawberry. Hortscience. 25:984-985.

Kirkwood, D.A. 1983. The mode of action of herbicides. In: Recent advances in weed research. W.W. Fletcher (ed.) 227-257.

Krause, G.H., E. Weis 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth-Res. 5(2): 139-157.

Lakso, A.N. , C. Pratt, R.C. Peasrson, R. M. Pool, R.C. Seen, and M.J. Welser. 1982. Photosynthesis, transpiration and water use efficiency of mature grape leaves with *Unincula necator* (powdery mildew). Phytopathology 72:232-235.

Lakso, A.N., G.B. Mattii, J.P. Nyrop, S.S., Denning. 1996. Influence of European red mite on leaf and whole-canopy carbon dioxide exchange, yield, fruit size, quality and return cropping in 'Starkrimson Delicious' apple trees. J. Amer. Soc. Hort. Sci. 121:954-958.

Layne, D. 1989. Damage thresholds for simulated trunk and leaf injury in 'Montmorency' sour cherry. Ms. Thesis. Michigan State University, East Lansing, MI.

Layne, D. 1991. Response of young, fruiting sour cherry trees to one-time trunk injury at harvest date. J. Amer. So. Hort. Sci. 116(5): 851-855.

Mahr, D.L., T.R. Roper, P.S. McManus, B.R. Smith, R.A. Flashinski. 2002. Strawberry and raspberry pest management in Wisconsin. University of Wisconsin Extension Bulletin A1934.

Mailloux, G., Bostanian, N.J., 1988. Economic injury level model for tarnished plant bug, *Lygus lineolaris* in strawberry fields. Environ-Entomol. College Park, Md. Entomological Society of America. 17(3):581-586.

Moreland , D.E. 1980. Mechanisms of action of herbicides. Ann. Rev. Plant Physiol. 31:597-638.

Osborne, C. 1997. Does Long-Term Elevation of CO_2 Concentration Increase Photosynthesis in Forest Floor Vegetation? Plant Physiology. 114:337-344.

Panneels, P. A., P. Van Moer, P. Reimer, A. Salis, R. Chouhiat, H. Figeys. 1987. Progress in Phot. Res. 3:827-830.

Pritts, M., M.J. Kelly. 1999. Strawberry cultivars compensate for simulated bud weevil damage in matted row damage. Hortscience. 34(1):109-111.

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

Roper, T.R., J.D. Keller, W.H. Loescher, C.R. Rom,1988. Photosynthesis and carbohydrate partitioning in sweet cherries: fruit effects. Plant Physiology. 72:42-47.

Schaffer, B., J.A. Barden, J.M. Williams. 1986. Whole plant photosynthesis and dry matter partitioning in fruiting and debloddomed dayneutal strawberry plants. J. Amer. Soc. Hort. Sci. 113:430-433.

Shaw, M. 1956. The physiology of host parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. Canadian Journal of Botany 34:(389-405).

Smith, P.R. and T.F. Neales. 1977. Analysis of the effects of virus infection on the photosynthetic properties of peach leaves. Aust. J. of Plant Physiol. 4:723-732.

Spotts, R.A., D.C. Ferree. 1979. Photosynthesis, transpiration and water potential of apple leaves infected by *Venturia inaequalis*. Phytopathology 69:717-719.

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

Strick, B.C., J.T.A. Proctor. 1986. Photosynthesis of strawberry genotypes differing yield. Hortscience 21:246.

Tresbt, A. 1981. Action mechanism of herbicides in photosynthetic electron transport. Photosynthesis VI. Photosynthesis and productivity, photosynthesis and environment. Pp. 507-530.

Unrath, C.R., 1981. An overview of environmental factors affecting orchard growth regulator response with special reference to apple. Acta Hort. 120:43-52.

Van Rensen, J.J.S. 1989. Herbicides interacting with photo system II. Herbicides and plant metabolism. Soc. Exp. Biol. 38: 21-28.

Voss M. , G. Renger, P. Graber, 1984. Fluorimietric detection of reversible and irreversible defects of electron transport induced by herbicides in intact leaves. In Advance in photosynthetic research 4:53-60.

Walsh, D.B., F.G. Zalom, D.V. Shaw. 1998. Interaction of the two spotted spider mite (Acari: *Tetranychidae*) with yield of day-neutral strawberries in California. Journal of economic entomology. 91(3): 678-685.

Wareing, P.F., M.M. Khalifa, K.J. Treharne. 1968. Rate limiting process in photosynthesis at saturating light intensities. Nature 220:453-457.

Zajac, M.A. , M.C. Wilson. 1984. The effects of nymphal feeding by the meadow spittlebug, *Philaenus spumarius*, on strawberry yield and quality. Crop Protection. 3(2): 167-175.

Chapter 1

DETERMINATION OF PHYSICAL DAMAGE THRESHOLDS TO LEAVES OF STRAWBERRY (*Fragaria* × *ananassa* cv. 'Honeoye')

ABSTRACT

Damage thresholds of strawberry plants (*Fragaria* × *ananassa*) were investigated by mechanical damage. Mechanical damage was applied such that 10%, 20% and 30% of the leaf area of a fully expanded single leaf was removed Strawberry leaves that were mechanically damaged did not recover their photosynthetic capacity at any damage level. Chlorophyll fluorescence (F_v/F_m) values were not affected from the mechanical damage. Increasing damage levels decreased the ability of the strawberry leaf to use light and carbon dioxide in photosynthesis. Difference in stomatal conductance and transpiration rates were insignificant. Internal CO_2 (C_i) levels were higher in damaged plants compared to the control plants.

INTRODUCTION

Damage threshold is defined as the level of pest damage above which plant growth or health affected negatively. Strawberry plants are perennial plants and the effect of such damage could be in the current year or in the subsequent crop.

Foliage damage threshold research studies have been performed in other plants, but such damage threshold level has not been determined for strawberry. Such threshold has been observed in wheat and barley (Shaw, 1956), soybean (Wareing 1986), lucerne (Hodgkinson, 1974), sour cherry (Layne, 1989), (Disegna, 1994). Determined threshold levels for other plants range from 5% - 20% depending on the crop. Crop load also affects the damage threshold levels (Disegna, 1994). Determination of such a value for strawberry plants would be useful for in strawberry production. These threshold levels can be used in IPM and pesticide application programs and in economical studies in strawberry production.

Biotic and abiotic damage can occur in strawberry plants. Biotic damage may be caused by insects, diseases and weeds. Abiotic damage may be caused by temperature, drought, anoxia etc. Biotic damage is a major concern in plant production. Growers need to intervene at different stages of growth and this intervention may differ depending on the level of the damage. Photosynthetic recovery levels may be different in different growth stages. Sink load can also be affected by the age of the plant. On the other hand leaf thickness is an important

factor that affects the resistance of the plants against pathogens and herbicides. Kirwood (1983) and Unrath (1981) found that cuticle of older leaves is less permeable and thicker.

Fruit load may also affect the photosynthetic recovery metabolism, since fruits are one of the major sinks for carbohydrates. Gucci and Flore (1990) found different responses in CO_2 assimilation rates in plum trees depending on the stage that fruits were removed from trees. Allocation of carbohydrates to fruit may affect the recovery process.

In perennial crops, damage can cause negative effects into the next season. This carry over effect may be in different forms such yield loss or decrease in cold hardiness. It has been found that early leaf loss caused a decrease in cold hardiness of the sour cherry buds and this effect was carried over into the next season (Howell, 1973).

The amount of the leaf damage may also have an affect on photosynthetic recovery. Damage occurs by different factors such as insects, diseases, nematodes and mammals. Some environmental factors also damage the leaves chilling from low temperature, wind, hail and fire. Cultural practices may also cause damage such as mechanical harvest and herbicide toxicity. Different parts (organs) of the plant can be damaged and each plant part may have different damage thresholds. Root damage may occur by nematodes in strawberry plants and different insects and diseases cause leaf damage. In woody plants trunk damage may occur by cold, small mammals or mechanical harvest. Trunk damage thresholds have been investigated in some trees (Layne, 1989).

The hypothesis tested in this research was "Leaf photosynthetic capacity will determine damage threshold levels for strawberry productivity".

For this purpose hole punching was used to simulate damage of insects in strawberry.

Hole punching is used to simulate insect damage in threshold studies (Kappel ,1986; Layne, 1989). Common pests that affect the strawberries are aphids, leaf rollers, mealybugs, leafhoppers, spittlebugs and spider mites (Hancock, 1999). In this study a mechanical method (hole punching) was used to simulate the injury to leaves.

The objective of this research was to determine the leaf damage threshold for strawberry (*Fragaria* × *ananassa* cv. Honeoye) by simulating leaf damage by hole punching. Because of the difficulty to calculate the dynamically changing canopy area of strawberry plants observations were conducted on single leaf.

MATERIALS AND METHODS

Plant Material

Strawberry plants (*Fragaria* × *ananassa* cv. Honeoye) were grown in the Michigan State University Plant Science Greenhouses, East Lansing, MI. Plants were planted in pots (2.2 L) containing a 1:1 (v/v) mix of sterilized greenhouse soil and BACTTO potting media (80% peat, 20% perlite). They were grown under long days to prevent flowering and encourage vegetative growth. Daylength was adjusted to 16 hour days and 8 hour nights using supplemental illumination provided by high pressure halogen lights (minimum of 110 $\mu mol.m^{-2}.s^{-1}$ PAR). Average greenhouse temperatures were 21°C during the day and 17°C during the night. Runners were removed from the plants every ten days. Plants were irrigated using a drip irrigation system that delivered water approximately 60 ml per pot three times during a 24 hours period. A soluble fertilizer (Peter's 20-20-20 N,P,K) was applied bi-weekly at the rate of 5 grams per plant by using the drip irrigation system. Pest management (Avid™ 0.49g/L and Strike™ 0.12g/L) was provided as necessary.

Leaf area was estimated by developing a regression equation formula based on measurements of the length and width of each of the leaflet triplet of strawberry. Fifty fully expanded leaves were measured at their widest points. Leaf area was measured by using the LI-COR (Lincoln, NE) LI-3000 leaf area

meter. Based on these measurements, the regression equation that was used to calculate the leaf area in these experiments was:

$A= -22.24 + (3.29W)+(4.97L)$ $(R^2=0.946)$

Where: W is width of the triplet leaf

L is length of the triplet leaf

A is area of each triplet in cm^2

Leaf area removal treatments were applied by removing 10% , 20% and 30% of the leaf area of recently fully expanded leaves using a paper hole punch (0.33 cm^2). One leaf was selected per plant and leaf discs were punched randomly throughout the lamina while avoiding the midrib of the leaflet. The number of punches was equal on either side of the midrib.

The experiment was arranged in a completely randomized design with seven plants in each treatment. Control plants had no damage (0%).

Gas exchange measurements

All measurements were conducted in the laboratory in a walk in growth chamber (Model PGV36, Conviron, Canada) using the open system described by Sams and Flore (1982) and Gucci (1988). The following modifications were made to the measurement system: a) A leaf cuvette was constructed from Verolite™ (Matra Industries Inc, Ontario, Canada) material which had dimensions of 30 cm (W) × 30 cm (L) × 21 cm (H) b) b) to construct the top of the cuvette Maylar® (DuPont Chemicals, US) was used c) The CIRAS-1 portable photosynthesis

system (PP Systems, Hertfordshire, UK) was used to measure differential in CO_2 concentration and partial water vapor pressure at the inlet and outlet of the leaf chamber. d) The air flow into the chamber was measured by using two Cole Parmer 10620 flow meters (each of them has 5 L/min maximum flow rate) f) A 110 V AC 12 cm fan was placed in the cuvette to provide uniform air circulation. g) A 15 cm × 20 cm cooling radiator was used which cools the leaf cuvette.

The pressurized air used in these experiments which was filtered and had a CO_2 concentration of 380±10 ppm. Fluorescent lights inside the growth chamber provided light at an intensity of 850 $\mu mol.m^{-2}.s^{-1}$. Air temperature inside the growth chamber was maintained at 22°C, while relative humidity was maintained at 75%.

Plant material was brought from the greenhouse to the growth chamber at 8:30 am on the day of the experiment and allowed to acclimate to growth chamber conditions for 30 minutes prior to initiation of measurements. A single treated leaf per plant was enclosed in the leaf chamber while still attached to the plant. Leaf temperature was monitored by a thermocouple that was in contact with the lower surface of the leaf. The temperature of the enclosed leaf ranged between 21.5 and 23°C. Air flow into the chamber was maintained at a rate of 8.6 L/min. Gas exchange measurements were made when CO_2 levels inside the cuvette stabilized. Gas exchange parameters (A, g_s, E and C_i) were calculated by using Photosyn Assistant Software, IRGA module, Version 1.1.2 (Dundee Scientific, Dundee, UK) . Measurements were made one day before the leaf damage treatments and 2, 4, 6, 8, 10, 12, 14 and 16 days after the treatments.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured on the same leaves that were used for gas exchange measurements. The Plant Efficiency analyzer (Hansatech Instruments Ltd, Norfolk, UK) was used for these measurements. Leaves were dark acclimated for 20 minutes using dark acclimation cuvetes. Leaves were then irradiated with actinic light for 5 seconds and chlorophyll fluorescence kinetics were recorded. Measurements were performed on the same leaves just before the treatments and 2, 4, 6, 8, 10, 12, 14 and 16 days after the treatments.

A/C_i curves

The effect of CO_2 concentration on assimilation rate was measured eight days after the initiation of the leaf damage treatment. Measurements were made as described in the gas exchange measurements section. CO_2 levels were adjusted by using an ADC GD600 (ADC Bioscientific Ltd, UK) gas dilutor and monitored by a CIRAS 1 unit (PP Systems, Hertfordshire, UK). 3000 ppm CO_2 was provided to gas dilutor. CO_2 scrubbing was performed using a column filled with lime when lower CO_2 levels were necessary. C_i levels were calculated using Photosyn Assistant Software, IRGA module, Version 1.1.2 (Dundee Scientific, Dundee, UK). CO_2 assimilation was calculated approximately at the following Ci levels (±20 ppm): 30, 40, 60, 70, 100, 130, 150,180, 200, 220, 240, 260, 330,

400, 500, 580, 640, 680 and 700 ppm. The actual C_i values were used in calculations.

Light response curves

Gas exchange in response to light response was determined eight days after the leaf damage treatments. Measurements were made as described in the gas exchange measurements section. Light intensity was adjusted raising of lowering the light bank in Conviron growth chamber (Model PGV36, Conviron, Canada). Light intensities used in this experiment were 0, 50, 100, 150, 350, 500, 650, 750, 850 and 1000 $\mu mol.m^{-2}. s^{-1}$. The highest light intensity 1000 $\mu mol.m^2.s^{-1}$ was obtained using supplemental portable high pressure sodium light. Light intensities were measured using a LI-COR quantum sensor (LI-COR, Lincoln, NE). Quantum efficiency and light compensation points were calculated using Photosyn Assistant Software, AQ Curve Analyis Module, Version 1.1.2 (Dundee Scientific,Dundee,UK).

Plot Design and Statistical Calculations

Completely randomized design was used in this experiment. There were seven plants in each treatment. Data were subjected to analysis of variance (ANOVA). Means were compared by Duncan's test or by standard deviation.

Any data represented in percentage was transformed by arcsin conversion before ANOVA.

Error bars in the figures represent standard deviation.

The SAS base statistical program (version 8.2, SAS institute, Cary, NC) was used for ANOVA.

RESULTS

Effects of foliar damage on CO_2 assimilation rate

Foliar damage caused a significant reduction in leaf CO_2 assimilation rates on leaf area basis (Figure 1). This reduction was apparent within two days of treatment in all foliar damage treatment levels. Plants exposed to 30% leaf damage suffered a statistically significant 44% reduction in CO_2 assimilation rate as compared to control plants (Figure 2). The CO_2 assimilation rate of these plants remained depressed, relative to the control, and fluctuated within a tight range of 5 to 6 $\mu mol.m^{-2}.s^{-1}$ over the two weeks following the treatment. In comparison, the average CO_2 assimilation rate in leaves of control plants ranged between 9.98 $\mu mol.m^{-2}.s^{-1}$ and 11.71 $\mu mol.m^{-2}.s^{-1}$ over the same period. The decrease in assimilation rate on day 16 of the experiment was observed in treated as well as untreated plants. CO_2 assimilation rates in plants exposed to 10% and 20% foliar damage exhibited patterns that were generally similar to the one observed in plants in the 30% damage treatment. Leaves exposed to 20% foliar damage suffered a significant reduction in CO_2 assimilation rates. The CO_2 assimilation rate in these plants ranged between 7.2 and 8 $\mu mol.m^{-2}.s^{-1}$, which were 28% to 35% lower than the rates measured in control plants. The 10% foliar damage treatment caused a decrease in CO_2 assimilation rate. However, the difference in assimilation rate between the damaged plants and control plants was significant only on days 2 and 14 following the treatment.

Gas exchange parameters

Foliar damage had no significant effect on stomatal conductance (g_s). Stomatal conductance in leaves of control and treated plants ranged between 129 and 220 $\mu mol.m^{-2}.s^{-1}$, with an exception that occurred on the fourth day after treatment in the 10% foliar damage treatment where g_s reached 329 $\mu mol.m^{-2}.s^{-1}$ (Table 3). Internal CO_2 (C_i) levels tended to be higher in treated plants than in control plants (Figure 3). C_i increased by up to 50% in leaves of damaged plants over the first 4 days after treatment then decreased through day 8 after the treatment. In comparison, C_i levels in control plants increased through day 8 by only 16% from initial levels of 220 ppm. Internal CO_2 levels in plants subjected to 30% foliar damage were significantly higher than C_i levels in control plants only on days 4, 12 and 14. Foliar damage had no significant effect on leaf transpiration rate (E) (Table 4) Transpiration rates ranged between 2.38 and 7 $mmol.m^{-2}.s^{-1}$.

Chlorophyll fluorescence

Chlorophyll fluorescence was evaluated as the ratio of F_v over F_m values (F_v/F_m).

The F_v/F_m was not affected by the foliar damage treatments (Figure 4). No significant differences in F_v/F_m values were found at any of the dates on which chlorophyll fluorescence was measured.

A/C_i curves

Foliar damage at the 20% and 30% levels altered the A/C_i relationship in the damaged leaves. At the 30% damage level, maximum assimilation rate was approximately 6 $\mu mol.m^{-2}.s^{-1}$, significantly lower than that of control plants which reached 17.5 $\mu mol.m^{-2}.s^{-1}$. At the 20% damage level, plants suffered a 38% reduction in maximum assimilation rate as compared to the control. Ten percent leaf damage caused only a small decrease in maximum assimilation rate.

The CO_2 compensation point was higher in damaged plants than in control plants (Table 1). The CO_2 compensation point in plants at the 30% damage level was 27 ppm higher than that of control plants. At the 20% damage level, the CO_2 compensation point increased by 14 ppm. The 10% leaf damage treatment had little effect on the CO_2 compensation point.

The carboxylation efficiency (C.E.), measured as the initial slope of the A/C_i curve, was also affected by leaf damage (Table 1). A substantial decrease in carboxylation efficiency, approximately 61%, was observed in the 30% damage treatment relative to the control plants. At the 20% damage level, the decrease in C.E. was approximately 32%, whereas there was little effect on C.E. in plants damaged at the 10% level.

Relative stomatal limitations (I_s) were calculated with sensitivity analysis method according to Jones (1998).

Damage level	I_s	C.E.	Internal CO_2 levels for A compensation (ppm)
0%	%37 a	0.62 a	82 c
10%	%34 a	0.59 b	83 c
20%	%23 b	0.42 c	96 b
30%	%18 c	0.38 d	109 a

Table 1. I_s, CE and CO_2 compensation values from the A/C_i curves. Means followed by different letters are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

Equations for the A/C_i curves were as follows

0% damage	y=7.8766Ln(x)-33.62	R^2= 0.96
10% damage	y=7.5186Ln(x)-32.28	R^2= 0.96
20% damage	y=5.0791Ln(x)-22.03	R^2= 0.96
30% damage	y=3.6672Ln(x)-16.48	R^2= 0.87

Light response curves

Leaf damage altered the light response relationship for photosynthesis. Plants in the 30% leaf damage treatment had the lowest CO_2 assimilation rates at all light intensities tested (Figure 6). In these plants, the maximum CO_2

assimilation rate achieved was 5.5 $\mu mol.m^{-2}.s^{-1}$, which was approximately 50% of the maximum rate attained by control plants. Plants subjected to damage levels of 10% and 20% also had diminished assimilation rates that were approximately 18% and 36% lower than that of the control. In all plants, CO_2 assimilation rates reached light saturation levels at a light intensity of approximately 850 $\mu mol.m^{-2}.s^{-1}$.

Photosynthetic quantum efficiency (Q.E.) was lower in damaged plants than in the control plants (Table 2). Calculated light compensation levels were higher in damaged plants than in the control plants (Table 2).

Damage level	Q.E.	Calculated light compensation levels for A ($\mu mol.m^{-2}.s^{-1}$)
0%	0.0421 a	24.4 b
10%	0.0378 a	23.9 b
20%	0.0205 b	31.3 b
30%	0.0136 c	53.8 a

Table 2. Q.E. and calculated light compensation values from the light response curves. Means followed by different letters are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

Equations for the light response curves were as follows

0% damage	$y = -0.000015x^2 + 0.025848x - 0.2936$	$R^2=0.98$
10% damage	$y = -0.00001x^2 + 0.01974x - 0.141$	$R^2=0.98$
20% damage	$y = -0.000006x^2 + 0.013966x - 0.313421$	$R^2=0.98$
30% damage	$y = -0.000004x^2 + 0.010321x - 0.551721$	$R^2=0.97$

	Days After Foliar Damage								
Damage Level	0	2	4	6	8	10	12	14	16
0%	195	185	164	183	166	135	129	148	121
10%	205	152	329	120	140	140	135	221	153
20%	153	174	220	149	164	138	130	155	120
30%	217	151	185	167	165	119	118	190	119

Table 3. Effects of the level of foliar damage on stomatal conductance (g_s). Means followed by different letters are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

	Days After Foliar Damage								
Damage Level	0	2	4	6	8	10	12	14	16
0%	2.48	2.48	2.80b	2.72	2.48	2.75	2.07	2.30b	4.57
10%	2.58	3.11	3.79b	3.07	2.75	2.22	2.14	2.89ab	3.43
20%	2.38	2.69	4.93ab	2.94	2.67	2.29	2.37	2.55ab	5.29
30%	2.40	2.56	7.09a	3.35	2.63	2.78	2.10	3.64a	3.44

Table 4. Effects of the level of foliar damage on transpiration rate (E). Means followed by different letters are significantly different by Duncan's Multiple Range Test ($P \leq 0.05$).

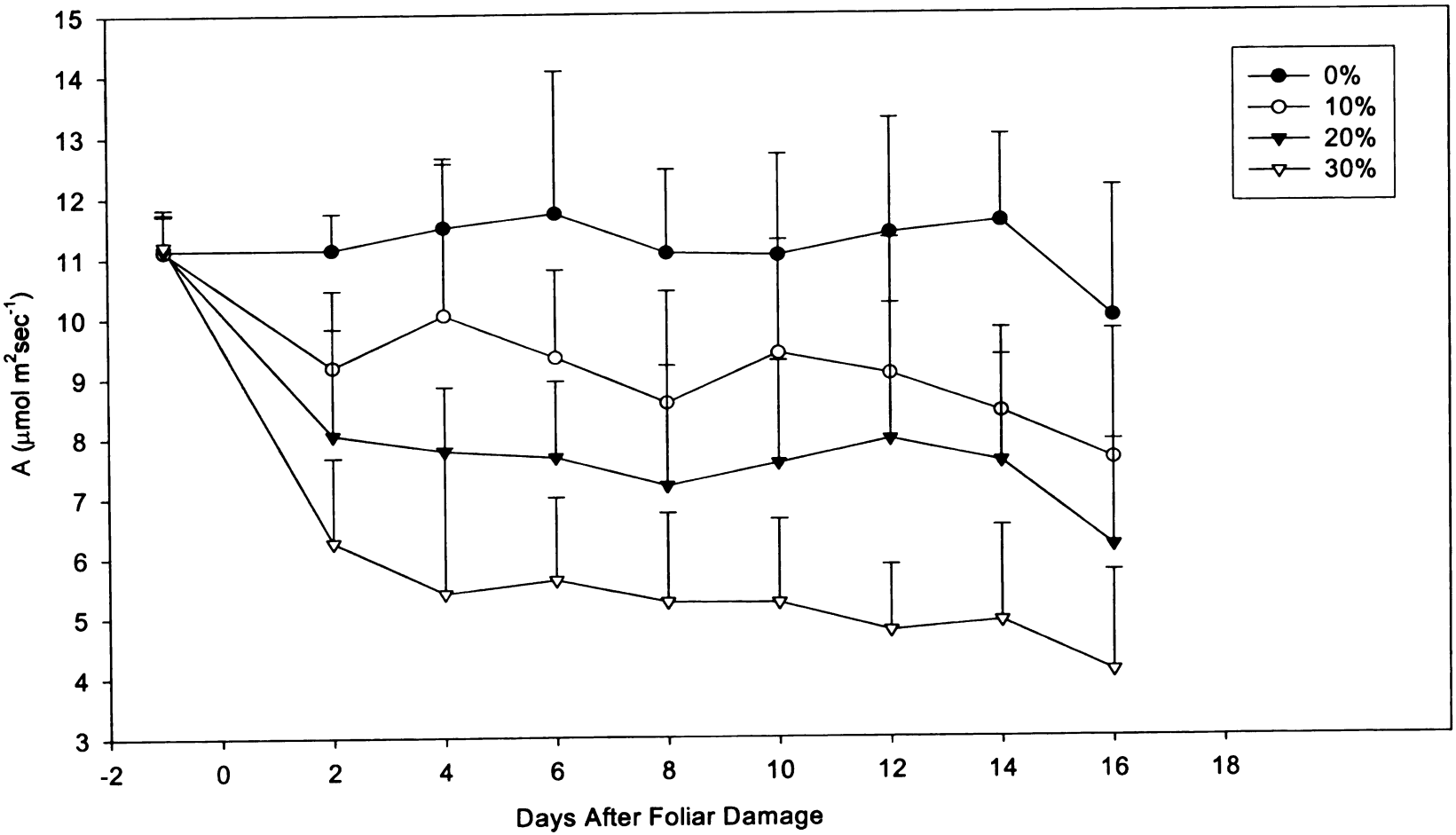

Figure 1. Effects of the level of foliar damage on CO_2 assimilation.

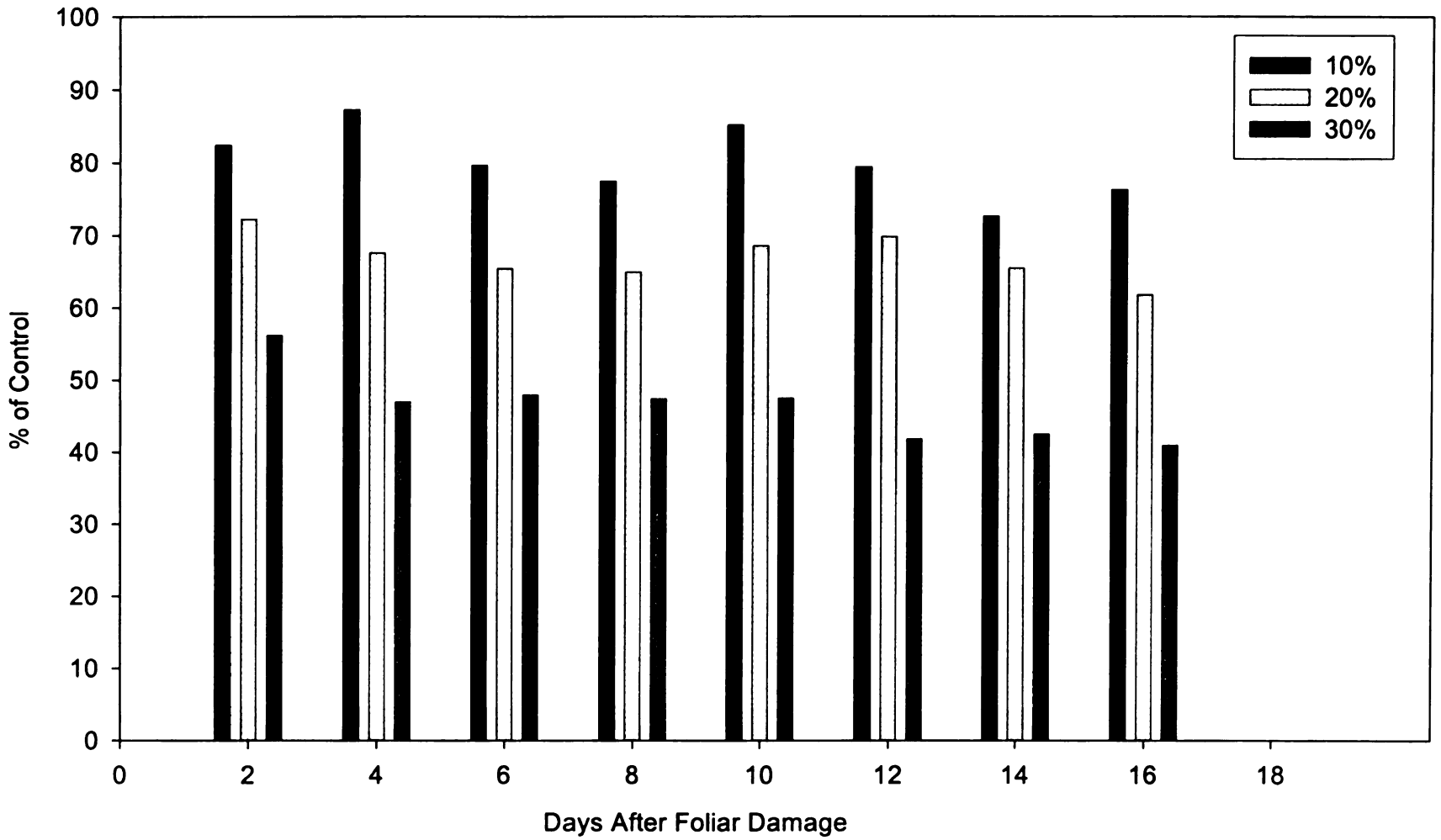

Figure 2. Effects of the level of foliar damage on CO_2 assimilation expressed as a percentage of control.

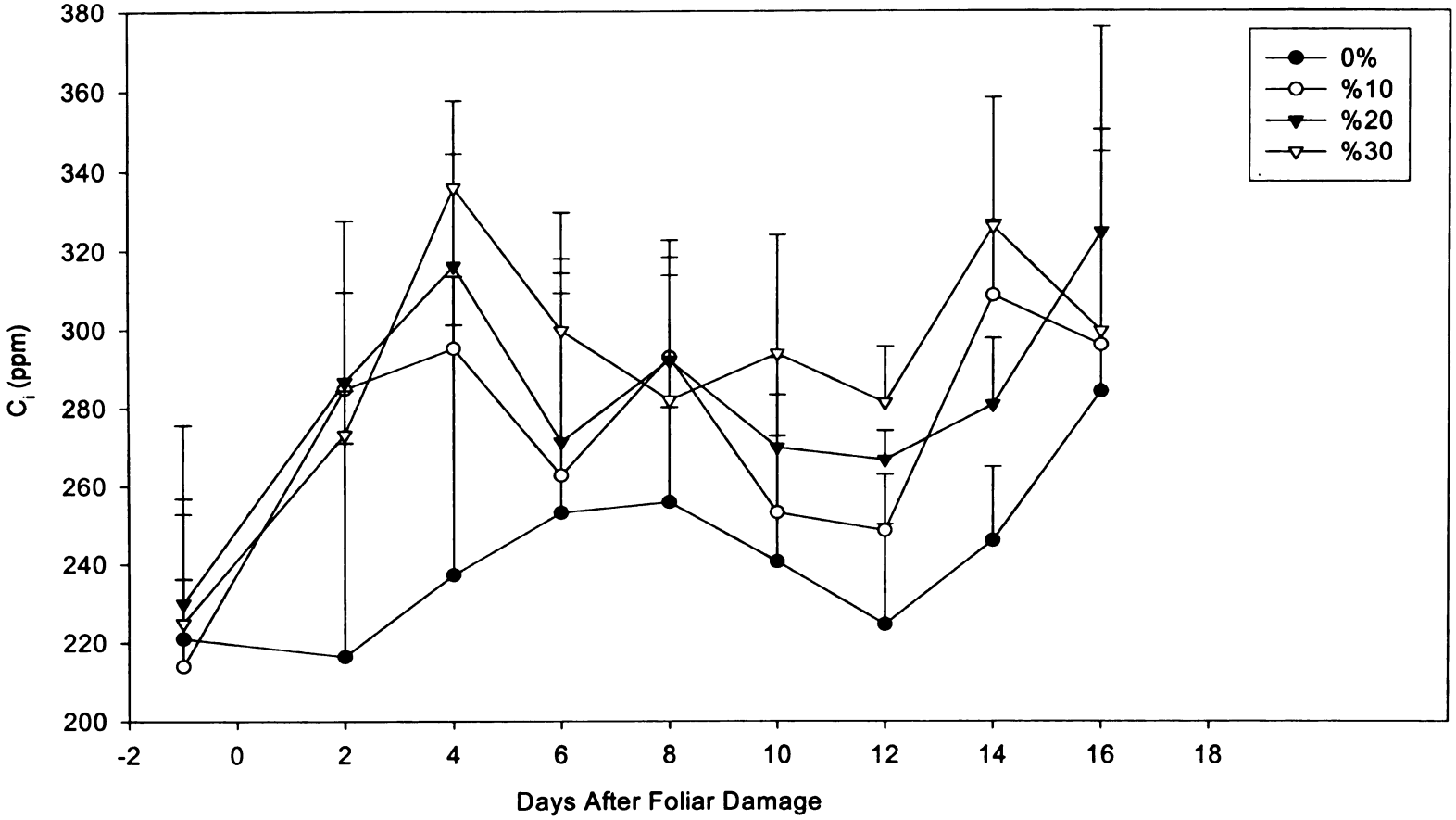

Figure 3. Effects of the level of foliar damage on internal CO_2 levels.

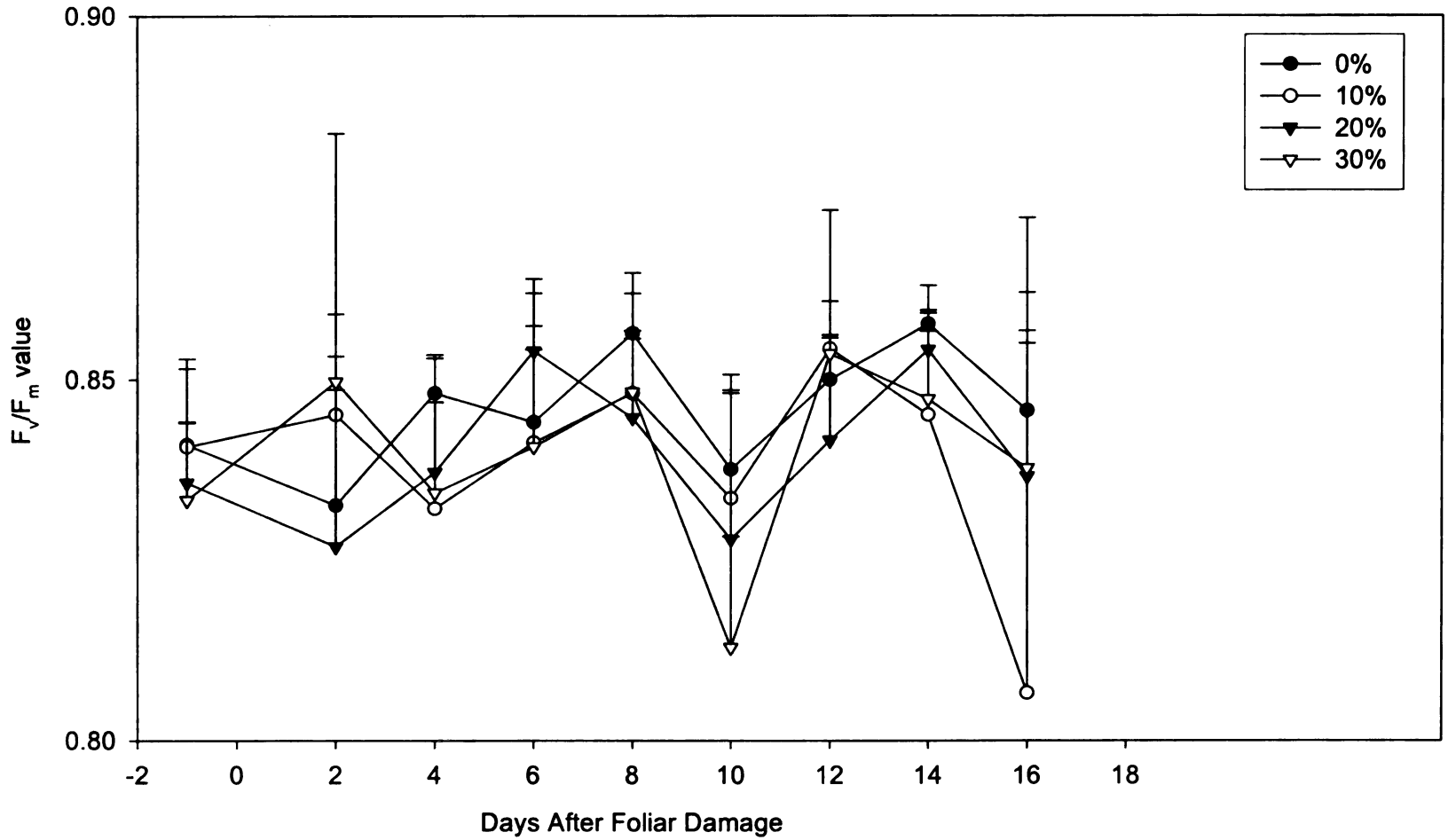

Figure 4. Effects of the level of foliar damage on F_v/F_m.

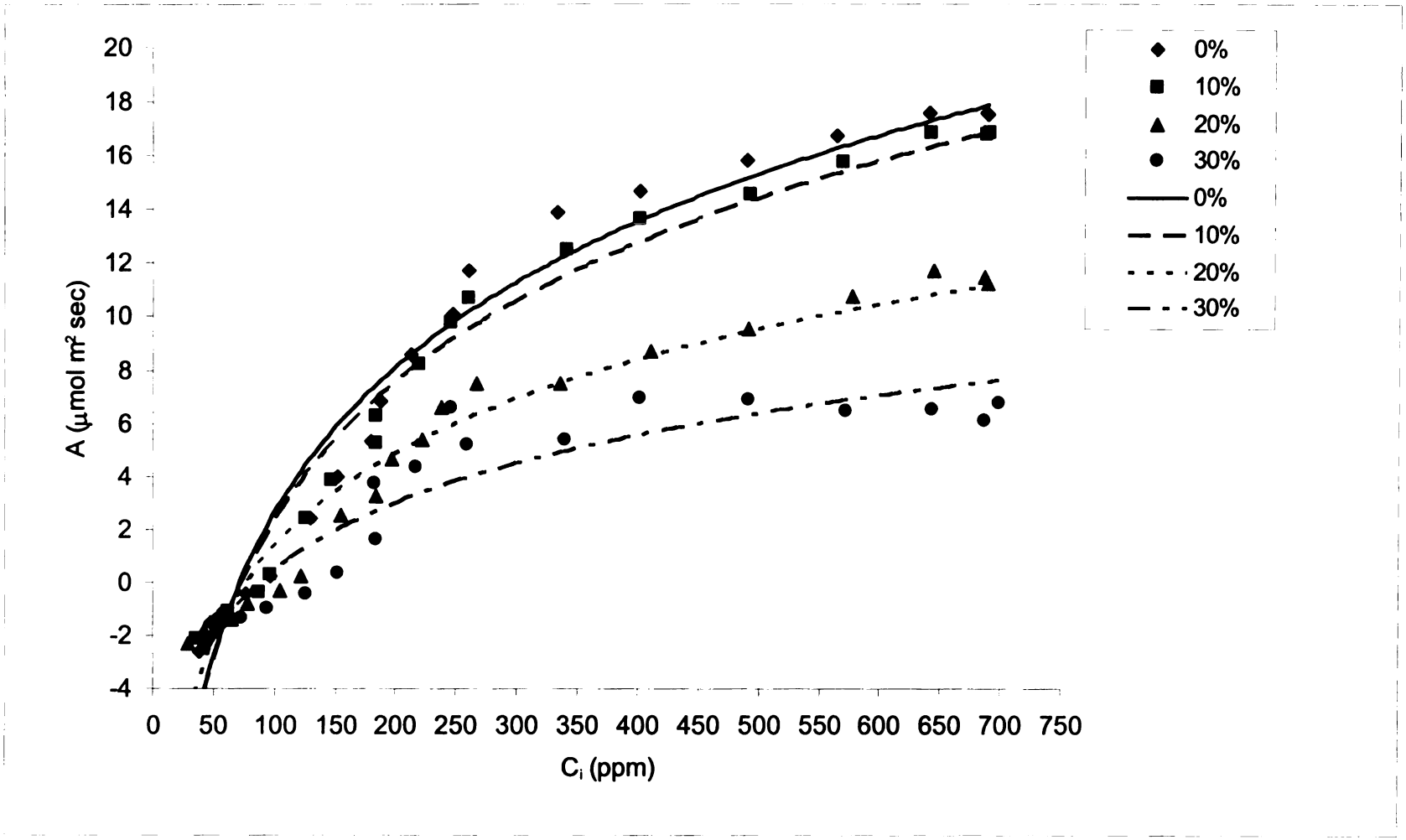

Figure 5. Effects of foliar damage on the A/C_i relationship.

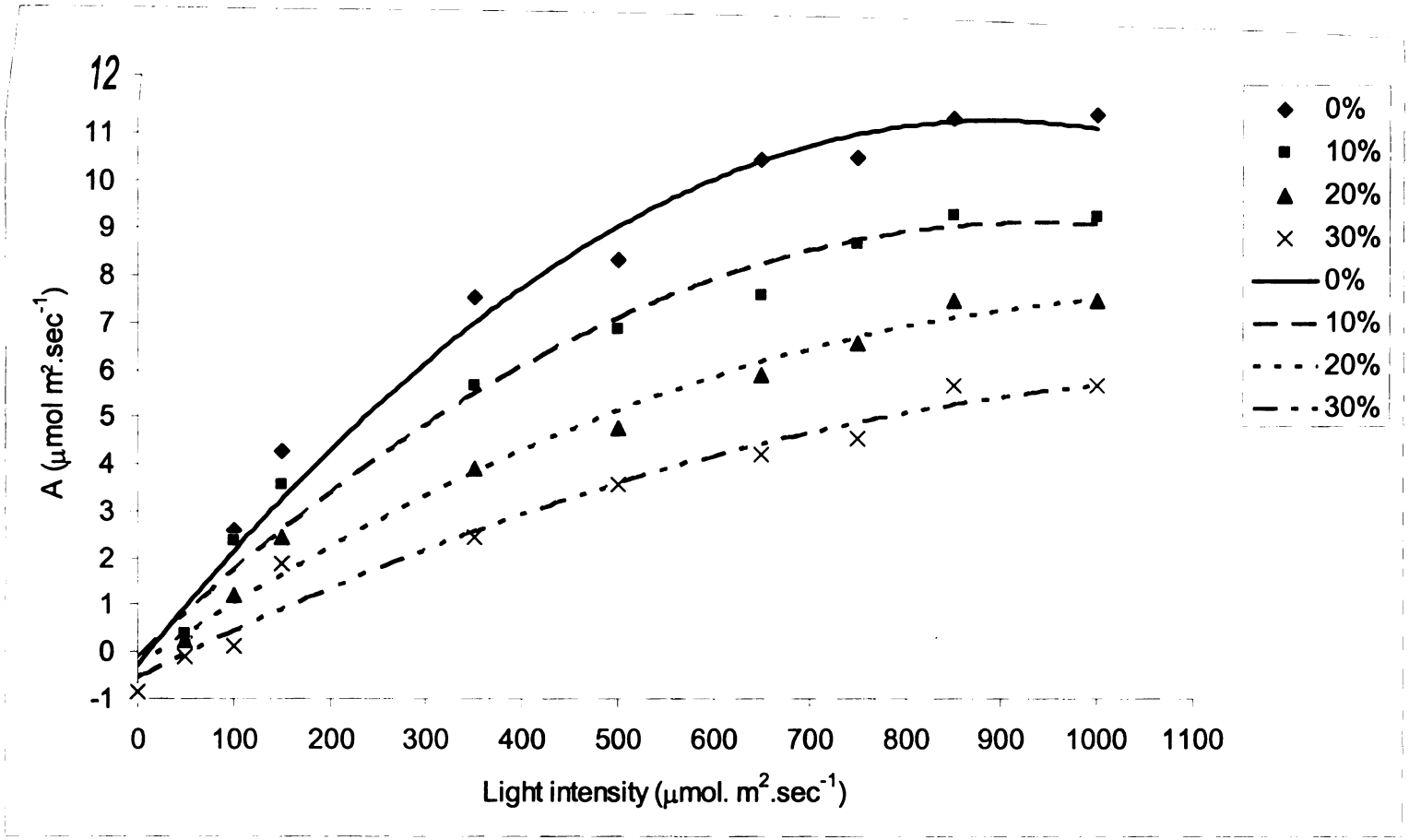

Figure 6. Light response curves for CO_2 assimilation rate in plants experiencing various levels of foliar damage.

Discussion

Foliar damage significantly reduced CO_2 assimilation rates in all of the treatments. Leaves exposed to 20% and 30% damage did not show any compensation comparable to control plants. Layne (1989) observed that sour cherry leaves were able to compensate with up to 20% foliar damage. In this research strawberry leaves did not show any compensation at this or any other damage level. Leaves exposed to 10% leaf damage could recover after two days following the treatments. However, this recovery was not maintained and the CO_2 assimilation rate was significantly lower after that day.

Compensation in CO_2 assimilation rates is generally due to changes in carboxylation efficiency and or RUBP regeneration rates (Farquhar, 1982; Jones 1985) . Based on data from the A/C_i curves, it was found that as the damage increased carboxylation efficiency decreased and CO_2 was not a limiting factor for the photosynthesis in damaged leaves. It is also found that as the damage increases carboxylation efficiency decreases and increases the CO_2 assimilation compensation point. These data indicated that strawberry leaves do not compensate photosyntheticly to leaf damage as in found in other plants.

Foliar damage also caused lower quantum efficiency (Table 2). Quantum efficiency decreased as the damage increase. Increasing light levels did not compensate for the foliar damage. Foliar damage also increased the light compensation values and decreased the light saturation points for CO_2 assimilation (Figure 5 and 6).

Chlorophyll fluorescence (F_v/F_m) was not affected from the foliar damage through out the measurements (Figure 4). Bounfeour (2002) also found that F_v/F_m values were affected by spider mites (*Tetranychus urticae* and *Tetranychus urticae*) feeding after two weeks of infestation (25 mites per leaflet). However, Iatrou (1995) found that chlorophyll fluorescence values were reduced in beans infested with *Tetranychus urticae*. At similar mite-days (Sances, 1979) found total chlorophyll content of strawberry leaves was not reduced by *Tetranychus urticae*. Since, chlorophyll content is related to the chlorophyll fluorescence values, it can be expected that chlorophyll fluorescence values would be similar.

Feeding habits of the pests may result in different results for chlorophyll fluorescence values. If the damage is limited to the spongy mesophyll and palisade layer is not damaged by the insects such results can be expected (Sances 1979). However, longer feeding time may decrease the chlorophyll fluorescence values as the damage increases proportional to the time. Population of the pests may also affect these values. In this study, damage was limited to the hole area so, the undamaged parts of the leaves were not affected by the foliar the damage. This may explain the lack of relationship between chlorophyll fluorescence and simulated foliar damage.

Internal CO_2 (C_i) values were higher in damage leaves than control plants and C_i levels increased as the level of foliar damage increased. This indicates that ability of leaf to use CO_2 was inhibited by the foliar damage.

Stomatal conductance (g_s) and transpiration (E) were not affected by the foliar damage at any level. On the fourth day of the measurements an increase in

stomata conductance (g_s) were observed in leaves which were damaged at 10% damage level. This result may explain the increase in the CO_2 assimilation rates on the fourth day. However, as indicated before this increases did not result in full compensation of the leaf photosynthesis. Layne (1989) and Proctor (1982) also found that simulated leaf and the leaf injury by 20 mines per leaf by damage by the *Phyllonorycter blancardella* did not affect the stomatal conductance and the transpiration rates of the leaves.

This study showed that photosynthetic compensation did not occur when damage occurred to single leaves. Since, in this study only single leaf was considered for measurements, to understand if a photosynthetic recovery metabolism exists in strawberry plants, whole plant photosynthesis measurements should be considered. However, the canopy of strawberry plants changes continuously as new leaves are formed from the crown and old leaves die. These are obvious limitations to calculate the canopy area of strawberry plants. Thus, it may be also difficult to apply simulated damage to strawberry plants.

LITERATURE CITED

Bounfour, M., L.K. Tanisgoshi, C. Chen, J. Cameron, S. Klauer. 2002. Chlorophyll content and chlorophyll fluorescence in red raspberry leaves infested with *Tetranychus urticae* and *Eoteranychus carpini borealis* (Acari: Tetranychidae). Phsiological and Chemical Ecology. 31: 215-220.

Disegna, E.J. 1994. The use terbacil as a tool to establish a photosynthetic threshold in apples. Michigan State Univ., East Lansing, Msc thesis.

Farquhar, G.D., T.D. Sharkey. 1982. Stomatal conductance and photosyntesis. Ann. Rev. Plant. Physiol. 33:317-345.

Gucci, R. 1988. The effect of fruit removel on leaf photosyntesis, water relations and carbohydrate partitioning in sour cherry and plum. Ph.D. Thesis. Michigan State University, East Lansing, MI.

Gucci, R., J.A. Flore. 1990. Net Photosynthesis and non-structural carbohydrates in leaves of field-grown plum trees following fruit removal. In: 3rd International meeting on "Regulation of photosynthesis in fruit crops". Perigia. Italy. P.127.

Jones, H.G. 1985. Partitioning stomatal and non-stomatal limitations to photosyntesis. Plant, Cell and Environment 8:95-104.

Jones, H.G. 1998. Stomatal control of photosyntesis and transpiration. Journal of Experimental Botany. 49:387-398.

Hancock, J.F. 1999. Strawberries. CABI publishing, Oxon, UK.

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

Howell, G.S., S.S. Stackhouse. 1973. The effect of defoliation time on acclimation and dehardining in tart cherry (*Prunus cerasus* L.) J. Amer. Soc. Hort. Sci. 98: 132-136.

Kappel, F. 1986. Influence of spotted tentiform leaf miner on apple leaf and tree physiology. Ph. D. Thesis. University of Guelph, Guelph, Ontario, Canada.

Kirkwood, D.A. 1983. The mode of action of herbicides. In: Recent advances in weed research. W.W. Fletcher (ed.) 227-257.

Layne, D. 1989. Damage thresholds for simulated trunk and leaf injury in 'Montmorency' sour cherry. Ms. Thesis. Michigan State University, East Lansing, MI.

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

Sams, C.E., J.A. Flore. 1982. The influence of age, position and environmental variables on net photosyntetic rate of sour cherries. J. Amer. Soc. Hort. Sci. 107:339-344.

Shaw, M. 1956. The physiology of host parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate

parasites including tobacco mosaic virus. Canadian Journal of Botany 34:(389-405).

Unrath, C.R., 1981. An overview of environmental factors affecting orchard growth regulator response with special reference to apple. Acta Hort. 120:43-52.

Wareing, P.F., M.M. Khalifa, K.J. Treharne. 1968. Rate limiting process in photosynthesis at saturating light intensities. Nature 220:453-457.

Chapter 2

THE USE OF TERBACIL AS A TOOL TO ESTABLISH A PHOTOSYNTHETIC THRESHOLD IN STRAWBERRIES (*Fragaria* × *ananassa* cv. 'Honeoye')

ABSTRACT

Damage thresholds of strawberry plants (*Fragaria* × *ananassa*) were investigated by terbacil application. Terbacil was applied to the field-grown plants during 2001 and 2002. In 2001, terbacil was applied at concentrations of 12.5, 25, 50, 100, 200 and 400 ppm levels. In 2002, a previously untreated group of two years old strawberry plants were sprayed with terbacil at concentration of 50, 100 and 200 ppm at two different growth stages (during fruit set and after harvest stages).

Strawberry plants which were treated with terbacil were able to recover at certain levels, except 400 ppm level during the first year experiment. CO_2 assimilation rate of the plants treated with 200 ppm were lower than the control plants 22 days after terbacil application. All other concentrations recovered to the level of control plants. Recovery occurred between 4 and 10 days after the terbacil treatment. Average fruit weight was adversely affected during the year following the 400 ppm terbacil treatment. Other concentrations of terbacil did not alter the response of the plants to terbacil.

Stage of the development did not alter the response of the plants to terbacil. Difference in stomatal conductance and transpiration rates were insignificant. Internal CO_2 (C_i) levels were higher in plants which were treated with high terbacil concentrations. Chlorophyll a and total chlorophyll content decreased following the terbacil treatment. However, chlorophyll a and total

chlorophyll increased 8 days after terbacil treatment. Plant dry matter values and chl b values were not affected from the terbacil treatments.

INTRODUCTION

Determination of damage thresholds is an important issue in plant science. Damage threshold is defined as the level of pest damage above which there are negative effects on the growth or the health of the plant.

In order to determine the plant damage thresholds different approaches are used. These methods are based on simulating damage in plants. Hole punching is used to simulate insect damage in threshold studies (Kappel, 1986; Layne, 1989). However, this method requires lots of time and labor. Herbicides that inhibits photosynthesis can also be used for threshold studies. Terbacil is a uracil type herbicide that blocks both the Hill reaction and photosytem II in the photosynthetic pathway (Ashton, 1973). It has been used by other researchers to simulate damage in other crops (Byers, 1990; Disegna, 1994).

Damage thresholds levels have been investigated for several plants. Such determinations were performed in wheat and barley (Shaw, 1956), soybean (Wareing 1968), Lucerne (Hodgkinson, 1974), sour cherry (Layne,1989) and (Disegna, 1994). Damage threshold levels for these crops range from 5% - 20% depending on the crop and the crop load (Disegna, 1994). However, damage threshold levels for strawberry plants have not been determined. Determination of such value would be useful in IPM and pesticide application programs, the assessment of environmental impacts and on economics studies.

In this research, terbacil is used as a tool to investigate the photosynthetic threshold of strawberry plants and to investigate other effects which may be related to photosynthesis (e.g. fruit yield, dry weight of the plant). Terbacil is

commonly used to control the weeds in strawberry production. It is usually applied before planting, in early season and after harvest renovation (Mahr et. Al, 2002).

The hypothesis tested in this research was "Leaf photosynthetic capacity will determine damage threshold levels for strawberry productivity".

For this purpose terbacil was used as a tool to establish a threshold in strawberry.

The objective of this research was to determine the leaf damage threshold for strawberry (*Fragaria* × *ananassa* cv. 'Honeoye') on whole plants under field conditions at different times during the growing season. Leaf damage was simulated by applying terbacil to the foliage at different concentrations and at different critical stages in crop development. The degree and duration of photosynthetic inhibition are dose dependant and crop specific. It was hypothesized that different levels of P_n reduction could reduce the production and storage of carbohydrates needed for growth and reduced carbohydrate production may affect yield and runner production negatively and the ability of the plant to resist environmental stress.

MATERIALS AND METHODS

2001 experiment

Strawberry plants (*Fragaria ananassa* cv. Honeoye) were planted in three raised beds (20 cm height, 50 cm width) at Michigan State University Horticulture Teaching and Research Center (HRTC), East Lansing, MI. Each bed had two rows of plants, 20 cm apart, and the distance between the plants within a row was 30 cm. The experiment was designed as a randomized complete block with three blocks with one bed per block. There were six plants per treatment. The treatments consisted of a single application of terbacil at concentrations of 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm and 400 ppm. X-77 (90%) surfactant (Alkylarylpolyoxyethlene, Alkylopolyoxyethylene, Fatty acids, Glycols and Dimethhypoly siloxane) was added to the herbicide at a concentration of 1.25 ml/L. Control plants were sprayed with water plus surfactant at 1.25 ml/L. Leaves were sprayed to drip point. Border plants were used to separate treatment plots. Root pruning was performed as needed and old leaves were removed before planting. Plants were drip irrigated as follows. One drip line placed per hill. Capacitiy of dripper was 4 L//h. Irrigation applied for 40 minutes at 7:30 am every day by a Torro irigation timer (Model 53331, Bloomington, MN). A 20-20-20 (N,P,K) fertilizer was applied three times during the growing season at a rate of 5 grams per plant. Straw mulch was used as the mulching material. Manual weeding was performed as necessary. No pesticides were applied to the

strawberry plants during the experiment and no significiant incest or disease damage was observed during the experiment.

Gas Exchange Measurements

Gas exchange measurements were made on three plants per treatment plot. One fully expanded leaf was selected for gas exchange measurements. The CIRAS-1 portable photosynthesis system (PP Systems, Hertfordshire, UK) was used to measure the gas exchange parameters which included CO_2 assimilation rate (A), stomatal conductance (g_s) and internal CO_2 (C_i). Gas exchange measurements were performed one day before terbacil treatments and 2, 4, 6, 10, 14, 18 and 22 days after the terbacil treatments. All gas exchange measurements were made between 8:30 am and noon.

Chlorophyll Fluorescence

Chlorophyll Fluorescence was measured on six plants per treatment plot. One fully expanded leaf was selected for gas chlorophyll fluorescence. The Plant Efficiency analyzer (Hansatech Instruments Ltd, Norfolk, UK) was used for these measurements. Leaves were dark acclimated for 20 minutes prior to measurements using dark acclimation cuvettes. These leaves were then irradiated with actinic light for 5 seconds and chlorophyll fluorescence kinetics were recorded (Krause,1984). Chlorophyll fluorescence measurements were

performed one day before terbacil treatments and 2, 4, 6, 10, 14, 18 and 22 days after the terbacil treatments.

2002 experiment

Two years old strawberry (*Fragaria* × *ananassa* cv. Honeoye) plants were used in this experiment which were planted in 2001 at Michigan State University Horticulture Teaching and Research Center (HRTC), East Lansing, MI. Cultural practices and planting distances were the same as described for the 2001 experiment. Terbacil treatments were applied at two different times. The first terbacil treatment was applied during fruit set and the second terbacil treatment was made after harvest. Based on the 2001 rates, terbacil was applied at rates of 50 ppm, 100 ppm and 200 ppm. X-77 (90%) surfactant (Alkylarylpolyoxyethlene, Alkylopolyoxyethylene, Fatty acids, Glycols and Dimethhypoly siloxane) was added to the spray solution at a concentration of 1.25 ml/L. Control plants were sprayed with an aqueous solution containing the surfactant only. Leaves were sprayed to the point of drip.

Gas Exchange Measurements

Four plants were selected for gas exchange measurements from each treatment plot with three replicates (blocks). Measurements were performed one day before terbacil treatment and 2, 4, 6, 8, 10, 14, 18 and 22 days after the

terbacil treatments. The same method was used for gas exchange measurements as described 2001 experiment. CO_2 assimilation rate, stomatal conductance and internal CO_2 parameters were recorded.

Chlorophyll Fluorescence

Measurements were conducted as described for the 2001 experiment. Measurements were performed one day before terbacil treatments and 2, 4, 6, 8, 10, 14, 18, and 22 days after the terbacil treatments.

Fruit Yield

Strawberry plants that were used in the 2001 experiment were harvested in 2002 in order to assess the effect of the previous seasons's damage on the following year's yield.

Plants that were treated during fruit set stage in 2002 experiment harvested. Fruit number and weight was collected on individual plants. Two harvests were performed.

Chlorophyll Content

Three leaf discs (0.33 cm^2) were removed from three different leaves on each plant using a paper punchhole. Chlorophyll was extracted by placing the

leaf discs in 7 ml N,N-dimethylformide for 36 hours in the dark at a temperature of 5°C. Absorbance of the extracts at wavelength of 664, 647 and 625 nm was measured using a Hitachi U-3110 spectrophotometer (Hitachi Ltd, Tokyo, Japan). The concentration of chlorophyll a, chlorophyll b and chlorophyll P was calculated according to the methods proposed by Moran (1982).

Chlorophyll content was determined one day before the treatments and 4, 8,12, 16 days after the terbacil treatments.

Dry Weight

Strawberry plants on which CO_2 assimilation rates were measured during the season (four plants per treatment), were removed from the field at the end of the growing season and separated into three parts (root, crown and leaves). Roots, crowns and leaves were placed in a forced air oven at a temperature of 60°C for four days until dry.

Plot Design and Statistical Calculations

A randomized complete block design was used in this experiment. Data were subjected to analysis of variance (ANOVA). Means were compared by Duncan test or by standard deviation.

Error bars in the figures represents standard deviation.

The SAS base statistical program (version 8.2, SAS institute, Cary, NC) was used for ANOVA.

RESULTS

2001 Experiment

Effects of Terbacil on CO_2 assimilation rate

Under the conditions of this experiment, the average CO_2 assimilation rate in leaves of control plants ranged between 12.5 and 18 $\mu mol.m^{-2}.s^{-1}$ (Figure 7) Terbacil, applied at a rate of 400 ppm, caused complete inhibition of CO_2 assimilation two days after treatment (Figure 7). At the 200 ppm rate, terbacil decreased leaf photosynthetic rates by 40% as compared to the untreated control plants. At rates of 12.5, 25 and 50 ppm, terbacil had no significant effect on leaf photosynthesis as indicated by measurements made over a period of 22 days following the treatment. Four days after treatment, leaf photosynthetic rate in the 200 ppm treatment decreased to 55% of the rate measured in control plants (Figure 8). At the same time, terbacil at 400 ppm continued to cause complete inhibition of photosynthesis. By the sixth day after treatment, CO_2 assimilation rates in the 200 ppm treatment had partially recovered to approximately 72% of the photosynthetic rate of control plants. CO_2 assimilation rates in the 400 ppm treatment also showed some recovery but remained at significantly lower levels than the control. Ten days after treatment, the recovery of photosynthetic activity continued in plants treated with 200 ppm of terbacil, whereas the recovery observed earlier in the 400 ppm treatment was not

apparent on this date. However, 14 days after treatment, CO_2 assimilation rates in plants treated with 400 ppm of terbacil recovered to about 50% of the rates measured in control plants. Leaf photosynthetic rates on this date for all other terbacil treatments were not significantly different from the control. On day 18, the CO_2 assimilation rate of plants in the 400 ppm treatment again decreased to less than 50% of the control, while plants in the 200 ppm treatment showed a smaller drop in assimilation rate to approximately 68% of the control level. The decrease in CO_2 assimilation rates became more severe by day 22, as leaf photosynthetic rates in the 100 ppm and 200 ppm treatments decreased by approximately 30% and 60%, respectively. In plants treated with terbacil at 400 ppm treatment photosynthetic activity appeared to have ceased completely by day 22 as leaves showed severe chlorosis.

Gas Exchange Parameters

At all rates tested in this experiment, terbacil had no significant effect on stomatal conductance (Figure 9). Stomatal conductance for the for all plants ranged between 125 and 375 $\mu mol.m^{-2}.s^{-1}$. Internal CO_2 levels were affected by the terbacil treatments (Figure 10). Plants treated with terbacil at 400 ppm consistently had the highest levels of internal CO_2 throughout the two weeks immediately following the treatment. The highest levels of internal CO_2, approximately 360 ppm, were observed in leaves of the 400 ppm treatment on days two and four after the treatment. Internal CO_2 levels in the 200 ppm

treatment were generally higher than those of the control and the other terbacil treatments; however, C_i levels in all but the 400 ppm treatment were similar by day 14.

Transpiration (E) was not affected by the terbacil treatments (Figure 11). No significant differences in E were found at any of the dates on which leaf gas exchange was measured.

Chlorophyll Fluorescence

Chlorophyll fluorescence was evaluated as the ratio of F_v over F_m values (F_v/F_m).

F_v/Fm value gradually decreased at the plants which were treated with 400 ppm. It was 56% of the control value after 2 days (Figure 12). At the end of the experiment F_v/Fm value was near to zero. At the 100 ppm and 200 ppm levels F_v/Fm values were lower than the control , 12.5, 25, 50 ppm levels after 2 days of the terbacil treatment F_v/Fm values were not significantly different between 2nd and 22nd of the treatments than each other except the 400 ppm level.

The relationship between F_v/Fm and assimilation (A) is given in figure 13 as a regression curve.

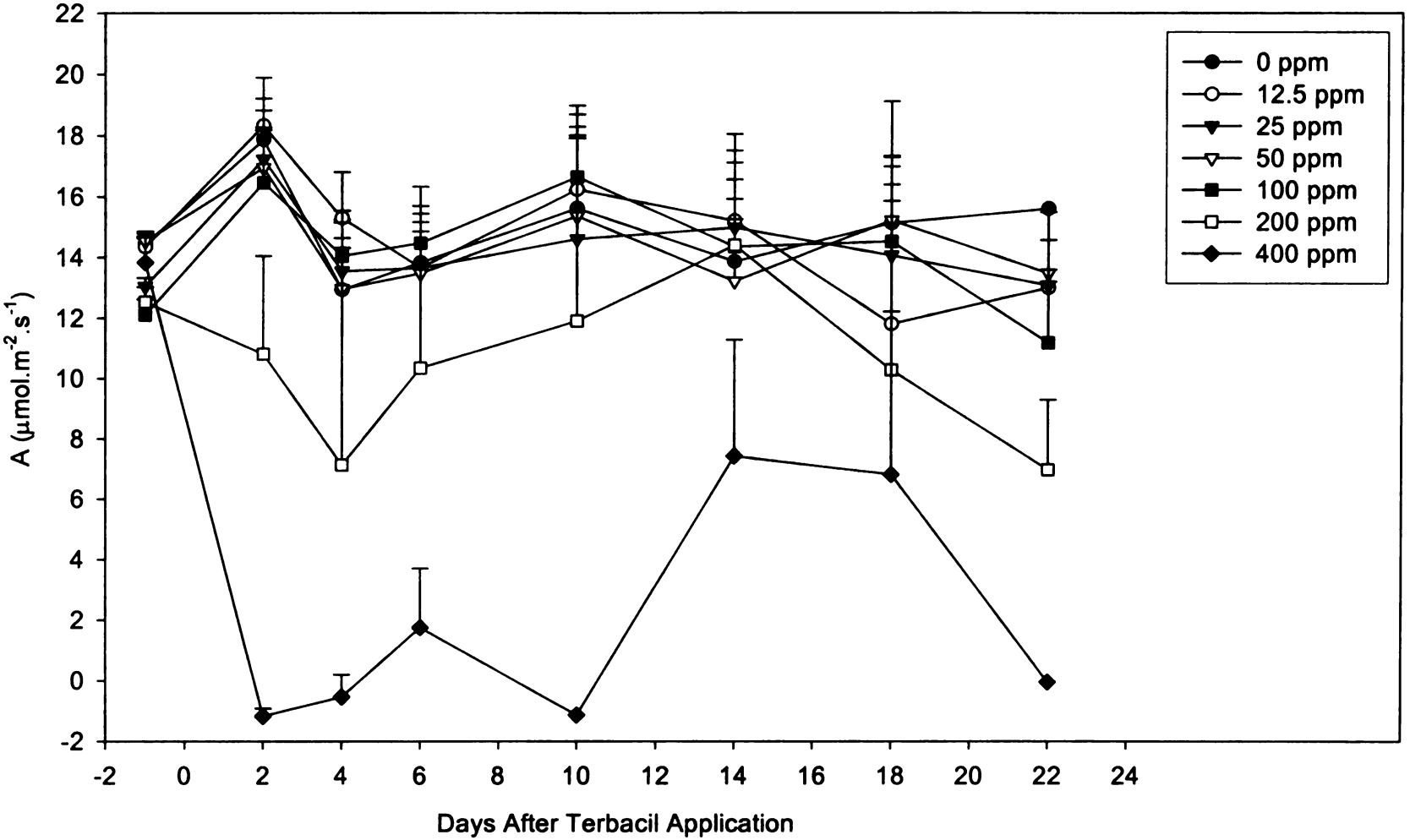

Figure 7. The effect of different terbacil concentrations on assimilation rate (A) in 2001 growing season.

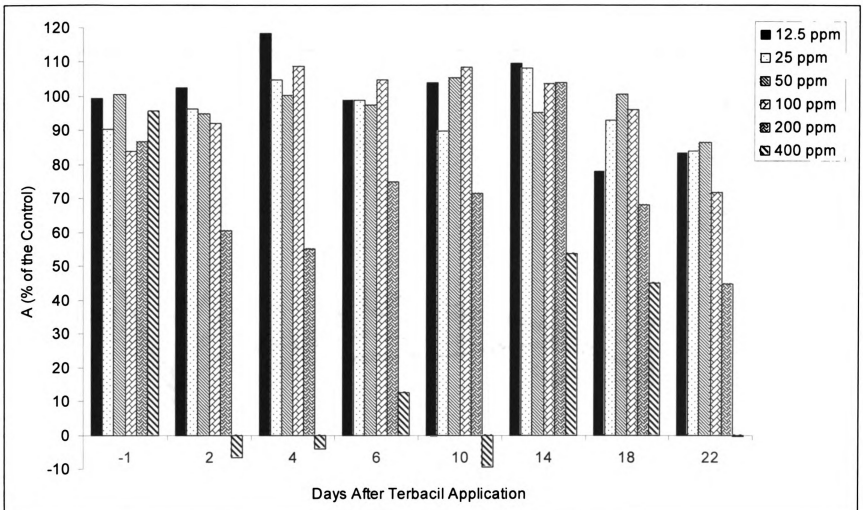

Figure 8. Change of assimilation rate (A) in percentage of control in 2001 growing season.

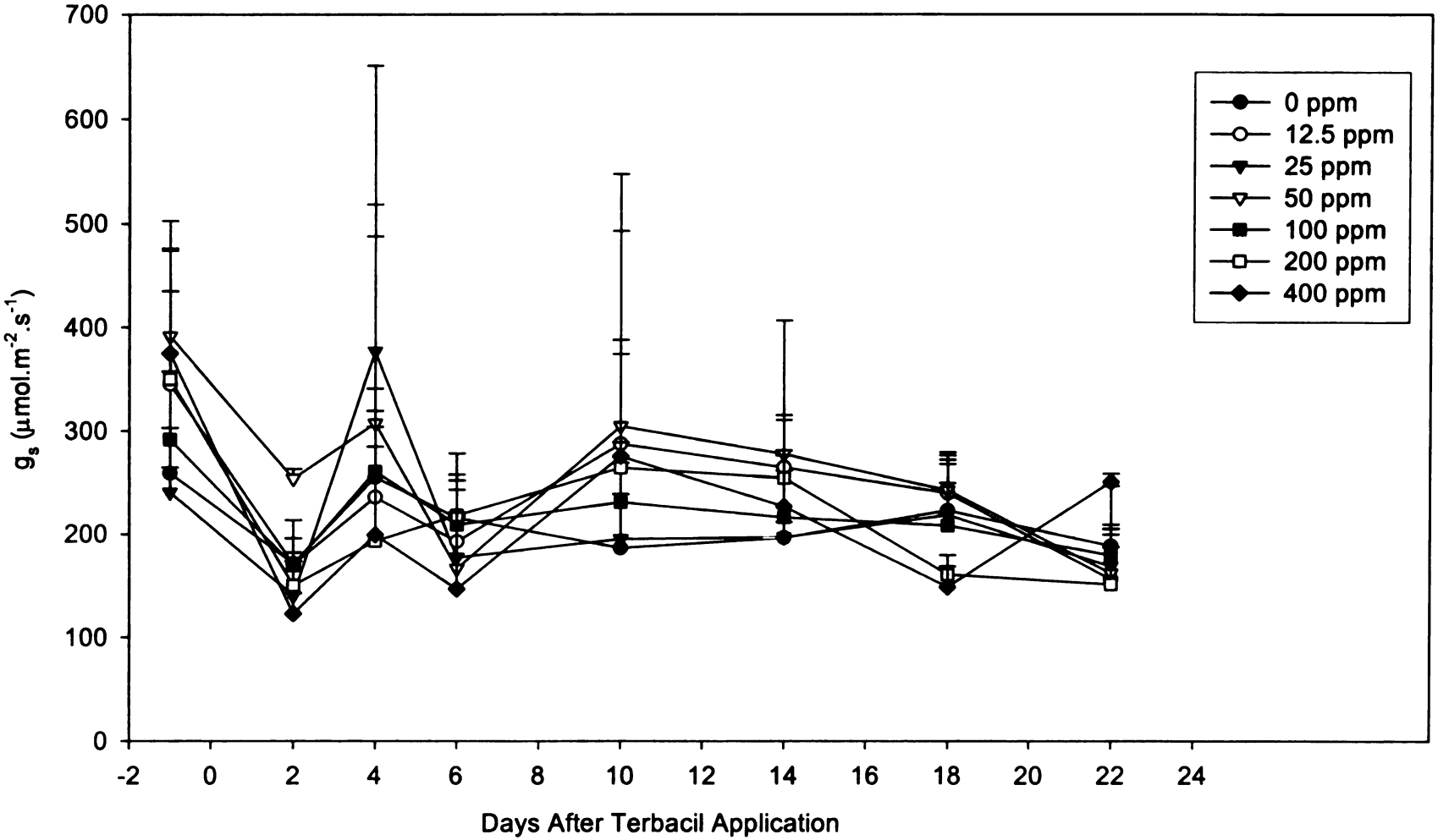

Figure 9. The effect of different terbacil concentrations on stomatal conductance (g_s) in 2001 growing season.

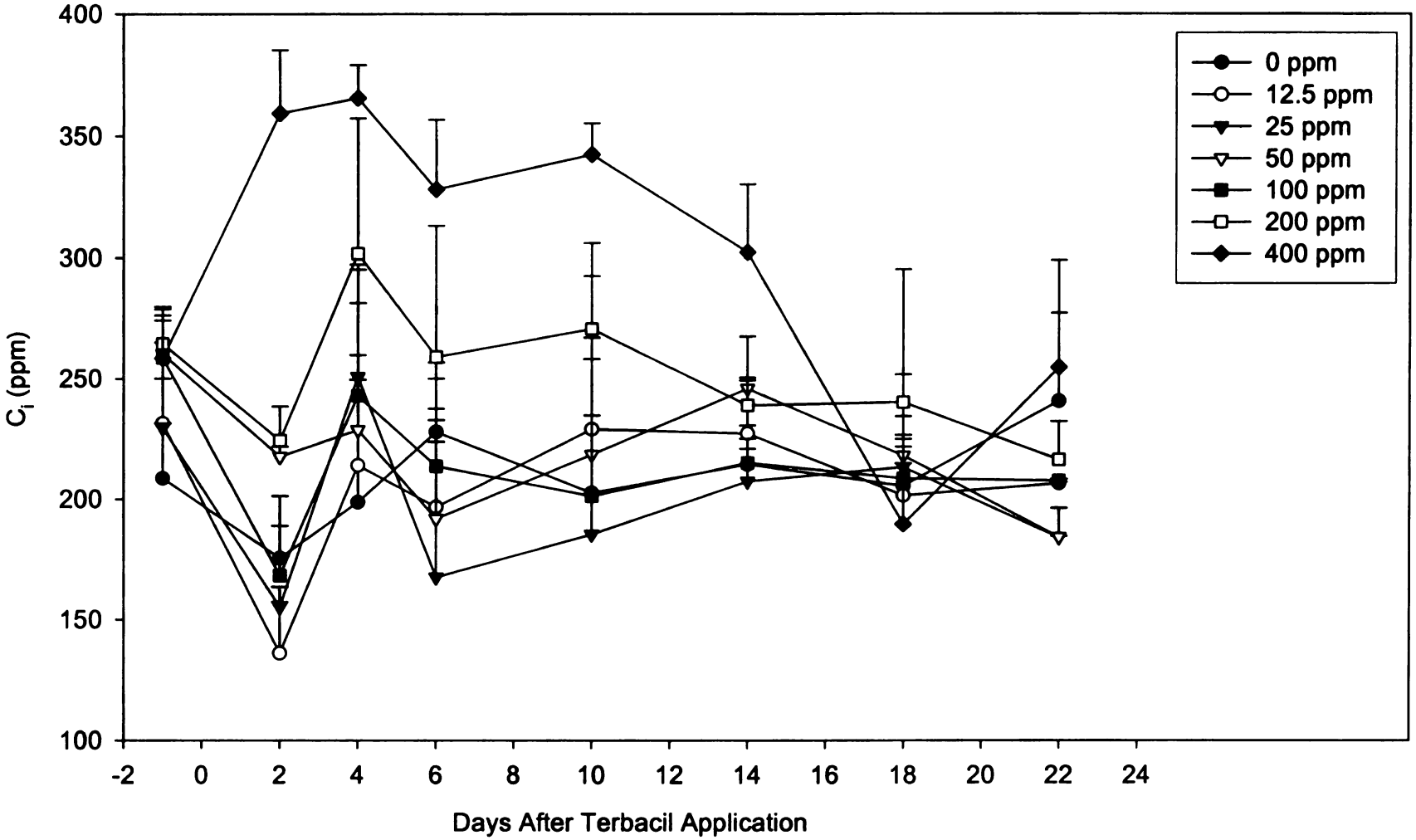

Figure 10. The effect of different terbacil concentrations on internal CO_2 (C_i) in 2001 growing season.

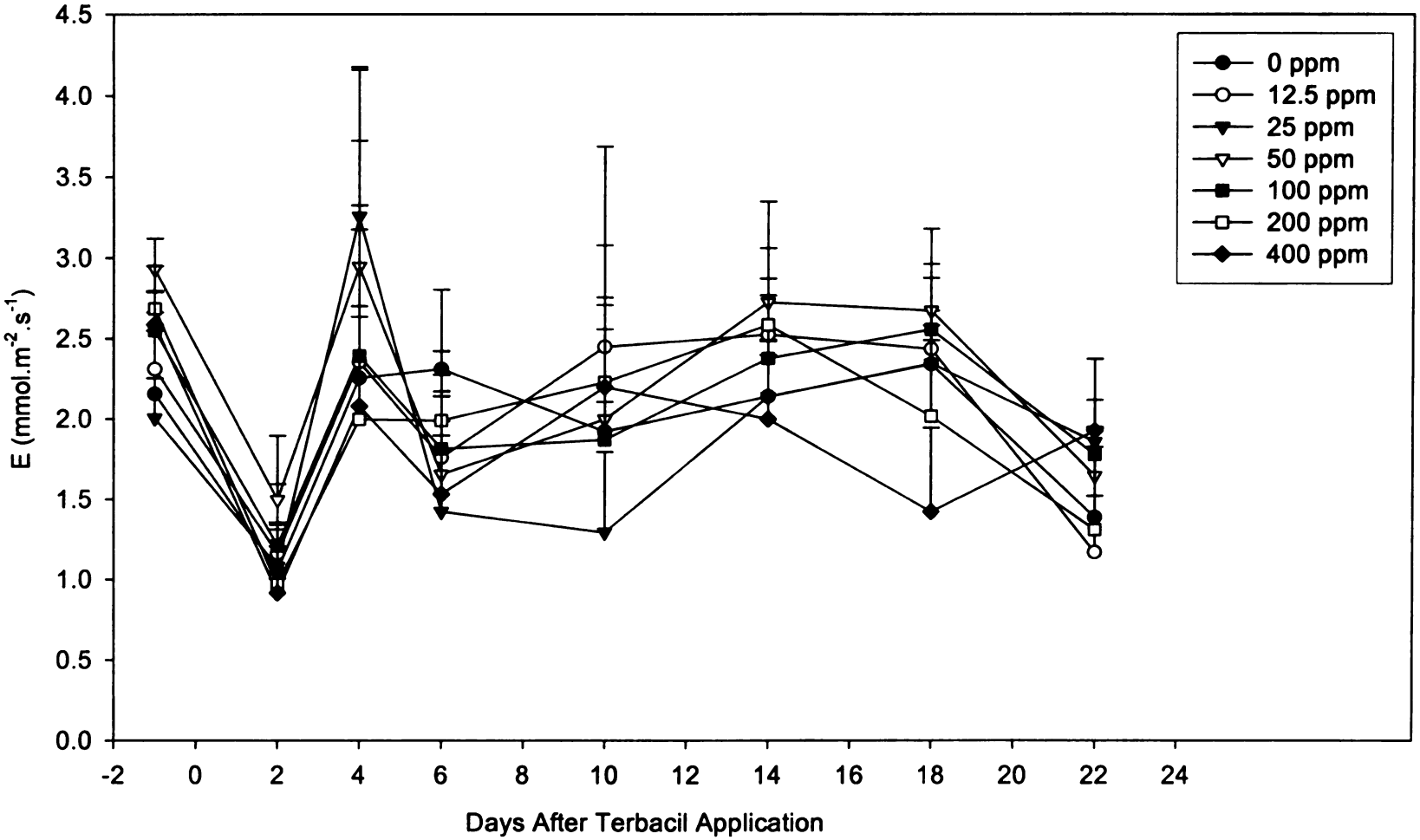

Figure 11. The effect of different terbacil concentrations on transpiration (E) in 2001 growing season.

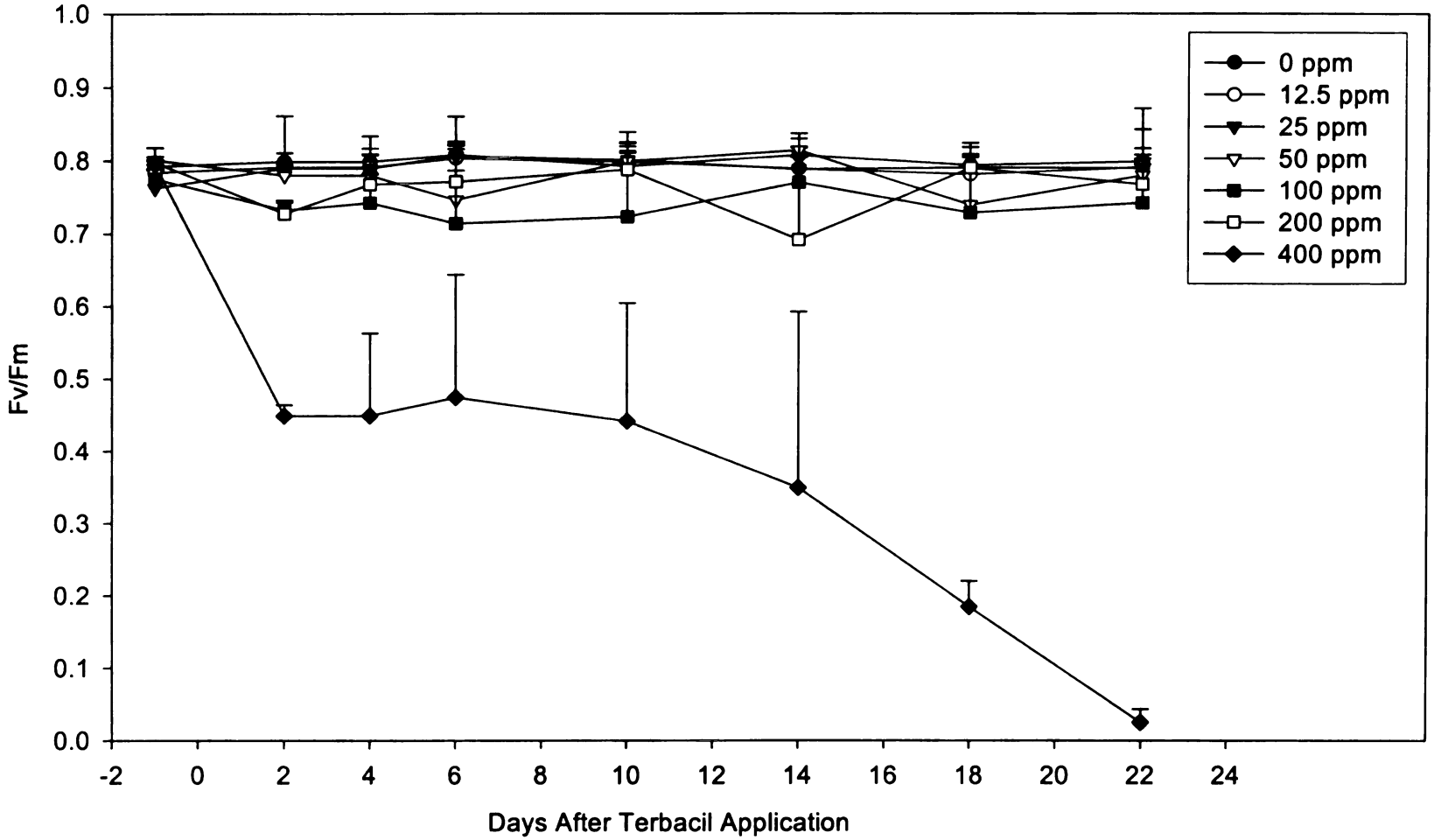

Figure 12. The effect of different terbacil concentrations on F_v/F_m in 2001 growing season.

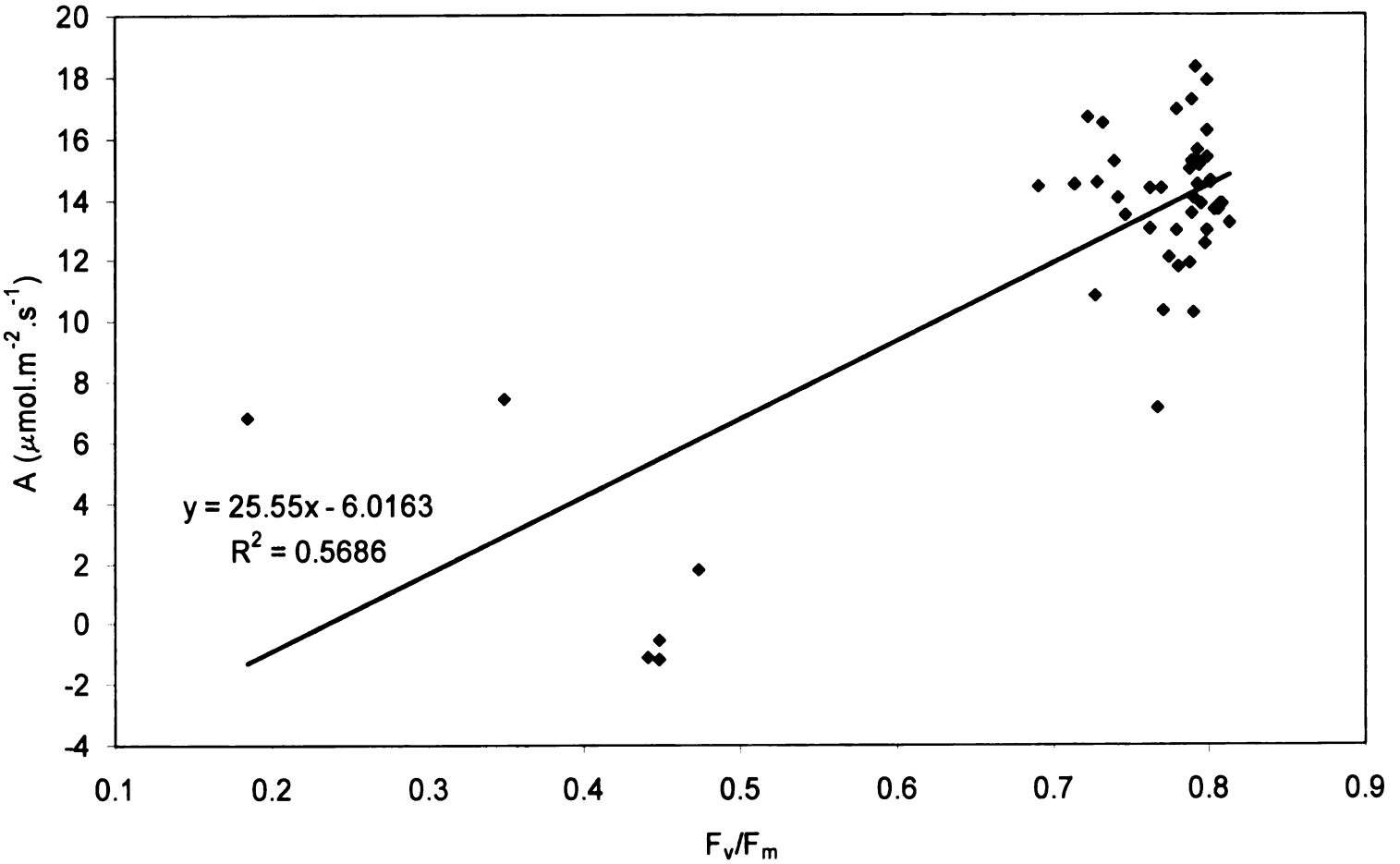

Figure 13. Relationship between F_v/F_m and assimilation in 2001 growing season.

2002 Experiment

Effects of Terbacil on CO_2 assimilation rate

The average CO_2 assimilation rate of control plants throughout the experiment ranged between 10.71 and 17.05 $\mu mol.m^{-2}.s^{-1}$ (Figure 14 and 15). Two days after treatment with terbacil at a rate of 200 ppm leaf photosynthetic rates decreased by 25% and 30% as compared to the untreated control plants at the "during fruit set" and "after harvest" stages, respectively. These values were 75% for 100 ppm two days after the treatments. Compared to the untreated control, reduction in CO_2 assimilation rate at the 100 ppm rate was 70% and 60% four days after the treatments (Figure 16 and Figure 17). Plants recovered 8 days after the treatments at both stages. A reduction in assimilation rate of control plants occurred after fourteen days, which may be due to leaf aging. After fourteen days, there were no significant differences in the assimilation rates of treated and untreated plants. This was observed between 14 and 22 days following the treatments at both stages of the plants.

The effect of application at different stages of development showed a small difference at 50 ppm level during the fruit set stage, 2 days after treatment. CO_2 assimilation rates were 78% of the photosynthetic rate of control plants and this value was significantly lower than the control value.

Gas Exchange Parameters

At all rates of terbacil tested at both growth stages, terbacil had no significant effect on stomatal conductance (Figure 18 and Figure 19). Stomatal conductance ranged between 108 and 209 $\mu mol.m^{-2}.s^{-1}$ (Figure 18 and Figure 19). Internal CO_2 levels were affected by the terbacil treatments as in the 2001 experiment. Plants treated with 200 ppm terbacil had the highest level of C_i two days after treatments. Six days after treatment, internal CO_2 levels in plants treated with 100 ppm terbacil were higher than those observed in control plants. C_i levels dropped to control levels after 8 days of the terbacil treatments. This was observed at both stages of the application (Figure 20 and Figure 21).

Transpiration (E) was not affected by the terbacil treatments. This was observed at the both application stages. No significant differences in E were found at any dates on which leaf gas exchange was measured (Figure 22 and Figure 23).

Chlorophyll Fluorescence

Chlorophyll fluorescence was evaluated as the ratio of F_V over F_m values (F_v/F_m).

Terbacil caused a significant decrease in F_v/F_m values within 2 days of treatment (Figure 24 and 25). The most severe decrease in F_v/F_m occurred in that plants were treated with terbacil at a concentration of 200 ppm, where F_v/F_m

decreased to 50% of F_v/F_m in control plants. In these plants, F_v/F_m remained at the depressed level until day 4, after which they increased significantly but remained below control levels until day 14. In plants treated with terbacil at 50 or 100 ppm the decrease in F_v/F_m was proportionate. A 10 to 30% decrease was observed in F_v/F_m, but still significantly below control levels. However, in these plants F_v/F_m had recovered partially 4 days after treatment and had recovered completely by day 6.

There was no apparent effect of plant growth stage on the response of F_v/F_m to terbacil.

The relationships between F_v/Fm and assimilation (A) are given figure 26 and 27 as a regression curve for both growing stages. R^2 values were found 0.52 and 0.53 respectively.

Plant Dry Weight

Plant dry weight expressed in total dry weight and dry weights of three parts of the plants (leaf, crown, root). Leaf dry weight values ranged between 10.33 g and 12.97 g . Crown dry weight values ranged between 5.1 g and 6.58 g and root dry weight value ranged between 3.88 g and 4.38 g. There was no significance difference due to treatment in dry weight based on the separation into different organs. (Figure 28 and 29)

Total dry weight ranged between 19.23 g and 23.71 g. Terbacil had no significant effect on plant dry weight.

Fruit Yield

Plants that were treated with terbacil were harvest in 2001 were harvested in 2002 to assess the carryover effect of terbacil on fruit yield. Terbacil did not have a significant effect on fruit yield in the two years of this study (Figure 30 and 31). Total yield per plant ranged between 55 g and 75 g. Average fruit weight was not affected by the terbacil treatments except at the 400 ppm level, which was 31% less than that of the control (Figure 32 and 33). The average fruit weight ranged between 8.8 and 9.6 g.

Chlorophyll Content

Terbacil reduced chl a and total chlorophyll content of the strawberry leaves. Total chl and chl a content increased in plant treated with terbacil 4 days after application and there was no significant difference after 12 days. Terbacil did not affect chl b and P chl content (Figure 34 and 35).

There was no apparent effect of plant growth stage on the chlorophyll content to terbacil.

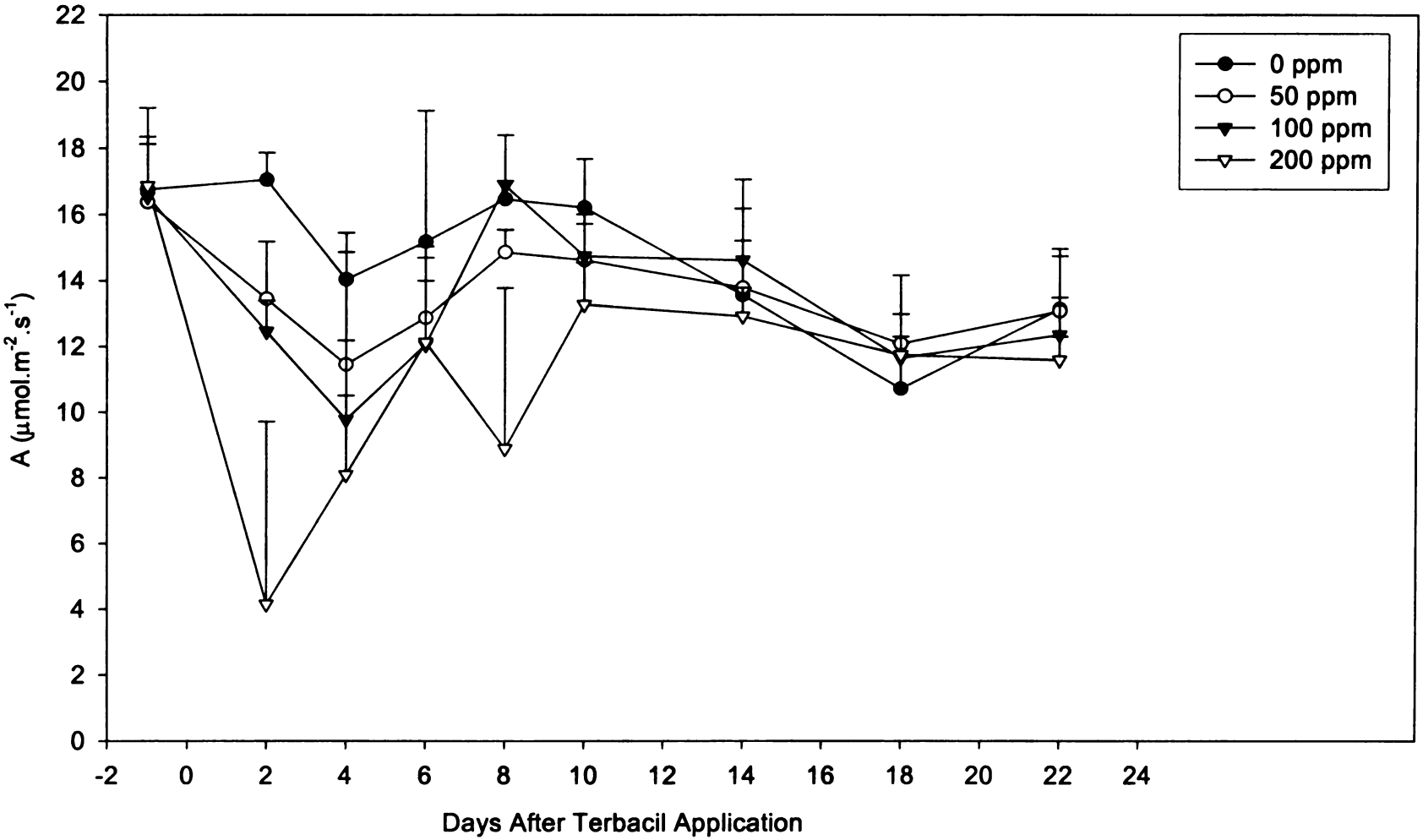

Figure 14. The effect of terbacil application during the fruiting stage on CO_2 assimilation rate (A) in 2002

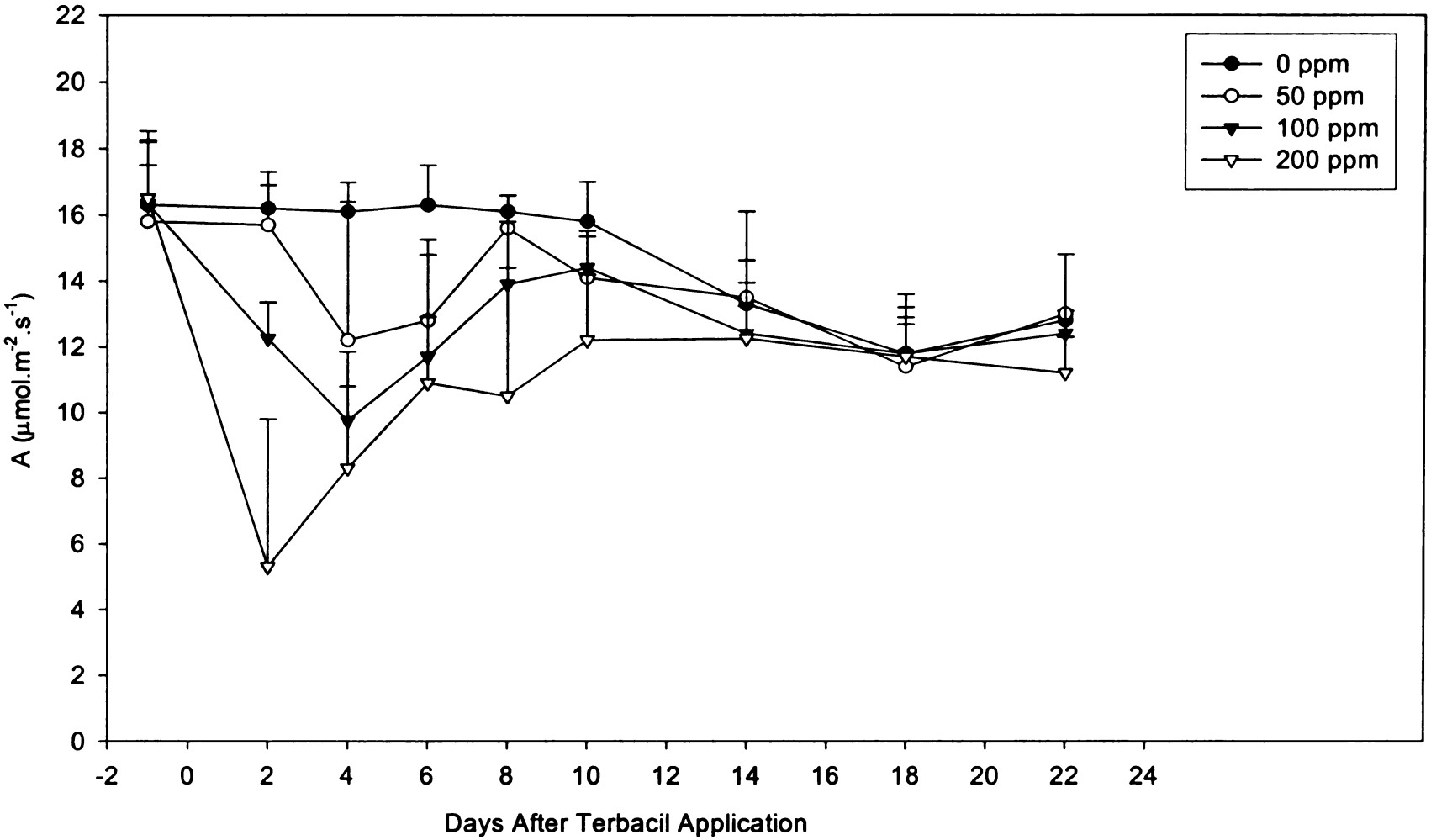

Figure 15. The effect of terbacil on assimilation rate (A) in 2002 growing season (After the harvest stage)

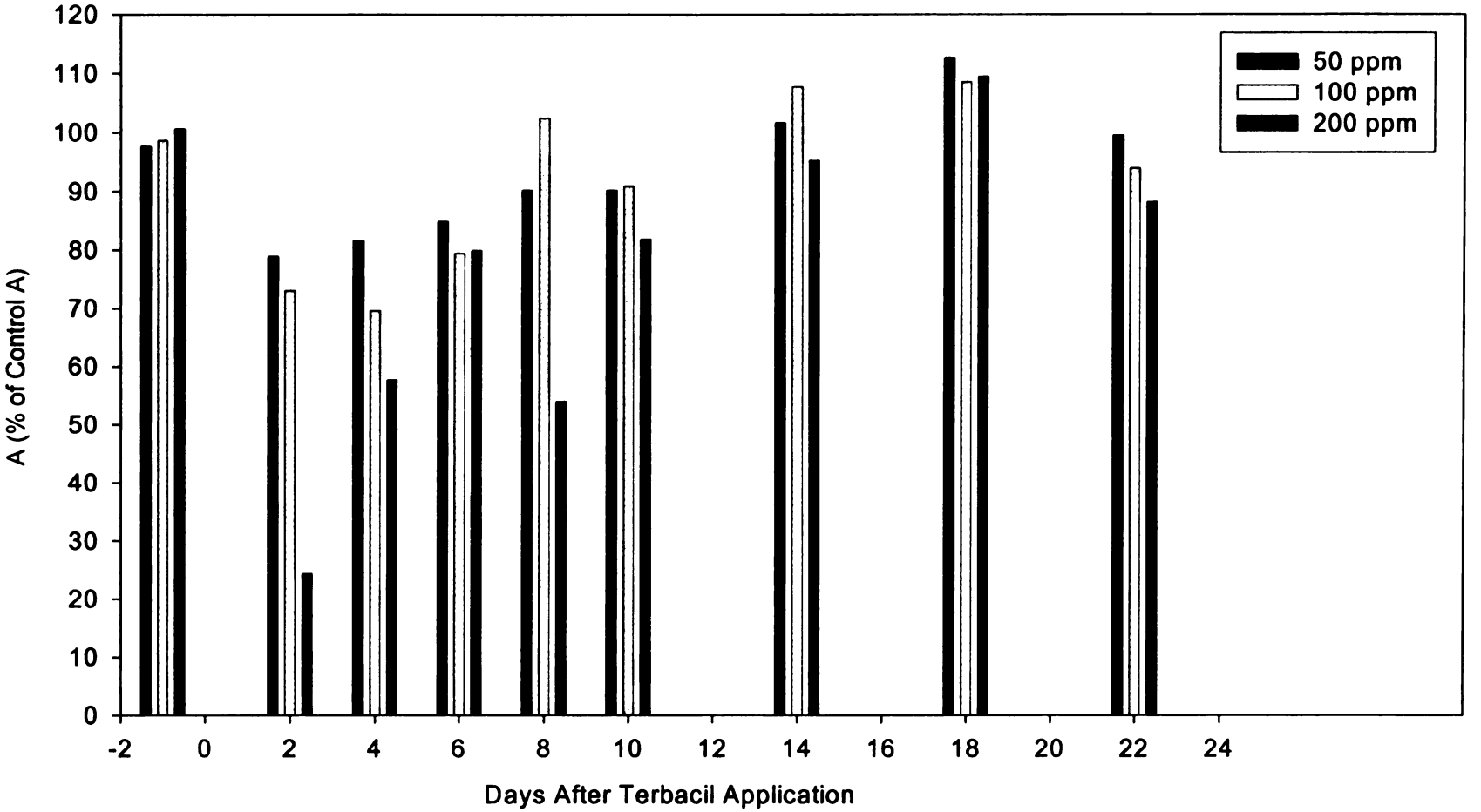

Figure 16. Effect of terbacil application during the fruting stage on assimilation rate (A) expressed as percentage of control in 2002

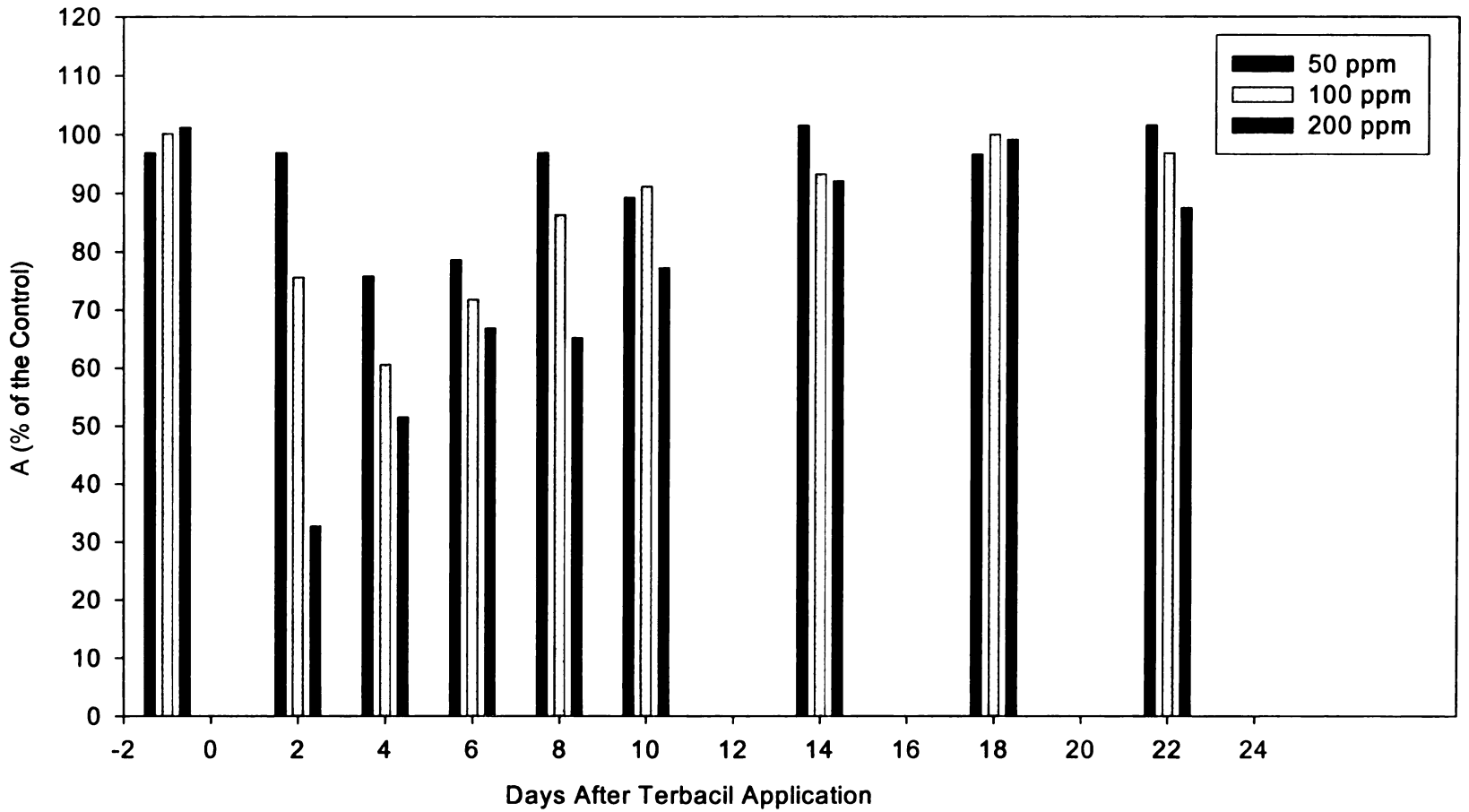

Figure 17. Effect of terbacil application during the after harvest stage on assimilation rate (A) expressed as percentage of control in 2002

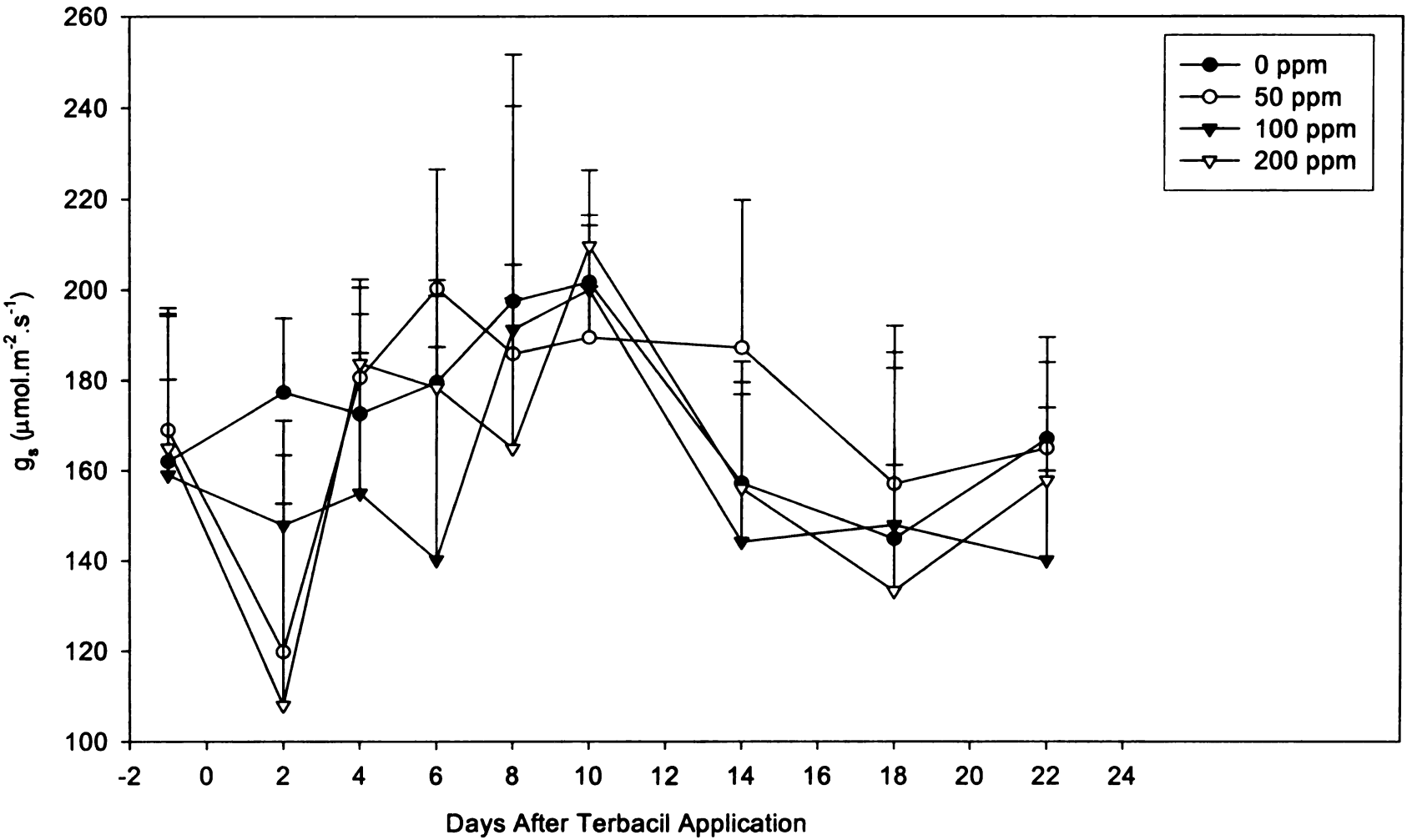

Figure 18. The effect of terbacil application during the fruiting stage on stomatal conductance (g_s) in 2002

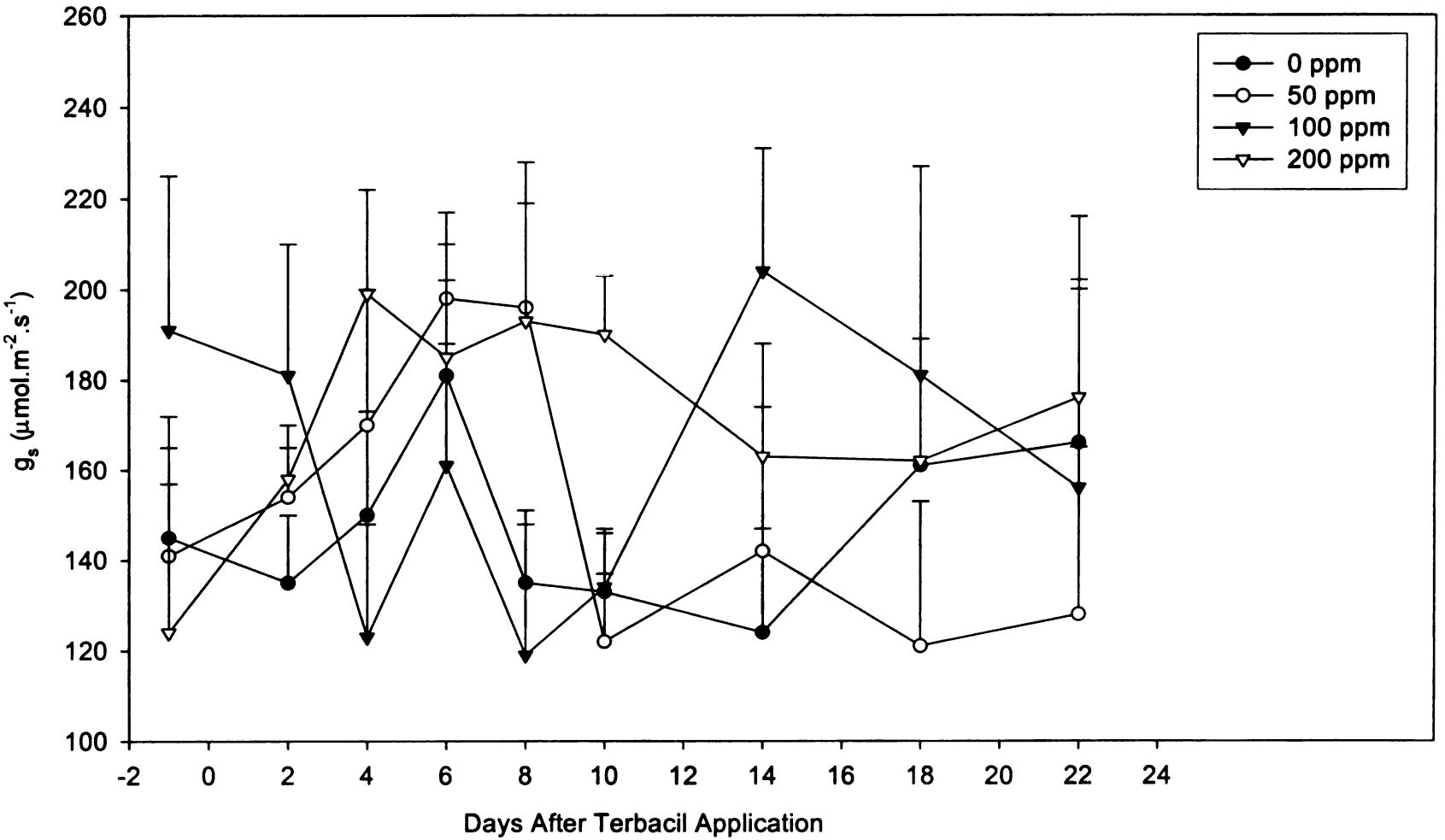

Figure 19. The effect of terbacil application on stomatal conductance (g_s) in 2002 (After harvest stage).

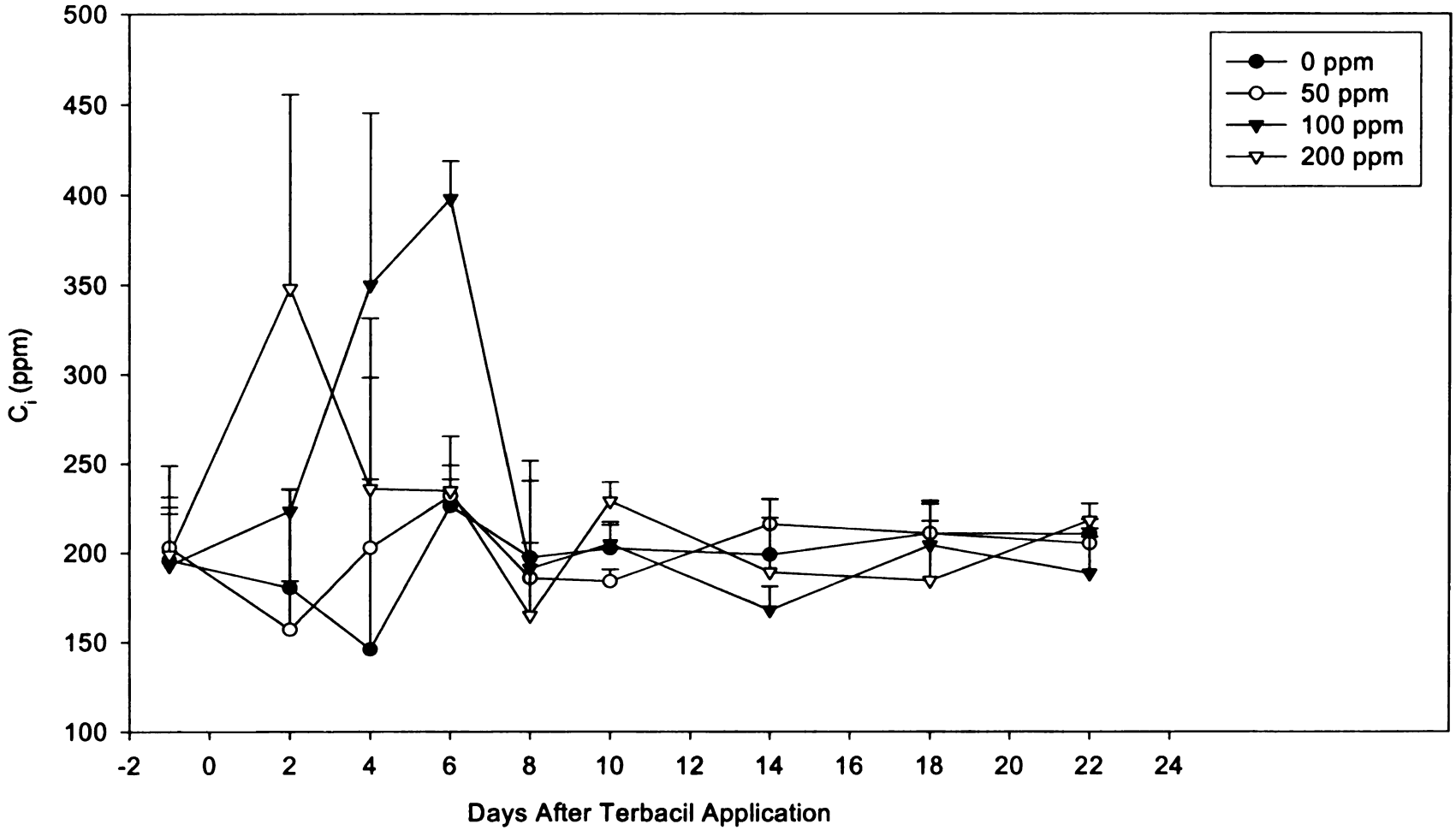

Figure 20. The effect of terbacil on internal CO_2 (C_i) in 2002 (During fruit set stage)

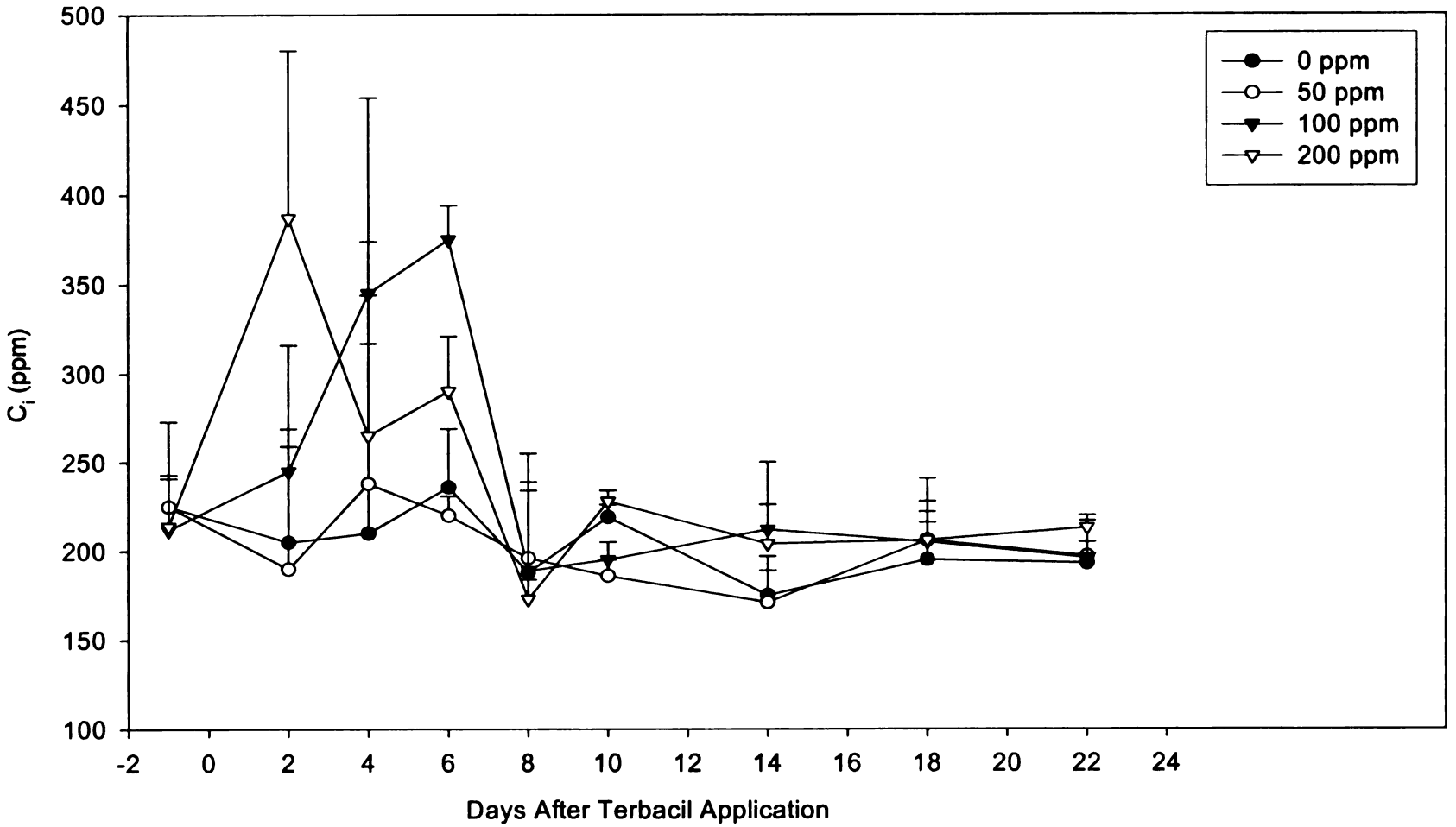

Figure 21. The effect of terbacil on internal CO_2 (C_i) in 2002 (After harvest stage).

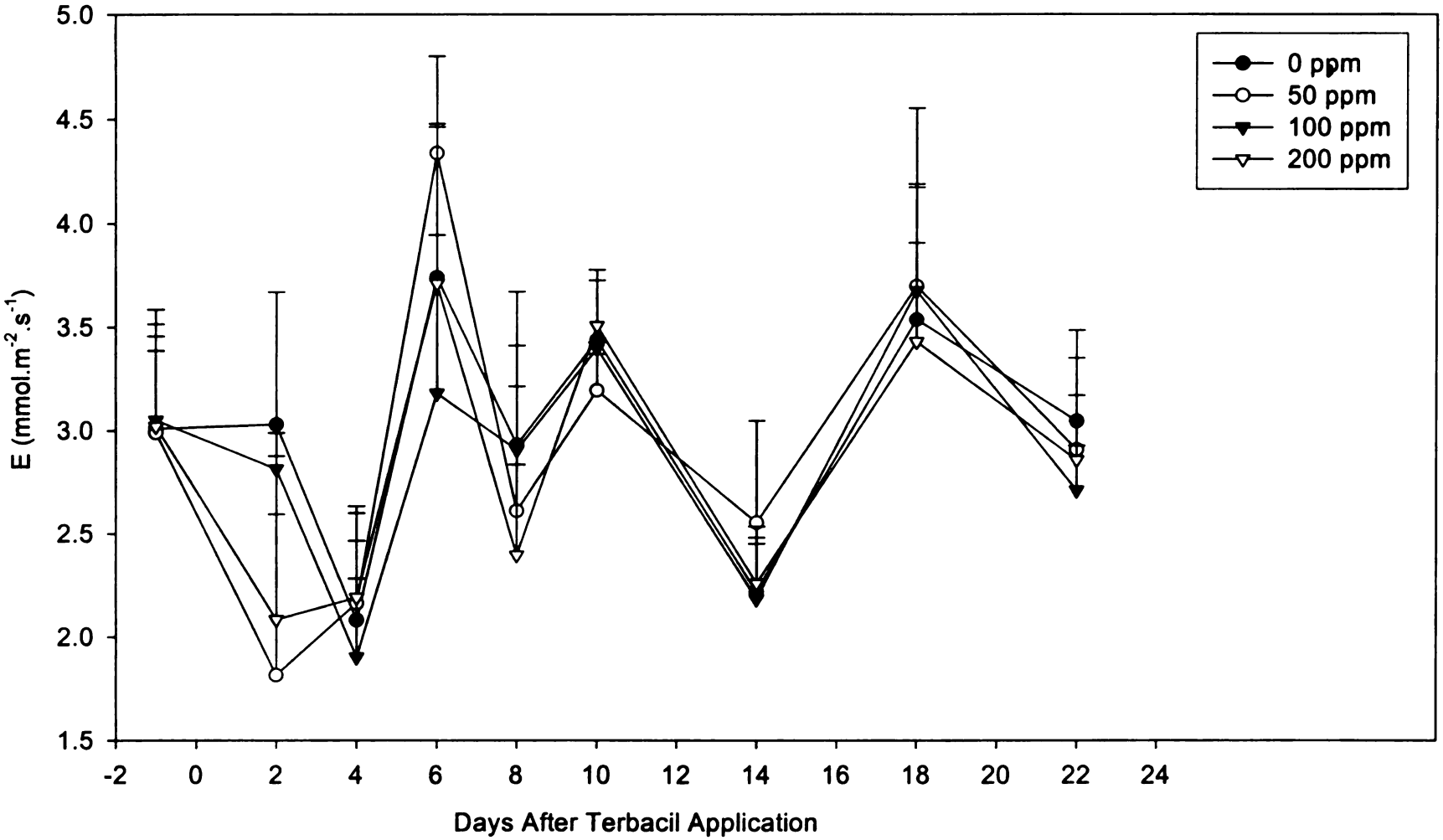

Figure 22. The effect of terbacil on transpiration (E) in 2002 (During fruit set stage)

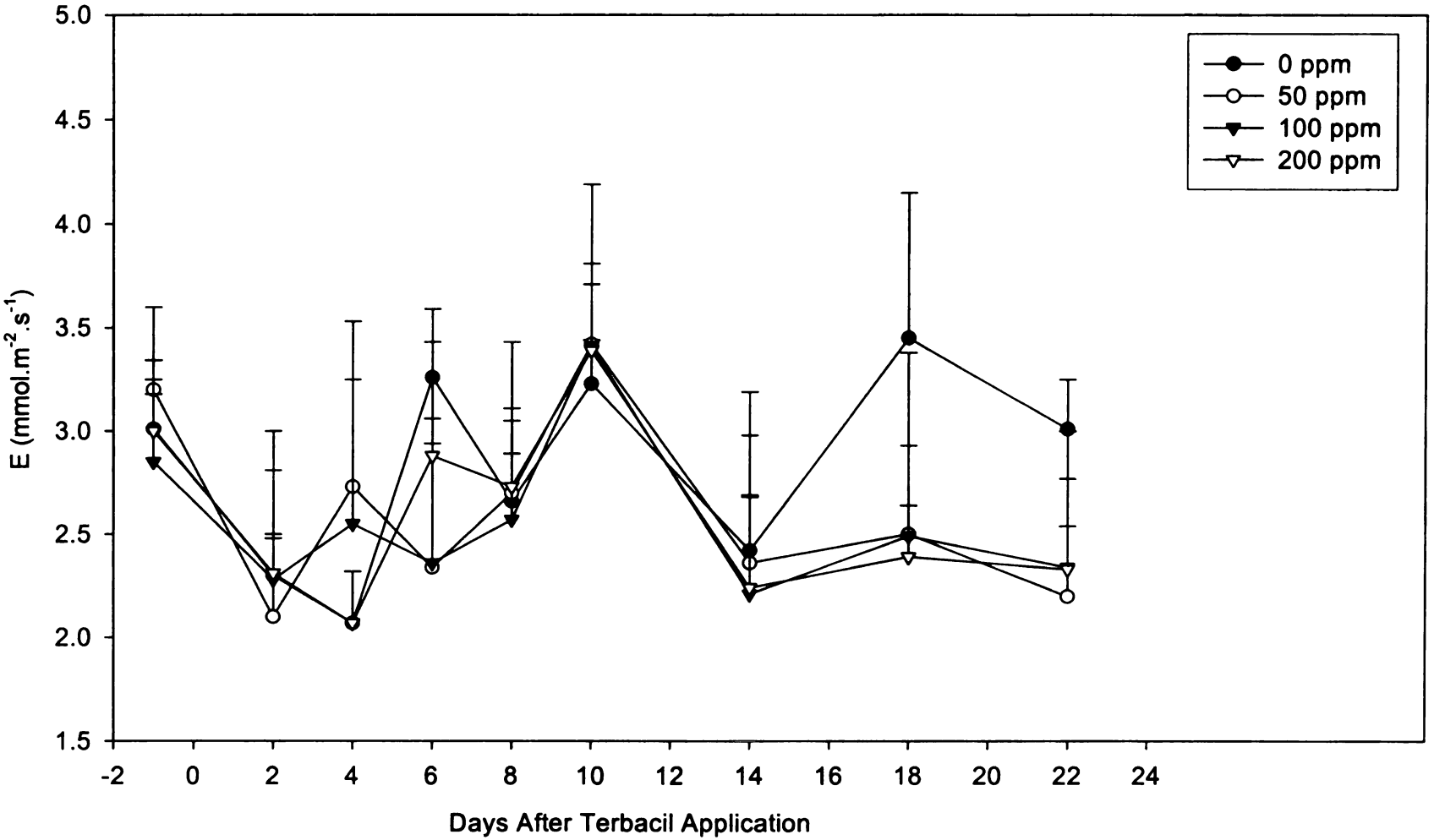

Figure 23. The effect of terbacil on transpiration (E) in 2002 (After harvest stage)

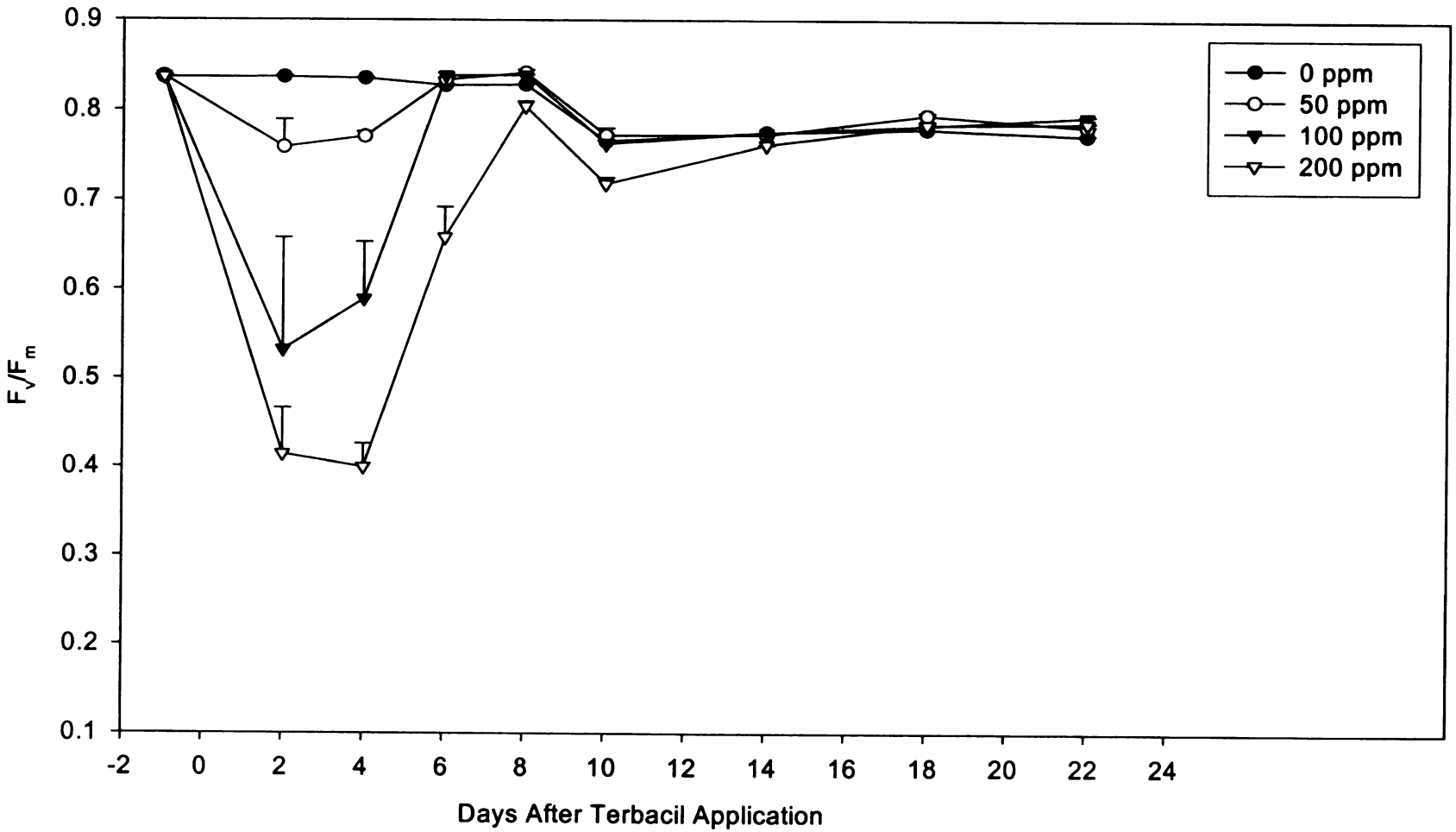

Figure 24. The effect of terbacil on F_v/F_m value in 2002 (During fruit set stage).

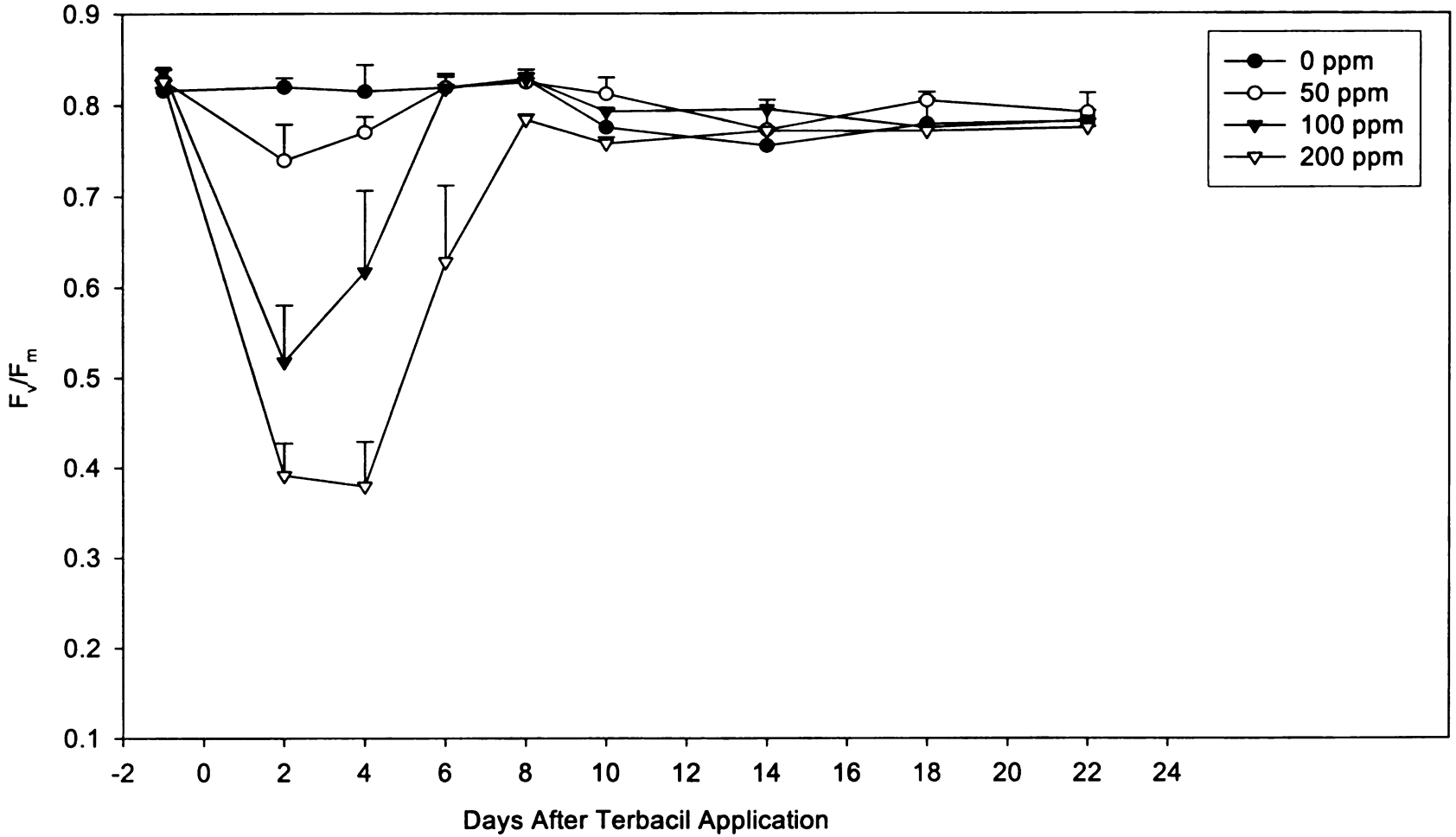

Figure 25. The effect of terbacil on F_v/F_m value in 2002 (After harvest stage).

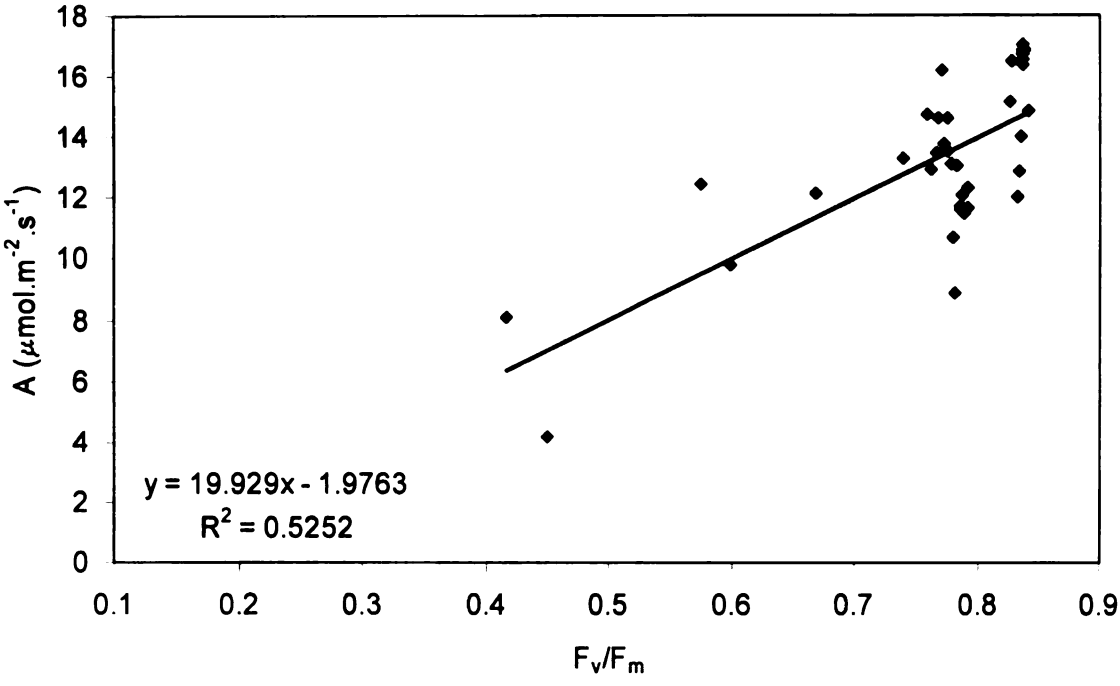

Figure 26. Relationship between F_v/F_m and assimilation in 2002 growing season (during fruit set stage).

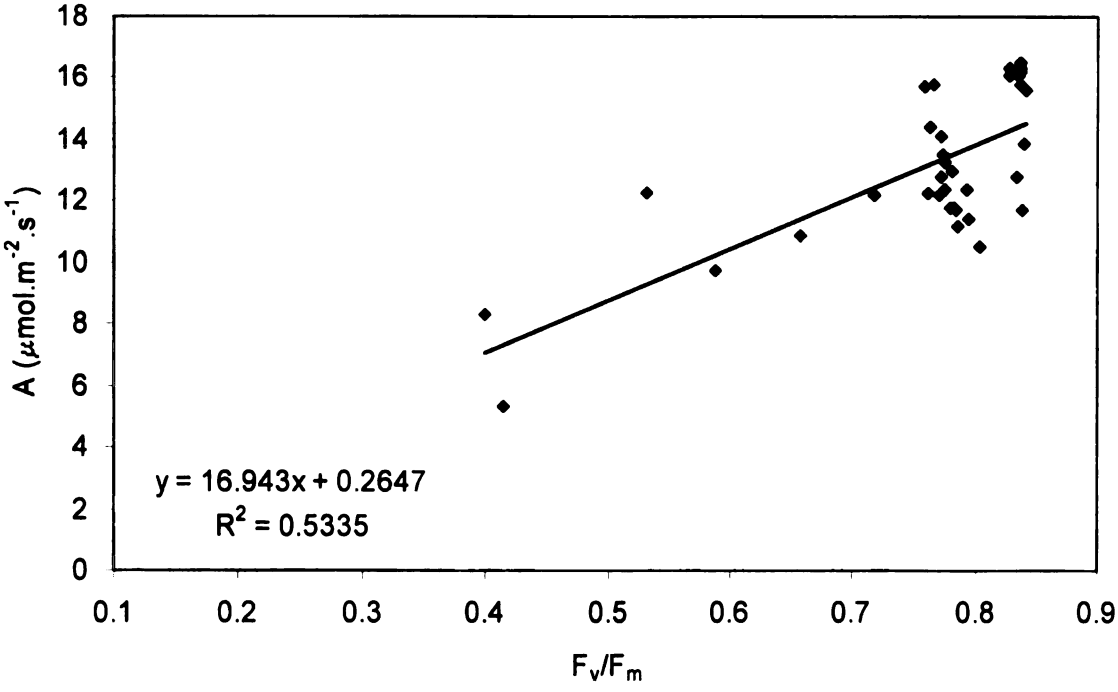

Figure 27. Relationship between F_v/F_m and assimilation in 2002 growing season (after harvest stage).

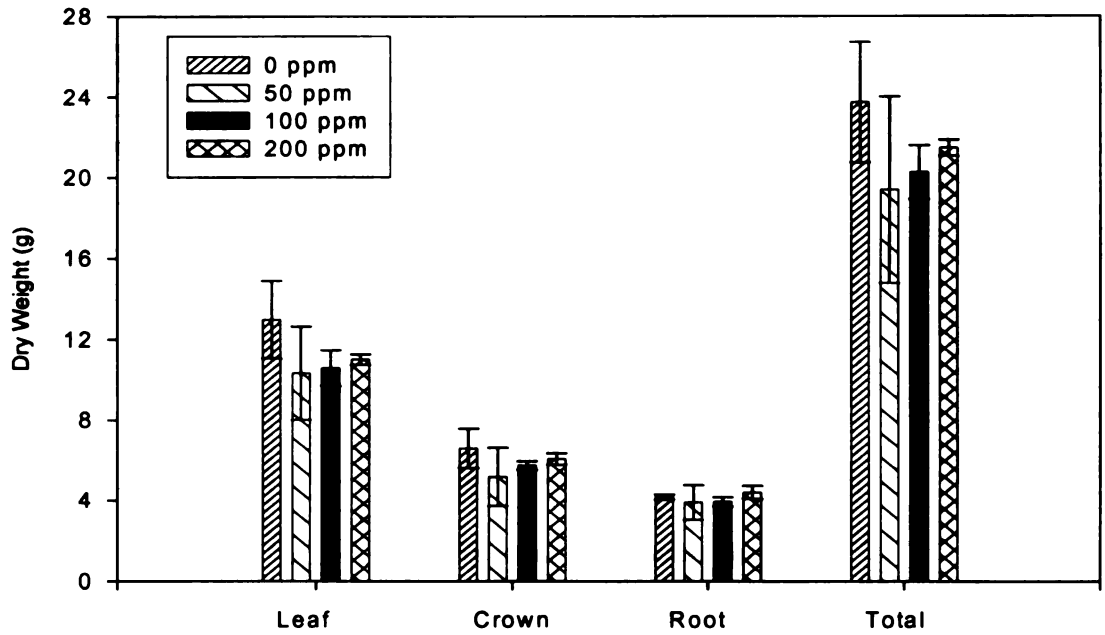

Figure 28. Effect of terbacil on root, crown, leaf and total plant dry weights. (During fruit set stage). Means followed by different letters are significantly different by Duncan's Multiple Range Test (P≤0.05).

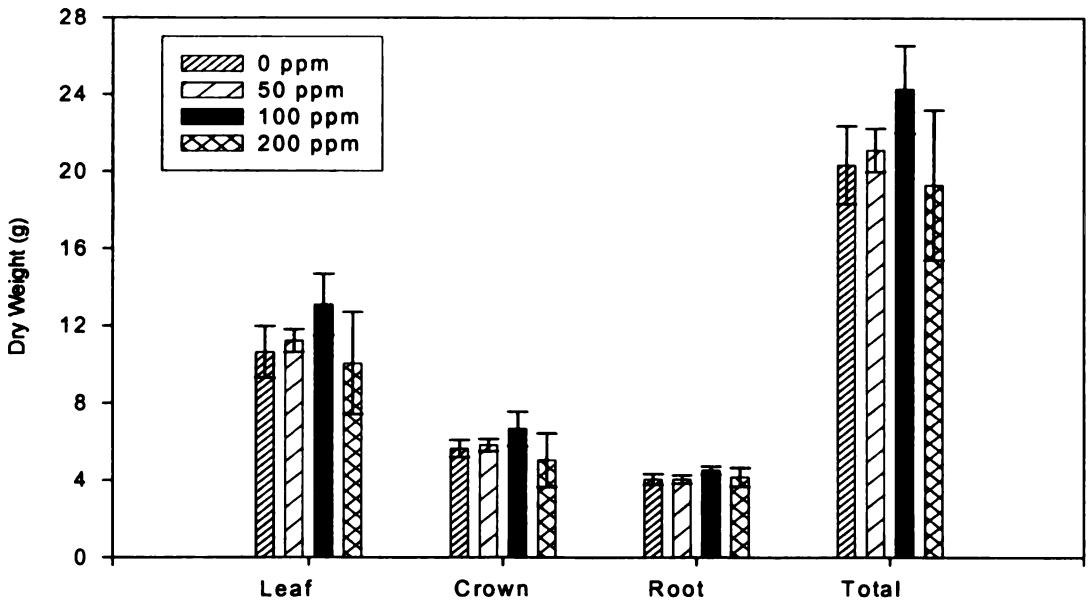

Figure 29. Effect of terbacil on root, crown, leaf and total plant dry weights dry (After harvest stage). Means followed by different letters are significantly different by Duncan's Multiple Range Test (P≤0.05).

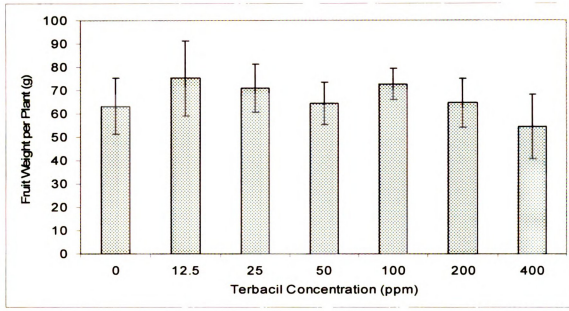

Figure 30. Effect of terbacil on fruit yield (Plants treated in 2001). Means followed by different letters are significantly different by Duncan's Multiple Range Test (P≤0.05).

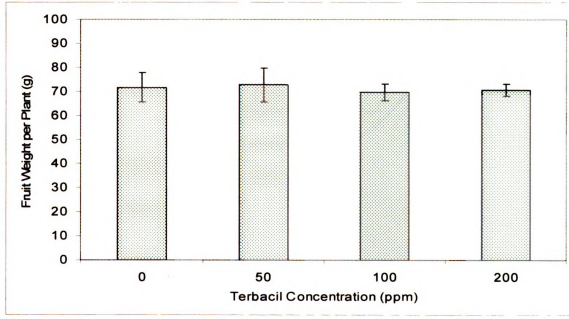

Figure 31. Effect of terbacil on total fruit yield (Plants treated in 2002). Means followed by different letters are significantly different by Duncan's Multiple Range Test (P≤0.05).

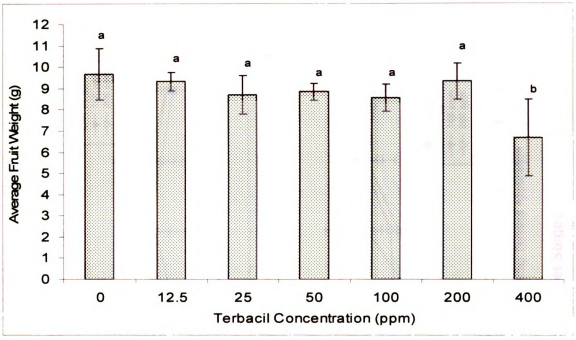

Figure 32. Effect of terbacil on average fruit weight (Plants treated in 2001) . Means followed by different letters are significantly different by Duncan's Multiple Range Test (P≤0.05).

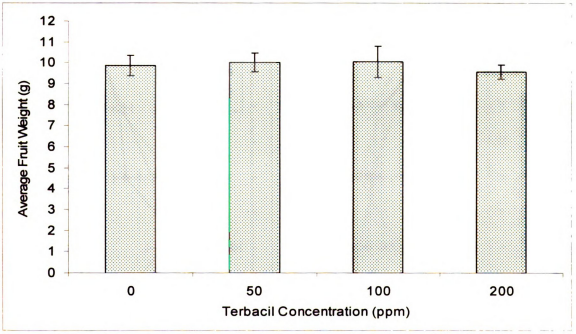

Figure 33. Effect of terbacil on average fruit weight (Plants treated in 2002). Means followed by different letters are significantly different by Duncan's Multiple Range Test (P≤0.05).

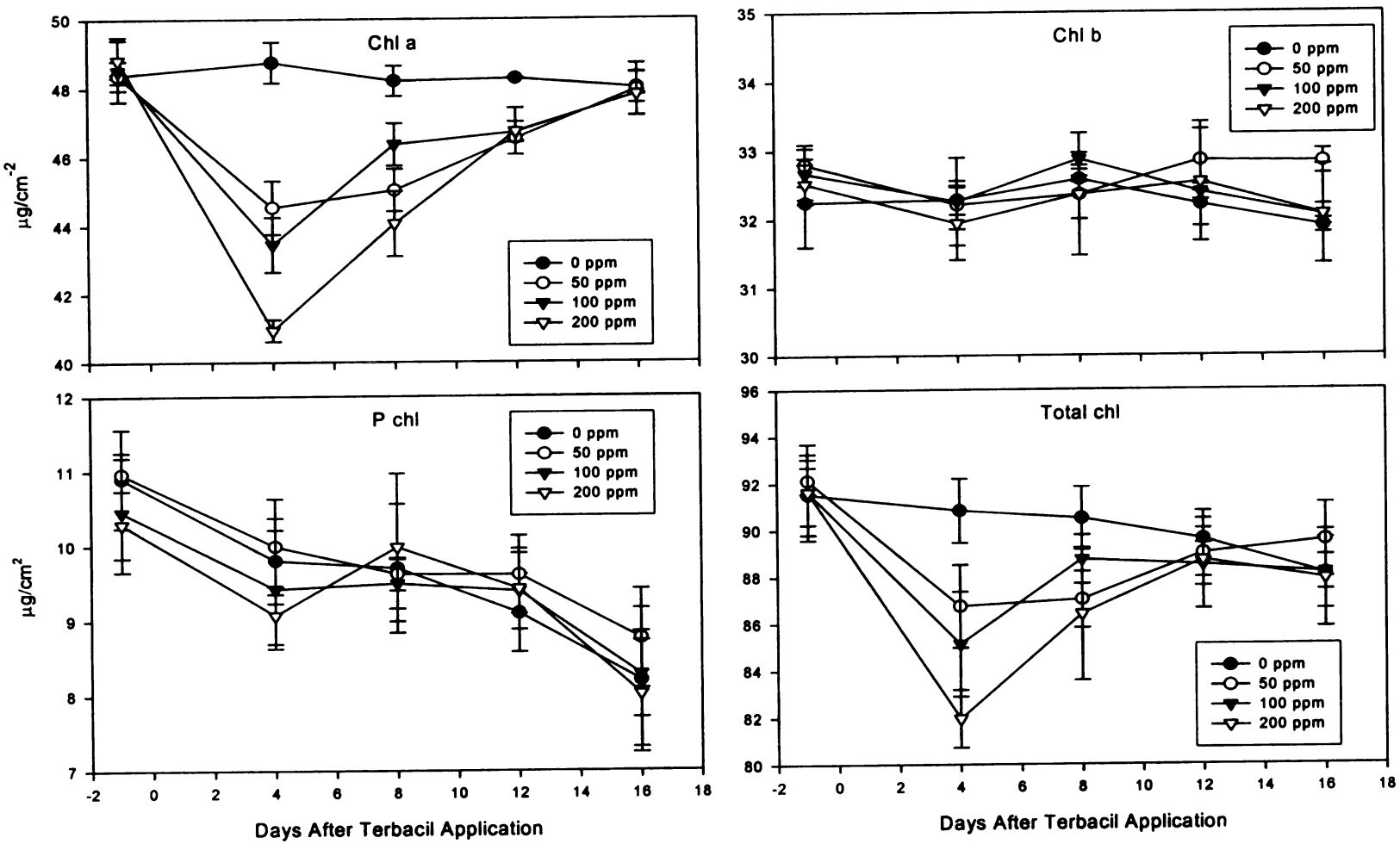

Figure 34. Effect of terbacil on total chl, chl a, chl b and P chl (During Fruit Set Stage)

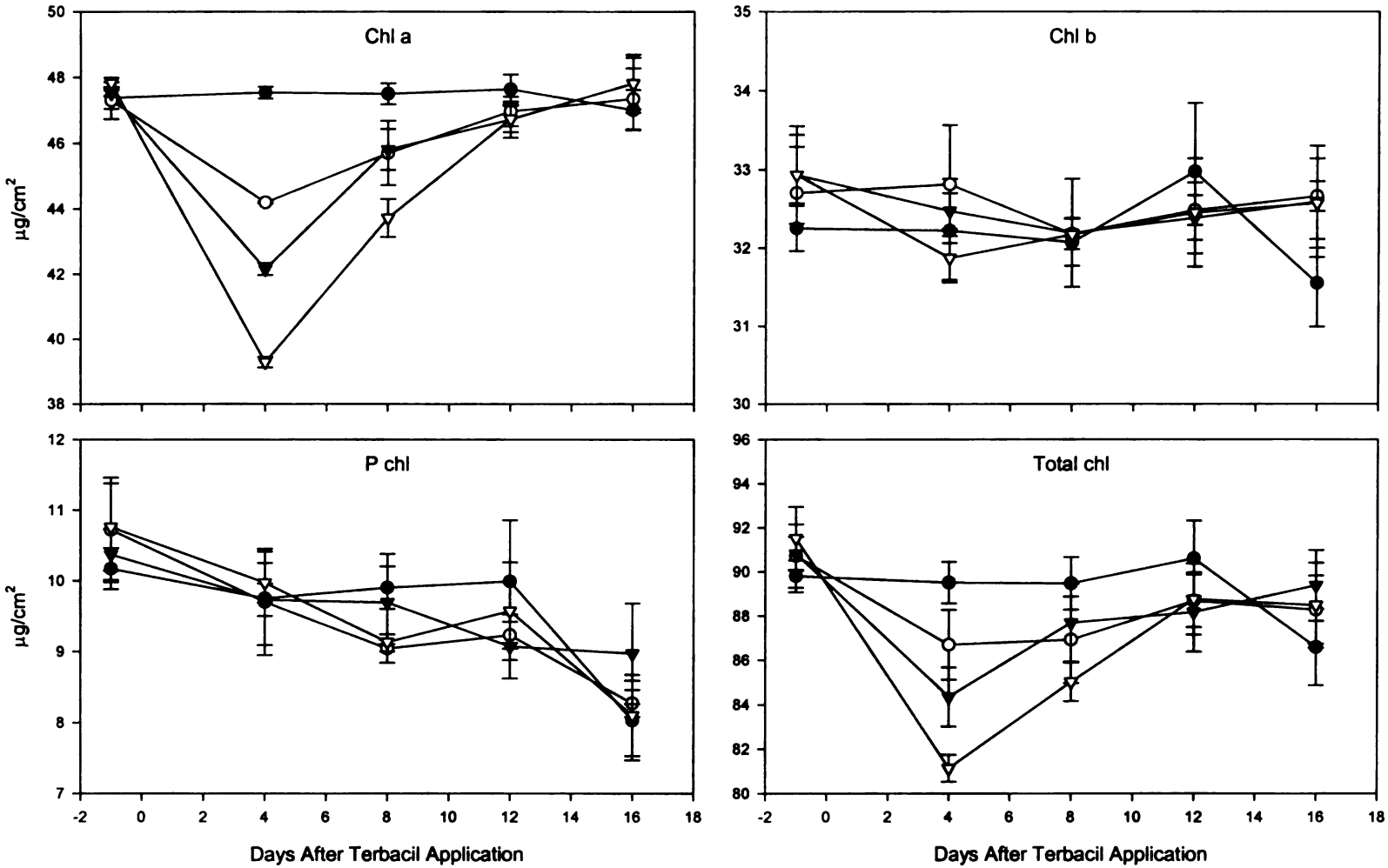

Figure 35. Effect of terbacil on total chl, chl a, chl b and P chl (After Harvest Stage)

Discussion

Terbacil was effective to limit photosynthesis in strawberry plants. Higher concentration of terbacil at 100, 200 and 400 ppm level, limited the photosynthesis in strawberry plants. However, 400 ppm level was toxic and leaves could not recover from the damage of 400 ppm terbacil. 400 ppm terbacil not only damage the photosynthetic apparatus but also it was phytoxic and damaged the leaves at the end of the experiment. However, since strawberry plants continued to form new leaves from the apical meristem of crown, plants survived from the 400 ppm terbacil experiment. This was a recovery mechanism for strawberry plants. Plants treated with 100 and 200 ppm were able to recover from the photosynthetic limitation. Photosynthetic recovery of the plants that were treated with 200 ppm was maintained after 14 days of terbacil application. Twelve and half, 25 and 50 ppm concentrations of terbacil did not limit the photosynthesis in 2001 experiment. In some species concentrations as low as 12.5 ppm terbacil treatment affected the photosynthesis of the plants. Disegna (1994) found that 12.5, 25, 50 and 63 ppm of terbacil may limit the photosynthesis in apple trees. Byers (1990) also found that photosynthesis was inhibited then recovered when terbacil was applied 50 ppm concentration. Catania (1993) reported 100 ppm terbacil treatment limit the photosynthesis in peach trees and photosynthesis recovered within 7-10 days. However, they did not test other concentrations of terbacil. So, these value may not be the threshold values that inhibit the photosynthesis. In this research 100 ppm of terbacil was

the lowest concentration that limits the photosynthesis. This may be due to the different cuticle structure and the epicuticular wax composition as suggested by Baker (1974).

Recovery of photosynthesis occurred between four and ten days after the terbacil treatment in 2001 experiment in plants which were treated with 200 ppm terbacil. Disegna (1994) reported that recovery time for the apple trees was 15 days in trees that treated with 12.5, 25 and 50 ppm terbacil. In peach trees recovery time was 7-10 days when terbacil applied at 100 ppm level. However, in this research treated plants could not sustain their CO_2 assimilation rates after 14 days of terbacil application.

Stages of the development of the plant which were tested in this research did not affect the CO_2 assimilation rates of the plants in 2002 experiments. Plants which were treated during fruit set stage did not show any effect of sink-source competition and their assimilation rates were similar to the plants which were treated after the harvest stage. This indicates that having sinks did not affect the CO_2 assimilation rates. This was consisted with results in cherries (Roper 1988).

Internal CO_2 levels (C_i) were higher in the plants that were treated with 400 ppm and 200 ppm terbacil in 2001 experiment. This indicates that the CO_2 in the plant is not being utilized by photosynthesis. Since photosystem II is inhibited by terbacil higher C_i values were expected as suggested by Moreland (1980).

Stomatal conductance (g_s) and transpiration (E) was not affected from terbacil treatments and they did not show any trend related to the terbacil

treatments. This may indicated that terbacil did not cause any stomatal limitations and any existing inhibition of photosynthesis was related to the inhibition of photo system II. Transpiration values were not affected by the terbacil treatment. Transpiration is affected by temperature and humidity and the weather conditions during measurements may have great effect on E. Even though, the CIRAS-1 has the ability to adjust the humidity and temperature during the measurements. Since, the plants were already acclimated to out side conditions any slight change in the humidity or temperature before or during the measurements may affect actual transpiration rate.

In 2001 experiment, chlorophyll fluorescence (F_v/F_m) measurements indicated the inhibition of photosynthesis in plants which had been treated with 400 ppm. Plants treated with 50, 100 and 200 ppm terbacil had lower values F_v/F_m values than plants treated with 0, 12.5 and 25 ppm terbacil. However, F_v/F_m measurements did not fully reflect the reduction in photosynthesis in the 2001 experiment in which plants treated with 50, 100 and 200 ppm. Regression analysis indicated that correlation between A and F_v/F_m was low (R^2=0.56).

In 2002 experiment F_v/F_m measurements indicated the inhibition of photosynthesis and recovery of the photosynthesis in both growing stages. Lower readings of F_v/F_m values in 2001 may be related with the growing stage of the plants. Measurements in 2001 experiments were performed late in the season August and September. However, measurements in 2001 experiment were performed in June and July. This may be related to thickness of cuticle of the end of the season. In this experiment cuticle thickness were not investigated.

If the cuticle the cuticle thickness is different in plants which were measured in August and September 2001 than plants measured in June and July in 2002, herbicide absorption may be different than each other. Kirwood (1983) and Unrath (1981) found that cuticle of older leaves is less permeable and thicker and this decreases herbicide absorption.

F_v/F_m measurements showed that plants treated with higher concentrations of terbacil recovered later than the plants treated with lower concentrations of terbacil. Plants treated with 200 ppm recovered after 6 days of terbacil treatments. Plants treated with 100 ppm recovered 4 days after terbacil treatments.

Disegna (1994) also found that late treatments of terbacil caused less F_v/F_m reduction in Apple trees.

Byers (1990) and Disegna (1994) found that development stages may affect the CO_2 assimilation response of the plants to the terbacil treatments in Apple and Peach trees. In 2001, CO_2 assimilation rates of plants which were treated with 100 and 200 ppm were higher compared to the plants treated in 2002 with the same concentrations. This may be related to different absorptions of herbicide at different stages.

On the other hand, unlike deciduous trees, strawberry plants continue to form new leaves through out the season. Further research may be needed to investigate the relationship between cuticle thickness and absorption of herbicide and the cuticle thickness of strawberry leaves in different growing stages.

Plants that were treated with 400 ppm in 2001, had low fruit yields when they are harvested in 2002. Since, photosynthesis was inhibited completely 22 days after the treatment. Carbohydrate storage for the following season may be lower and this may affect the next years yield.

In 2002 experiment, yield was not affected from the terbacil treatments in which plants treated were during fruit set stage. This may indicate that when photosynthesis was inhibited there was enough carbohydrate supply to maintain carbohydrate demand to sinks (fruits). Disegna (1994) found that cropping apple trees could not maintain enough carbohydrate to sinks when treated with terbacil. So, fruit yield was lower in apple tress depending on the fruit load. However, since fruit formation time and photosynthetic recovery times were short in strawberry plants, they may have enough carbohydrates stored to compensate the demand for a short time.

Dry matter content was not affected from the terbacil treatments. This was not expected, because photosynthesis was inhibited for a limited time. Photosynthetic recovery occurred 14 days after the treatment for all of the concentrations. Dry matter contents were not significant when crown, leaf and roots were compared individually. This indicates that treatments did not cause any alteration to allocation of carbohydrates due to the terbacil treatments. Designa (1994) reported that 20 days of reduction of photosynthesis due to the terbacil did not alter wood carbohydrate storage in apple trees. This was also the case in strawberry considering the main carbohydrate storage organ are crowns, there were significant difference in dry matter content of the crowns.

Total chlorophyll content was affected from the treatment and temporary chl a reduction occurred. This was consisted with results of Izawa (1965) and Disegna (1994). An incerase in chl a followed the reduction of chl a and total chl indicating that chl a generated in the recovery process.

Terbacil was useful to study the damage thresholds in strawberry plants. It has advantages to simulate effects of disease and insects. Using insects and disease infestation have difficulties and limitations. Such research may require lots of time, insect or disease material. This usually increases the cost. Terbacil application is easy and cheap. So terbacil can be applied to simulated such disease and insects. Damage can be regulated by using different concentrations of terbacil.

Use of terbacil as a herbicide is common and this research was useful to determine to figure out the threshold levels for strawberry. However, since terbacil is applied on area basis to soil. This threshold levels may vary depending on soil type and conditions. Genotype is also an important factor that may affect the threshold levels.

LITERATURE CITED

Ashton, F.M. , O.T. De Villiers, R.K. Glenn, W.B. Duke, 1977. Localization of metabolic sites of action of herbicides. Pestic. Biochem. Physiol. 7:122-124.

Byers, R.E., J.A. Barden, R.F. Polomski, R.W. Young, D.H. Carbaugh. 1990. Apple thinning by photosynthetic inhibition. J. Amer. Soc. Hort. Sci. 115:14-19.

Catania, M., J.A. Flore. 1993. When does photosynthesis limit yield of peach?. Hortscience Abstr. 28(5):121. (Abstr: 238).

Disegna, E.J. 1994. The use terbacil as a tool to establish a photosynthetic threshold in apples. Michigan State Univ., East Lansing, Msc thesis.

Gucci, R., J.A. Flore. 1990. Net Photosynthesis and non-structural carbohydrates in leaves of field-grown plum trees following fruit removal. In: 3rd International meeting on "Regulation of photosynthesis in fruit crops". Perigia. Italy. P.127.

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

Howell, G.S., S.S. Stackhouse. 1973. The effect of defoliation time on acclimation and dehardining in tart cherry (*Prunus cerasus* L.) J. Amer. Soc. Hort. Sci. 98: 132-136.

Izawa, S., S.S. Stackhouse. 1965. The number of sites sensitive to 3 – (3,4 – dichlorophenly) – 1, 1 – dimethylurea, 3 (4 – chlorophenly) -1, 1-dimentylurea and 2 – chloro – 4 – (2 – propylamino) 6 – ethylamino-s-triazine in isolated chloroplasts. Acta 102:20-26.

Krause, G.H., E. Weis 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth-Res. 5(2): 139-157.

Kirkwood, D.A. 1983. The mode of action of herbicides. In: Recent advances in weed research. W.W. Fletcher (ed.) 227-257.

Layne, D. 1989. Damage thresholds for simulated trunk and leaf injury in 'Montmorency' sour cherry. Ms. Thesis. Michigan State University, East Lansing, MI.

Mahr, D.L., T.R. Roper, P.S. McManus, B.R. Smith, R.A. Flashinski. 2002. Strawberry and raspberry pest management in Wisconsin. University of Wisconsin Extension Bulletin A1934.

Moran, R. 1982. Formulae for determination of chlorophyllous pigments extracted with N,N- Dimethylformamide. Plant Physiol. 69:1376-1381.

Moreland , D.E. 1980. Mechanisms of action of herbicides. Ann. Rev. Plant Physiol. 31:597-638.

Roper, T.R., J.D. Keller, W.H. Loescher, C.R. Rom,1988. Photosynthesis and carbohydrate partitioning in sweet cherries: fruit effects. Plant Physiology. 72:42-47.

Shaw, M. 1956. The physiology of host parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. Canadian Journal of Botany 34:(389-405).

Unrath, C.R., 1981. An overview of environmental factors affecting orchard growth regulator response with special reference to apple. Acta Hort. 120:43-52.

Wareing, P.F., M.M. Khalifa, K.J. Treharne. 1968. Rate limiting process in photosynthesis at saturating light intensities. Nature 220:453-457.

SUMMARY AND CONCLUSIONS

Use of terbacil was more practical than mechanical damage determine the threshold levels in strawberry plants. Use of terbacil was easy and it was possible to test many different of damage levels. Simulated leaf injury is time consuming to study the damage thresholds.

Strawberry leaves which were damaged mechanically could not recover photosynthetically due to a lack of compensation by carboxylation. Increasing damage limited the carboxylation efficiency. It may also be possible that even the 10% damage level was too severe for strawberry leaves to recover. Under severe damage strawberry plants may compensate to damage by simply forming new leaves instead of increasing photosynthetic rate. However, there are certain difficulties to calculate the changing area canopy. In order to calculate net photosynthesis whole plant leaf area must be calculated precisely. On the other hand aging of the older leaves should also be considered. Aging effect may hide any photosynthetic compensation in younger leaves. Thus, further research is needed to study whole plant photosynthesis of strawberry plants and how it affects the yield and dry weight of the strawberry plants.

Since, the yield and dry weight values were not affected by terbacil 200 ppm concentration, we may consider that the threshold level for strawberry plants were greater than that caused by this level of damage. Terbacil used between 100 and 200 ppm concentration found to be useful to study the threshold level of

strawberry plants. However, these concentrations may be different depending on the cultivar used.

APPENDIXES

Appendix 1. Daily weather data for the period that measurements were performed in year 2001.

Date	Maximun Temperature (C)	Minimum Temperature (C)	Precipitation (mm)	Solar Flux (KJ/m^2)
August 28, 2001	26.2	13.4	0.76	22974
August 29, 2001	27.2	10.2		23009
August 30, 2001	29.2	13.2		19278
August 31, 2001	25.4	12.9		15641
September 1, 2001	22.1	6.4		23691
September 2, 2001	25.2	6.8		21957
September 3, 2001	28.2	12.8		20933
September 4, 2001	23	10.8		21925
September 5, 2001	25	7.5		22403
September 6, 2001	27.8	8.7		18923
September 7, 2001	30.6	18	3.56	15917
September 8, 2001	28.5	18.7	10.16	11465
September 9, 2001	25.4	15.4	17.53	10954
September 10, 2001	22.9	12.3		20136
September 11, 2001	23.9	9.4		21058
September 12, 2001	26.9	10.2		20877
September 13, 2001	18.7	8.1	2.03	9472.2
September 14, 2001	17.7	4.8		16639
September 15, 2001	18.8	5		15801
September 16, 2001	22	4.8		20273
September 17, 2001	22.1	7		11716
September 18, 2001	23.6	10.6		12956
September 19, 2001	19.8	14.5	23.11	3289.8
September 20, 2001	19.1	12.2	3.81	9552.6

Appendix 2. Daily weather data for the period that measurements were performed in year 2002 (During fruit set stage).

Date	Maximum Temperature (C)	Minimum Temperature (C)	Precipitation (mm)	Solar Flux (KJ/m^2)
June 21, 2002	27.9	20.1		12375
June 22, 2002	32	20		22295
June 23, 2002	32.1	18.7		23130
June 24, 2002	32.6	18.2		24344
June 25, 2002	33.4	16.7		21726
June 26, 2002	29.1	20.8	0.76	20549
June 27, 2002	27.3	20.4		18550
June 28, 2002	29.1	13.4		27514
June 29, 2002	31.1	13.8		26475
June 30, 2002	32.2	16.8		22556
July 1, 2002	33.6	19.9		25224
July 2, 2002	32.9	19.4		27532
July 3, 2002	33.5	18.7		26180
July 4, 2002	32.6	21.2		25578
July 5, 2002	24.8	15		24839
July 6, 2002	27.8	10.7		24921
July 7, 2002	31.8	12.5		27891
July 8, 2002	32.7	15.2	5.08	23389
July 9, 2002	27.1	19.4	28.96	10679
July 10, 2002	24.2	15.5		27176
July 11, 2002	25.2	10.2		26343
July 12, 2002	26.7	8.4		29078
July 13, 2002	30.2	9.7		27989
July 14, 2002	29.5	11.7		28486
July 15, 2002	31.3	12.9		24021

Appendix 3. Daily weather data for the period that measurements were performed in year 2002 (After harvest stage).

Date	Maximum Temperature (C)	Minimum Temperature (C)	Precipitation (mm)	Solar Flux (KJ/m^2)
July 16, 2002	32.4	18.6		25810
July 17, 2002	30.4	17.9		24379
July 18, 2002	29.5	18.1	8.13	15258
July 19, 2002	28.5	17		19579
July 20, 2002	30	14.5		26749
July 21, 2002	32.9	18.9	0.25	15258
July 22, 2002	33.5	21.9	4.32	20035
July 23, 2002	23.5	13.9		28118
July 24, 2002	25.6	9.4		27153
July 25, 2002	26	12.6		20401
July 26, 2002	29.5	17.8	17.02	21276
July 27, 2002	27.6	18.1	1.52	12205
July 28, 2002	28.6	20.9	9.91	10968
July 29, 2002	30.2	20.8	19.81	13970
July 30, 2002	29.8	19.1		26690
July 31, 2002	32.2	19.1		26388
August 1, 2002	31.2	19		21983
August 2, 2002	27.5	16.2	1.52	25501
August 3, 2002	30.4	13.4		26328
August 4, 2002	31.1	19.7	0.25	15108
August 5, 2002	27.6	18.7	0.25	18739
August 6, 2002	21.8	11.7		25927
August 7, 2002	23.9	8		23930
August 8, 2002	25.5	8.1		25246