This is to certify that the
thesis entitled

MANIPULATING GAS EXCHANGE RATES OF KENTUCKY BLUEGRASS (POA PRATENSIS L.) USING SURFACTANTS AND FUNGICIDES

presented by

DANE R. WILLIAMSON

has been accepted towards fulfillment
of the requirements for the

MS degree in CROP & SOIL SCIENCES

Major Professor's Signature

July 20, 2009

Date

MANIPULATING GAS EXCHANGE RATES OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) USING SURFACTANTS AND FUNGICIDES

By

Dane R. Williamson

A THESIS

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

MASTER OF SCIENCE

Department of Crop and Soil Science

1983

ABSTRACT

MANIPULATING GAS EXCHANGE RATES OF KENTUCKY BLUEGRASS (*POA PRATENSIS L.*) USING SURFACTANTS AND FUNGICIDES

By

Dane R. Williamson

Cultures of Kentucky bluegrass were used to measure the effects on gas exchange of various chemicals commonly used on turfgrass. The surfactants Aqua-Gro and Hydro-Wet or the fungicides benomyl (Tersan 1991), iprodione (Chipco 26019), triadimefon (Bayleton) and CGA 64251 were applied as soil drench treatments at various concentrations. The gas exchange rates were measured on the second youngest fully expanded leaf with an open infrared gas analysis system. Benomyl and Hydro-Wet applied at 500 ug ml^{-1} biweekly reduced stomatal and epidermal cells per unit area after three weeks. This reduced gas exchange by a similar amount. A single application of benomyl at field rates did not alter gas exchange. Aqua-Gro and iprodione applied biweekly at 500 ug ml^{-1} slightly reduced gas exchange, but did not significantly reduce cell number. CGA 64251 increased CO_2 assimilation and improved the transpiration/assimilation ratio at a single 500 ug ml^{-1} application. Triadimefon did not alter gas exchange rates from the control.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER 2 (continued)

CHAPTER 1

CHEMICAL MANIPULATION OF STOMATAL NUMBER AND BEHAVIOR IN MERION KENTUCKY BLUEGRASS (*POA PRATENSIS* L.)

ABSTRACT

Turfgrass swards have been over-watered in some areas to supply needed amounts to other areas. Chemical manipulation of plant water consumption would help reduce irrigation needs and provide a more uniform sward during hot, dry periods. Cultures of Merion Kentucky bluegrass (*Poa pratensis* L.) were treated bi-weekly for three weeks with a full nutrient solution containing 500 ug ml^{-1} of the surfactant Aqua-Gro (50% polyoxyethylene ester and 50% polyoxyethylene ether) or Hydro-Wet or the fungicides Benomyl (Tersan 1991, methyl-N- (1-butylcarbamoyl)-2-benzimidazole) carbamate) or Iprodione (Chipco 26019, 3-(3,5-dichloropenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide). Leaves were analyzed for stomatal number, stomatal conductance, photosynthesis, and transpiration. Hydro-Wet and benomyl significantly reduced stomatal and epidermal cell numbers per unit area. The ratio of stomata to epidermal cells was also significantly reduced. Photosynthesis and transpiration were reduced by a similar percentage. Aqua-Gro and iprodione did not alter stomatal number, but did reduce transpiration rates. The data pro-

vided evidence that surfactants and systemic fungicides may be translocated from soil into turfgrass plants where micro-morphological development and physiological processes were altered.

INTRODUCTION

Dry hot periods during the summer in the cool-humid region of the United States present problems to practically all turfgrass areas. Irrigation water has been available in sufficient quantitiy to allow over-watering some areas in order to supply needed amounts to other areas. Problems, often associated with traffic, occur in over-watered areas.

A number of golf superintendents have suggested that where systemic fungicides such as benomyl have been used on fairways for anthracnose control, less irrigation was needed for turfgrass survival (private communications). Other superintendents have reported that wetting agents have been instrumental in water management, thus reducing traffic problems associated with over-watering (B. Williams, 1980). However, most of the attributes cited for wetting agents are associated with improved mobility of water and pesticides in the soil (Huggenberger et al., 1973) and improved wetting of hydrophobic soils (Rieke, 1975).

The idea of chemically reducing water consumption is not new. Antitranspirants that coat the leaves with a chemical layer impervious to water vapor have been used

successfully on woody species for some time (Comar and Barr, 1944). However, because of mowing and traffic, these layers do not persist long on actively growing turfgrasses (Beard, 1973). Also, while they persist, water vapor cannot escape causing leaf temperatures to rise on hot days (Tanner, 1963), often to the point where damage might occur. Carbon dioxide exchange also appears to be limited, resulting in a greater reduction in photosynthesis than transpiration (Woolley, 1967).

Chemicals which translocate throughout a plant may reduce water consumption by altering the physiology of the plant. Surfactants were shown to be toxic to turfgrasses growing in solution cultures, but were not toxic in soil solutions where absorption apparently binds the surfactant to the soil (Endo et al., 1969). The author suggests root toxicity causes the injury, but does not rule out systemic action in the plant. Systemic fungicides are known to translocate throughout the plant. Several of these fungicides have a chemistry similar to kinetin, and have been observed to have kinetin-like activity (Thomas, 1974). Thus, these chemicals may affect cell division in developing turfgrass plants. The objective of this investigation was to determine the effect of two surfactants and two systemic fungicides on transpiration, photosynthesis and stomatal number of Merion Kentucky bluegrass leaves.

MATERIALS AND METHODS

Mature Kentucky bluegrass, cultivar Merion, was obtained

from the Michigan State University Experimental Field Laboratory in October, 1980 and acclimated in 0.3 L styrofoam containers in the greenhouse for two weeks. A Hoagland's nutrient solution was supplied two times per week in the check cultures while treatments included the addition of 500 ug ml^{-1} of Aqua-Gro, Hydro-Wet, benomyl, or iprodione. These treatments continued biweekly for three weeks. Even though the application rate was excessive compared to field application, no phytotoxicity was observed throughout and for three weeks following the study.

For gas exchange measurements, the second youngest fully expanded leaf of a tiller was kept intact and placed into a waterjacketed aluminum chamber that had a Plexiglas window to admit light. The chamber allowed an air stream to pass over 1.19 cm^2 area of leaf tissue. There was a separate air stream for the upper and lower surface of the leaf, each with a flow rate of 50 L hr^{-1}.

The air stream was humidified and then passed through a glass condenser in a water bath kept at 18 C to keep the dew point of the air constant. Also, the air was passed through two soda lime towers, after which CO_2 was added to give the desired CO_2 concentration. An infrared gas analyzer (URAS 2, Hartmann & Braun, Frankfort A.M., W. Germany) was used to monitor the CO_2 concentration. The gas exchange of CO_2 and H_2O for both the upper and lower leaf surfaces were measured with four additional gas analyzers used as

differential analyzers to increase the sensitivity. The temperature of the leaf was measured with a copper-constantan microthermocouple pressed against the non-illuminated side. Throughout all analyses the temperature and the water vapor pressure deficit across the leaf were held at 25 C $\pm$ 0.5 and 15.0 ml L^{-1} $\pm$ 0.5, respectively.

White light was provided by an Osram XBF 6000 W water cooled xenon arc lamp shining through a Corning No. 4600 infrared-absorbing glass filter. The irradiance was reduced with neutral density Plexiglas filters (No. 800 and 838, Rohm and Hass, Darmstradt, Germany). Irradiance was monitored with a calibrated silicon cell placed in the same plane as the leaf chambers. Irradiance was controlled at 130 W m^{-2} throughout the investigation. Assimilation and transpiration rates, stomatal conductance, and intercellular CO_2 concentration were calculated by computer.

The figures are fourth level multiple regression analysis curves of stomatal aperture and gas exchange monitored every 2 minutes for the first 30 minutes after irradiance was initiated. Each quartic curve was based on three replications for a total of 45 observation points.

For stomatal and epidermal cell counts, a 1 cm section of transparent mending tape was fixed to the second youngest fully expanded leaf one-third the distance from the ligule to the tip. Clear nail polish was painted on a small portion of the adjacent 2 cm portion of the leaf and allowed to dry for 10 minutes. The tape and 2 cm section of dry polish was

pulled gently from the leaf, placed on a slide and viewed under a photographing microscope at 100X. The leaf imprint was positioned to permit photographing of 0.72 mm by 0.49 mm section of the leaf immediately adjacent and parallel to the row of bulliform cells on either side of the midvein. Photographs of the underside of the leaf always included 2 small parallel veins for which cells were not counted.

The number of stomatal and epidermal cells was calculated for a 1 mm^2 area and the stomata cell to epidermal cell(S/E) ratio was determined by dividing the number of stomata by the number of epidermal cells. The data is the mean of three replications. Means were separated by Duncan's Multiple Range Test.

RESULTS

Maximum conductance (stomatal aperture) was found in the non-treated check 15 minutes after the light was turned on (Figure 1). Maximum conductance for Aqua-Gro, iprodione, and Hydro-Wet occurred at a similar time as the check. Maximum stomatal aperture of plants treated with benomyl did not occur until 28 minutes after light initiation.

In Figure 2, assimilation of CO_2 was greatest in the non-treated check. Maximum photosynthesis occurred within 10 to 15 minutes in all treatments except in plants treated with benomyl which did not reach maximum until the end of

the 30 minute analysis period. The Aqua-Gro and iprodione treatments resulted in the least reduction of photosynthesis, while Hydro-Wet and benomyl reduced peak photosynthesis by 50% or more compared to the check. Ten minutes of light was required before photosynthesis overcame respiration in the plants treated with benomyl.

The quartic curves for transpiration in Figure 3 are nearly identical to the stomatal conductance curves in Figure 1. Since gas exchange is the basis for determining stomatal aperture, and since up to 1000 times as much water as CO_2 passes through stomatas it is not surprising that these curves are similar.

Table 1 is a summary of the effects of the chemicals. The percentages were calculated on the average conductance, transpiration or assimilation of each treatment during the second half of the 30 minute analysis period. Benomyl and Hydro-Wet exhibited the greatest reduction of gas exchange including a large reduction in photosynthesis. Iprodione reduced transpiration to 58% while lowering photosynthesis to 76% of the untreated check. Aqua-Gro reduced transpiration by 32% while reducing photosynthesis by only 13%.

Figure 4 is a photomicrograph of the upper and lower surfaces of the leaf imprint. The upper surface of leaves treated with each chemical is also shown. Data from the leaf imprints shown in Table 2 indicate the micro-morphology of the upper leaf surface was altered to a greater extent

Figure 1. The effect of four chemicals on stomatal conductance of intact leaves of Merion Kentucky bluegrass. Quartic regression analysis yielded R^2 values of Control-0.94, Aqua-Gro- 0.91, Iprodione- 0.89, Hydro-Wet- 0.83, and Benomyl- 0.95.

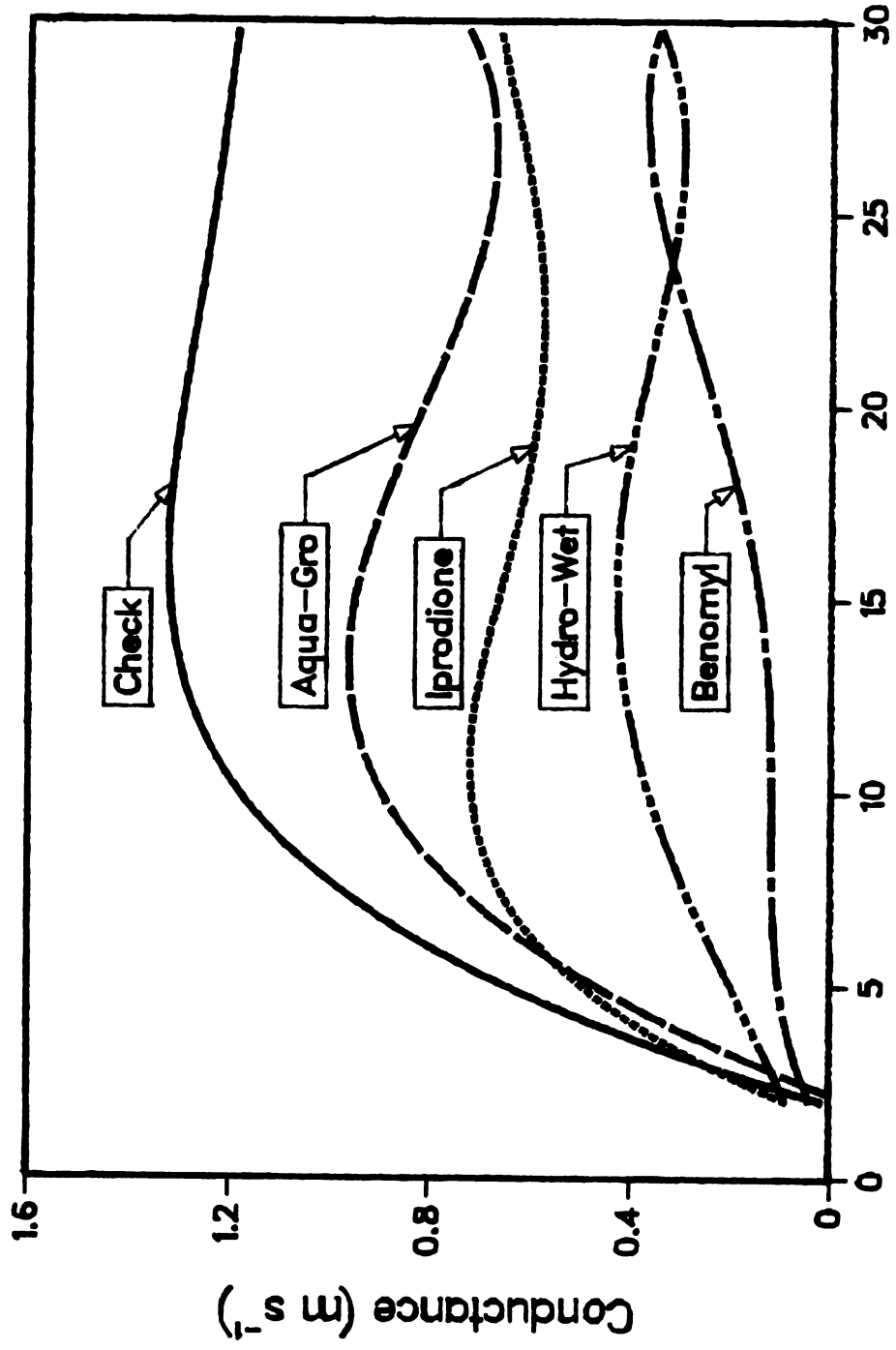

Conductance (m s⁻¹)
1.6
1.2
0.8
0.4
0
0
5
10
15
20
25
30
Time after activating radiation source (min)
Check
Aqua-Gro
Iprodione
Hydro-Wet
Benomyl

Figure 1

Figure 2. The effects of four chemicals on assimilation of intact leaves of Merion Kentucky bluegrass. Quartic regression analysis yielded R^2 values of Control- 0.95, Aqua-Gro- 0.81, Iprodione- 0.85, Hydro-Wet- 0.31, and Benomyl- 0.96.

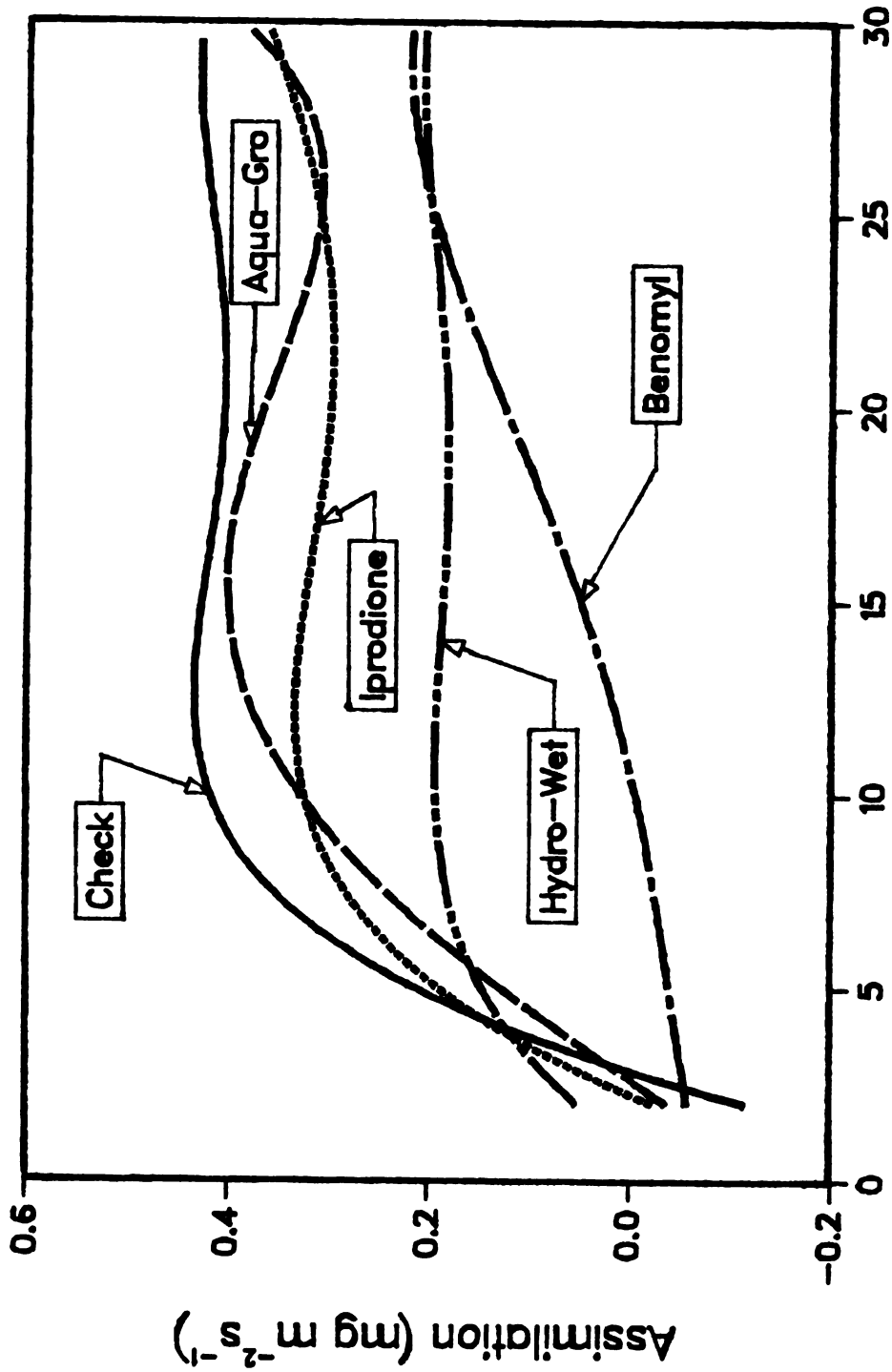

Assimilation (mg m⁻²s⁻¹)
0.6
0.4
0.2
0.0
−0.2
0
5
10
15
20
25
30
Time after activating radiation source (min)
Check
Aqua-Gro
Iprodione
Hydro-Wet
Benomyl

Figure 2

Figure 3. The effects of four chemicals on transpiration of intact leaves of Merion Kentucky bluegrass. Quartic regression analysis yielded R^2 values of Control- 0.94, Aqua-Gro- 0.90, Iprodione- 0.90, Hydro-Wet- 0.77, and Benomyl- 0.95.

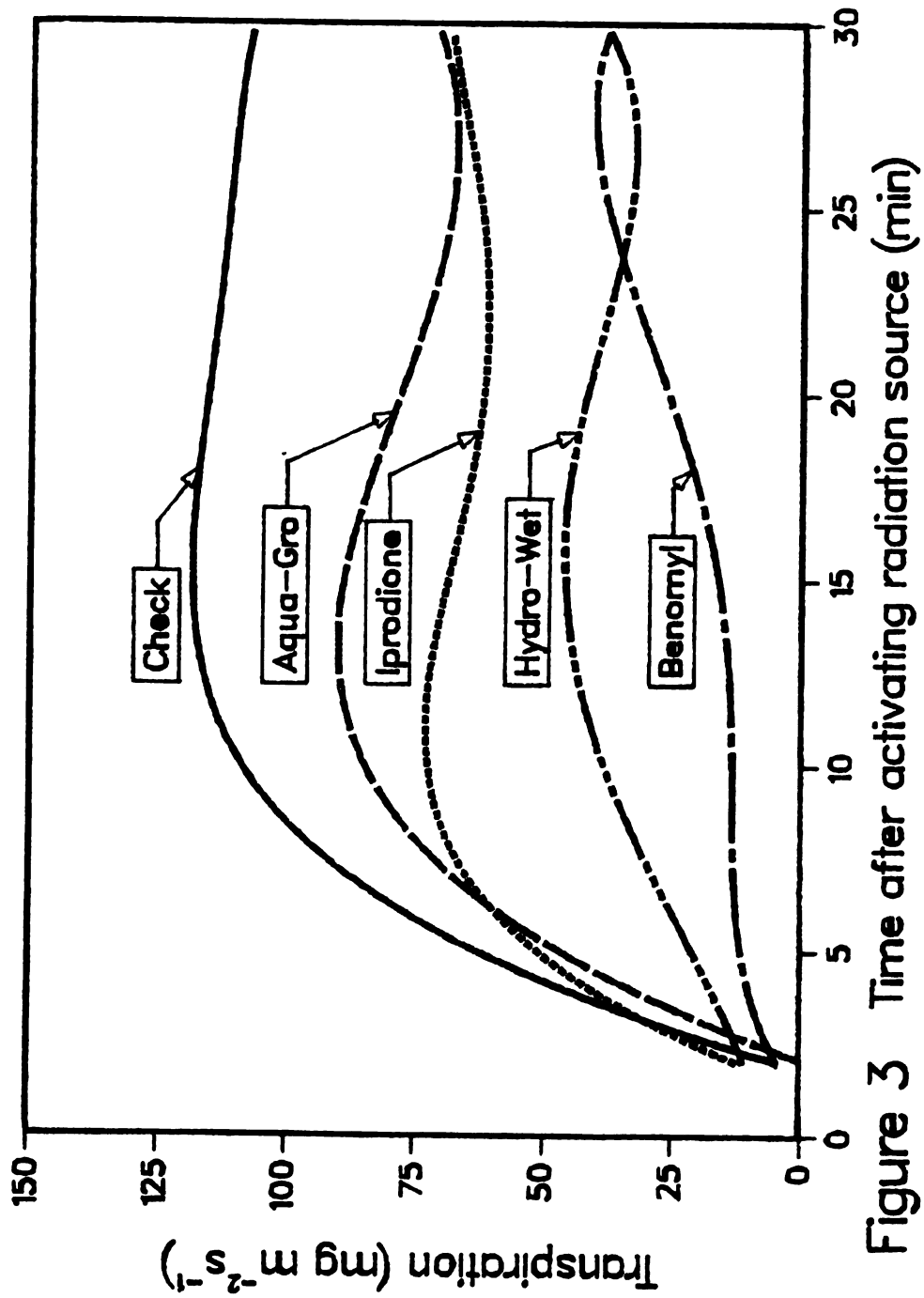
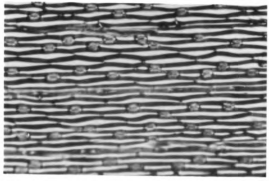
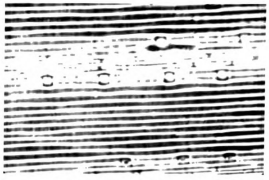

Transpiration (mg m⁻²s⁻¹)
150
125
100
75
50
25
0
0
5
10
15
20
25
30
Time after activating radiation source (min)
Check
Aqua-Gro
Iprodione
Hydro-Wet
Benomyl

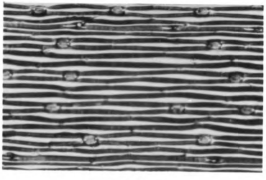
Figure 3



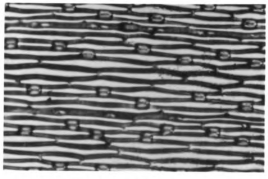
Check (u)



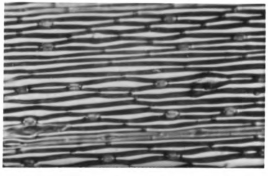
Check (l)



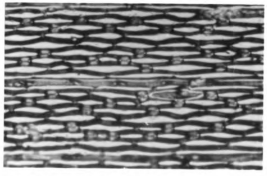
Tersan 1991 (u)



Chipco 26019 (u)



Hydrowet (u)



AquaGro (u)

Fig. 4. Photomicrographs of 0.72-x 0.49-mm section of Kentucky bluegrass leaf imprints of upper (u) and lower (l) surfaces.

TABLE 1. The effect of four chemicals on stomatal conductance, transpiration and photosynthesis of Merion Kentucky bluegrass after three weeks from initial application.

Chemical	Percent of untreated check		
	Conductance	Transpiration	Photosynthesis
1. Aqua-Gro	62	68	87
2. Hydro-Wet	28	36	46
3. Benomyl	21	34	31
4. Iprodione	49	58	76
5. Check	100	100	100

TABLE 2. The effect of four chemicals on the number of stomata and epidermal cells on the upper and lower leaf surface of Merion Kentucky bluegrass after three weeks from initial application.

Treatment	Upper Leaf Surface			Lower Leaf Surface		
	Stomata per mm^2	Epidermal cells/mm^2	S/E* ratio	Stomata per mm^2	Epidermal cells/mm^2	S/E* ratio
Check	119 a**	333 a	0.36 a	37 a	201 ab	0.19 a
Aqua-Gro	119 a	298 ab	0.40 a	33 a	187 b	0.18 a
Iprodione	89 b	266 bc	0.34 ab	37 a	225 a	0.16 a
Hydro-Wet	62 c	244 c	0.26 bc	30 a	185 b	0.17 a
Benomyl	52 c	230 c	0.22 c	32 a	155 c	0.21 a

* S/E equals stomatal number divided by epidermal cell number.

** Treatments having the same letter within vertical columns are not significantly different. Mean separation is by Duncan's Multiple Range Test at the 5% level.

than the lower surface. The density of stomata on the upper surface were significantly lower in leaves that developed during the iprodione treatment. Leaves of plants treated with Hydro-Wet and benomyl exhibited a significantly lower density of stomata than the iprodione treatment. A similar reduction was noted in the number of epidermal cells. However, the S/E ratio indicates that the stomatal number was lowered to a significantly greater extent than the epidermal cell number per unit area. The lower side of the leaf only exhibited a reduced epidermal cell number.

DISCUSSION

The data indicate that high but not toxic rates of Hydro-Wet and benomyl reduce transpiration, photosynthesis and stomatal density of Merion Kentucky bluegrass. Field application of these two chemicals at rates similar to this study would likely be more costly than irrigation. Research needs to be conducted to determine the effect found for rates that are normally applied in the field. However, the data of benomyl and Hydro-Wet indicate the potential for reducing water consumption while maintaining adequate photosynthesis.

Since the plants were treated with a soil drench, root impairment, particularly for high rates of benomyl, might have caused a water deficit and subsequent low transpiration measurements. However, the plants were periodically examined throughout the treatment period and did not exhibit the

the xeromorphic conditions typical of a water deficient Kentucky bluegrass. Additionally the surfactant Aqua-Gro, shown by Endo (1968) to cause root damage in solution culture, exhibited the least transpiration reduction of any of the chemicals.

Since the reduction of transpiration and photosynthesis per unit leaf area correlates well with the reduction in the number of stomata per unit leaf area, the response to benomyl is suggested to be primarily micro-morphological rather than physiological. Benomyl is known to have kinetin-like properties (Thomas, 1974). Kinetins are involved in cytokinesis or cell wall formation during cell division. During a three week growth period, the second youngest leaf at the time of analysis likely initiated and grew after the first chemical treatment. The high rates of benomyl within the plant during initiation and growth may have altered cell division.

When the quartic curves were studied, the curve for Hydro-Wet indicates that the stomata, although fewer in number, were opening to light initiation at a similar but reduced rate compared to the check. However, the stomata of the plants treated with benomyl were much slower to respond. This indicates that there may be a physiological as well as a micro-morphological response to benomyl.

Thus, there is evidence to suggest that the surfactant Hydro-Wet was translocated from soil solutions into turfgrass plants where stomatal development was altered, and that the

systemic fungicide benomyl may alter host resistance to a disease such as Fusarium Blight by reducing water consumption and the subsequent severity of drought stress.

ACKNOWLEDGEMENT

Appreciation is expressed to the United States Golf Association Green Section for their support in this investigation.

LITERATURE CITED

1. Beard, J.B. 1973. Turfgrass: Science and Culture. Prentice Hall, New Jersey, pp. 1-658
2. Endo, R.M., J. Letey, N. Valoras, and J.F. Osborn. 1969. Effects of nonionic surfactants on monocots. Agron. J., Vol. 61:850-854
3. Comar, C.L. and C.G. Barr. 1944. Evaluation of foilage injury and water loss with use of wax and oil emulsions. Plant Physiology 19:90-104.
4. Huggenberger, F., J. Letey, and W.J. Farmer. 1973. Effect of two nonionic surfactants on absorption and mobility of selected pesticides in a soil-system. Soil Sci. Amer. Proc., Vol. 37: 215-219.
5. Ricke, P.E. 1975. Soils research: N. carriers, potassium studies and rewetting of a hydrophobic soil. 45th Annual Michigan Turfgrass Conference Proceedings, Vol. 4:3-5.
6. Tanner, C.B. 1963. Plant temperatures. Agron. J., Vol. 55: 210-211.

7. Thomas, T.H. 1974. Investigations into the cytokinin-like properties of benzimidazole derived fungicides. Annals of Appl. Biol., Vol. 76:237-241

8. Woolley, J.T. 1967. Relative permeabilities of plastic films to water and carbon dioxide. Plant Physiol., Vol. 42(5):641-643

CHAPTER 2

MANIPULATING GAS EXCHANGE RATES OF ADELPHI KENTUCKY BLUEGRASS (*POA PRATENSIS* L.)

ABSTRACT

Adelphi Kentucky bluegrass (*Poa pratensis* L.) was used to examine the effects on gas exchange rates of applications of three systemic fungicides. Triadimefon (Bayleton or 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triaxol-1-yl)--2-butanone), benomyl (Tersan 1991 or methyl-N-(1-(butylcarbamoyl)-2-benzimidazole) carbamate), and CGA 64251 (1-((2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl)methyl--1H-1,2,4-triazole) were applied at rates of 0, 50, 500 ug ml^{-1} a.i. as soil drench applications. Further studies on benomyl at field rates were also conducted. CGA 64251 at the 500 ug ml^{-1} rate increased assimilation over the control, but did not increase transpiration. Benomyl increased gas exchange rates at both 50 and 500 ug ml^{-1} concentrations by increased stomatal aperture. Benomyl did not alter the gas exchange rate of the turfgrass plant when applied at field rates. Triadimefon did not alter gas exchange rates from the control. The use of benomyl or triadimefon at field rates to reduce gas exchange is not recommended based on the results of this study. The effect of CGA 64251 on assimilation needs further investigation.

INTRODUCTION

Fusarium Blight is a turfgrass disease caused by a combination of factors. The pathogens Fusarium roseum and Fusarium tricinctum are believe to be weak parasites of Kentucky bluegrass because they cause symptom development only when the host plant is already under stress (Smiley and Craven, 1977).

Triadimefon at low concentrations does not control in vitro cultures of Fusarium roseum or Fusarium tricinctum (Smiley and Craven, 1977). However, it controls Fusarium blight symptoms in turfgrass if applied before infection. Applications after infection will not control the disease. Therefore triadimefon may be altering the plan to prevent infection by the fungi, possibly by conserving water usage by altering the gas exchange rates and reducing water stress. Triadimefon has been shown to exhibit growth regulating properties in Kentucky bluegrass resulting in darker green foliage (Hardison, 1974): Sanders et al, 1978). Dicots showed reduced internode length as well as darker green foliage (Buchenauer and Grossman, 1977). Triadimefon is a gibberellin (GA) biosynthesis inhibitor. Dark green leaves are a typical response of plants to other compounds that inhibit GA biosynthesis such as CCC, AMO 1618 or ancymidol.

CGA 64251, a Ciba Giegy experimental fungicide, is a highly effective broad-spectrum fungicide (Gilpatrick, 1979; Staub et al, 1979). When used on apple trees for the

control of apple scab, the compound causes inhibition of internode elongation of lateral shoots (Kelley and Jones, 1981). The leaves are smaller, thicker and darker green than controls. Cross sections of the leaves revealed treated leaves have 3-5 layers of palisade cells, while controls have 2-3 layers of cells. Thus CGA 64251 exhibits growth regulator properties and color changes similar to triadimefon. The exact mode of action of CGA 64251 in the plant has not been established.

High rates of benomyl applied biweekly to the soil for three weeks have been shown to reduce the gas exchange rate of Kentucky bluegrass by at least 50% (Kaufmann and Williamson, 1981). This was accounted for by a reduction in the number of stomata per unit leaf area. A corresponding reduction in the number of epidermal cells per unit leaf area resulted in larger cells in the treated plants than cells of the control plants. It was concluded that benomyl can act as a growth regulator by changing the micro-morphological structure of the turfgrass plant.

This investigation was designed to determine if the systemic fungicides benomyl, triadimefon and CGA 64251 which exhibit some growth regulating properties, alter the gas exchange rates of Kentucky bluegrass. The first experiment examines the gas exchange rates of turfgrass plants treated with the three systemic fungicides at field rates and extremely high rates applied only once. The second experiment further examines the gas exchange rate of

turfgrass treated with benomyl at field rates.

MATERIALS AND METHODS

In the first experiment, 9 month old plants of Adelphi Kentucky bluegrass (Poa pratensis L.) grown from seed in the greenhouse were transplanted into 300 ml styrofoam containers. The soil mixture was a 1:1:1 mixture of sand, loam and peat provided by the MSU Plant Science Greenhouses. A modified nutrient solution (Hoagland's No. 1 solution with a 2:1 potassium to nitrogen ratio, see Appendix Tablet 1) was applied byweekly throughout the experiments. Ten plants were transplanted to each of the styrofoam and allowed to establish for four weeks before treatment applications. Plants were maintained in the greenhouse throughout the experiment which occurred in October when day temperatures reached 32 C and night temperatures 7 C. Solutions of 0, 50, and 500 ug ml^{-1} a.i. of all three fungicides in 50 ml of water applied in a single soil drench application. No leaching occurred with this volume. Irrigation was applied throughout the experiment to moisten the soil thoroughly, but not enough to allow drainage from the bottom of the container to prevent leaching. Gas exchange was then measured 1, 4, and 8 days after treatment on the second youngest fully expanded leaf. Measurements were made with an open infrared gas analysis system until leaves maintained a steady state for 30 minutes. Readings were then averaged

for the last 30 minutes to obtain a single value. The gas analysis system is described in a previous paper by Kaufmann and Williamson (1981). Leaf temperatures during the measurements were maintained at 25 C $\pm$ 0.5, relative humidity at 50% and light intensity at 200 W m^{-2}. The transpiration/assimilation ratio (T/A) was calculated by dividing the transpiration rate by assimilation rate (Gale and Hagan, 1966).

In the second experiment mature plants of Adelphi Kentucky bluegrass were handled as described in experiment 1. After four weeks of establishment the plants were transferred to a growth chamber and allowed to acclimate for 8 days before treatments with benomyl. The growth chamber was maintained at a 16 hour day length, day-night temperatures of 25-19 C respectively, a relative humidity of 40% and a light intensity of 200 W m^{-2}.

After being placed in the growth chamber the plants were grown under two moisture regimes and watered four times a day. Containers in the wet regime received a total of 200 ml water a day which kept the soil well saturated without excess leaching. Containers in the dry regime received a total of 80 ml of water a day which was enough to keep the plants from wilting between waterings. The modified Hoagland's nutrient solution was applied twice a week to all treatments.

Benomyl was applied as a soil drench solution at the rates of 0.0, 9.1, 18.2, and 36.5 kg ha^{-1}. Gas exchange was

measured 24 hours after treatment and then every other day for two weeks. Relative humidity of the airstream during the first test period was controlled at 85% and 50% in the second test period. Leaf temperatures were 25 C $\pm$ 0.5 and light intensity was 200 W m^{-2}.

RESULTS

The treatment with triadimefon showed no significant difference in transpiration from the control (Table 1), CO_2 assimilation or T/A ratio. Highest CO_2 assimilation and lowest transpiration occurred 4 days after treatment with 500 ug ml^{-1} triadimefon.

CGA 64251 caused no significant differences in transpiration from the control (Table 2). The assimilation rate of the 500 ug ml^{-1} treatment showed a significant increase over the control on day 4. The treatments were averaged over time. The 500 ug ml^{-1} treatment showed a significant increase over the control for assimilation, but not for transpiration. The T/A ratio when averaged over time shows a significant decrease from the control for the 500 ug ml^{-1} treatment.

Benomyl at the 50 ug ml^{-1} treatment significantly increased transpiration from the control 24 hours after application and then showed no differences from the control by the 8th day (Table 3). The 500 ug ml^{-1} treatment increased the transpiration rate significantly above the control on the 8th day. Transpiration averaged over time showed a

TABLE 1. The effects of triadimefon on gas exchange of Adelphi Kentucky bluegrass leaves.

Variable	Conc ug ml^{-1}	Days After Treatment 1	4	8	Ave
Transpiration ($mg\ m^{-2}s^{-1}$)	0	66.2a *	55.8a	63.0a	61.7a
	50	66.4a	62.8a	71.1a	66.8a
	500	64.4a	53.1a	59.4a	59.0a
Assimilation ($mg\ m^{-2}s^{-1}$)	0	.285a	.253a	.246a	.261a
	50	.267a	.275a	.257a	.267a
	500	.272a	.290a	.220a	.260a
T/A Ratio	0	232a	221a	256a	237a
	50	249a	228a	277a	250a
	500	237a	183a	270a	227a

* Different letters in columns for each variable for days 1, 4, and 8 show significance at the 0.05 level using Duncan's Multiple Range Test. Data are means of three replications.

TABLE 2. The effects of CGA 64251 on gas exchange of Adelphi Kentucky bluegrass leaves.

Variable	Conc ug ml^{-1}	Days After Treatment			
Transpiration ($mg\ m^{-2}s^{-1}$)	0	67.5a *	60.8a	64.8a	64.3a
	50	50.9a	65.7a	54.9a	57.1a
	500	57.8a	64.8a	51.7a	58.0a
Assimilation ($mg\ m^{-2}s^{-1}$)	0	.243ab	.225b	.234a	.234b
	50	.198b	.238b	.223a	.220b
	500	.271a	.325a	.270a	.289a
T/A Ratio	0	278a	270a	277a	275b
	50	257a	276a	246a	259b
	500	213a	199a	191a	210a

* Different letters in columns for each variable for days 1, 4, and 8 show significance at the 0.05 level using Duncan's Multiple Range Test. Data are means of three replications.

TABLE 3. The effects of benomyl on gas exchange of Adelphi Kentucky bluegrass leaves.

Variable	Conc ug ml^{-1}	Days After Treatment 1	4	8	Ave
Transpiration (mg $m^{-2}s^{-1}$)	0	62.1b	55.6a	52.9b	56.9b
	50	74.5a	62.5a	54.5b	63.9a
	500	58.1b	56.3a	75.8a	63.4a
Assimilation (mg $m^{-2}s^{-1}$)	0	.400a	.179a	.197a	.259a
	50	.325a	.284a	.268a	.292a
	500	.361a	.260a	.313a	.312a
T/A Ratio	0	155a	311a	269a	220a
	50	229a	220a	204a	219a
	500	161a	217a	242a	203a

* Different letters in columns for each variable for days 1, 4, and 8 show significance at the 0.05 level using Duncan's Multiple Range Test. Data are means of three replications.

significant stimulation over the control for both treatments of benomyl. The assimilation rates were generally above the control, but showed no significant differences at the 5% level. The T/A ratio showed no significant differences at any time.

The second set of experiments with benomyl at field rates measured at 85% relative humidity showed no differences in any of the treatments in transpiration or assimilation (Figures 1,2,3,&4). The treatments follow the controls quite closely. Three weeks after the treatments there were still no differences between treatments and controls.

The second series of tests with benomyl where responses measured at 50% relative humidity, showed greater variation. The transpiration rates varied slightly from the control for the first three days in the wet regime, then showed no variation from the control by the 5th day (Figure 5). The dry regime showed variation throughout the 7 days (Figure 6). However, there were no significant differences at the 5% level. The assimilation curves follow the same general patterns as the transpiration curves. The T/A ratios are not shown because there were no significant differences at the 5% level.

DISCUSSION

From a previous study (Kaufmann and Williamson, 1981) in which the plants were treated biweekly for a period of 3

Figure 1. The effect of benomyl on transpiration of Adelphi Kentucky bluegrass leaves maintained under a wet regime and measured at 85% relative humidity. Each point is an average of three replications.

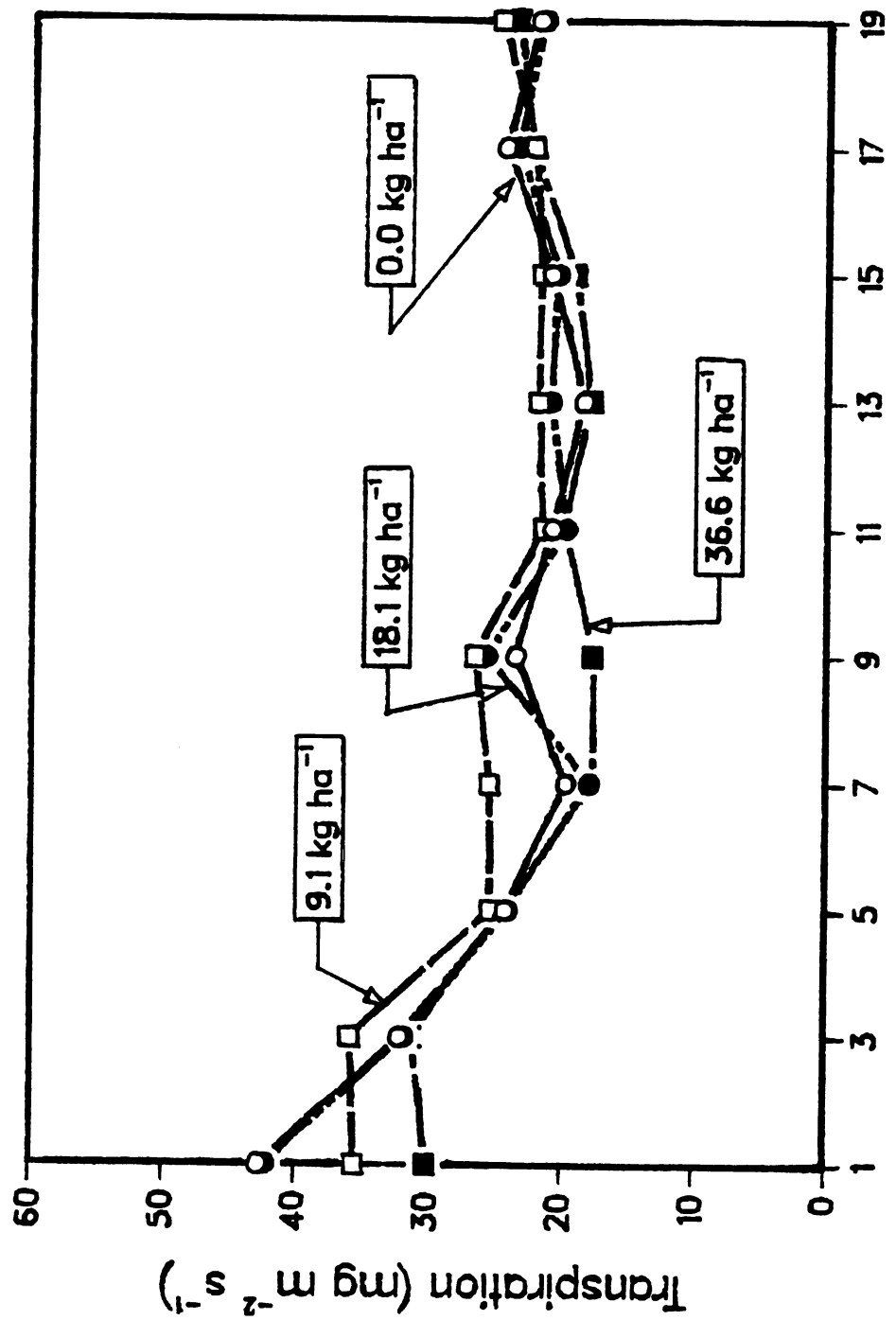

Transpiration (mg m^{-2} s^{-1})
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
Days After Treatment
0.0 kg ha^{-1}
9.1 kg ha^{-1}
18.1 kg ha^{-1}
36.6 kg ha^{-1}

Figure 1

Figure 2. The effect of benomyl on transpiration of Adelphi Kentucky bluegrass leaves maintained under a dry regime and measured at 85% relative humidity. Each point is an average of three replications.

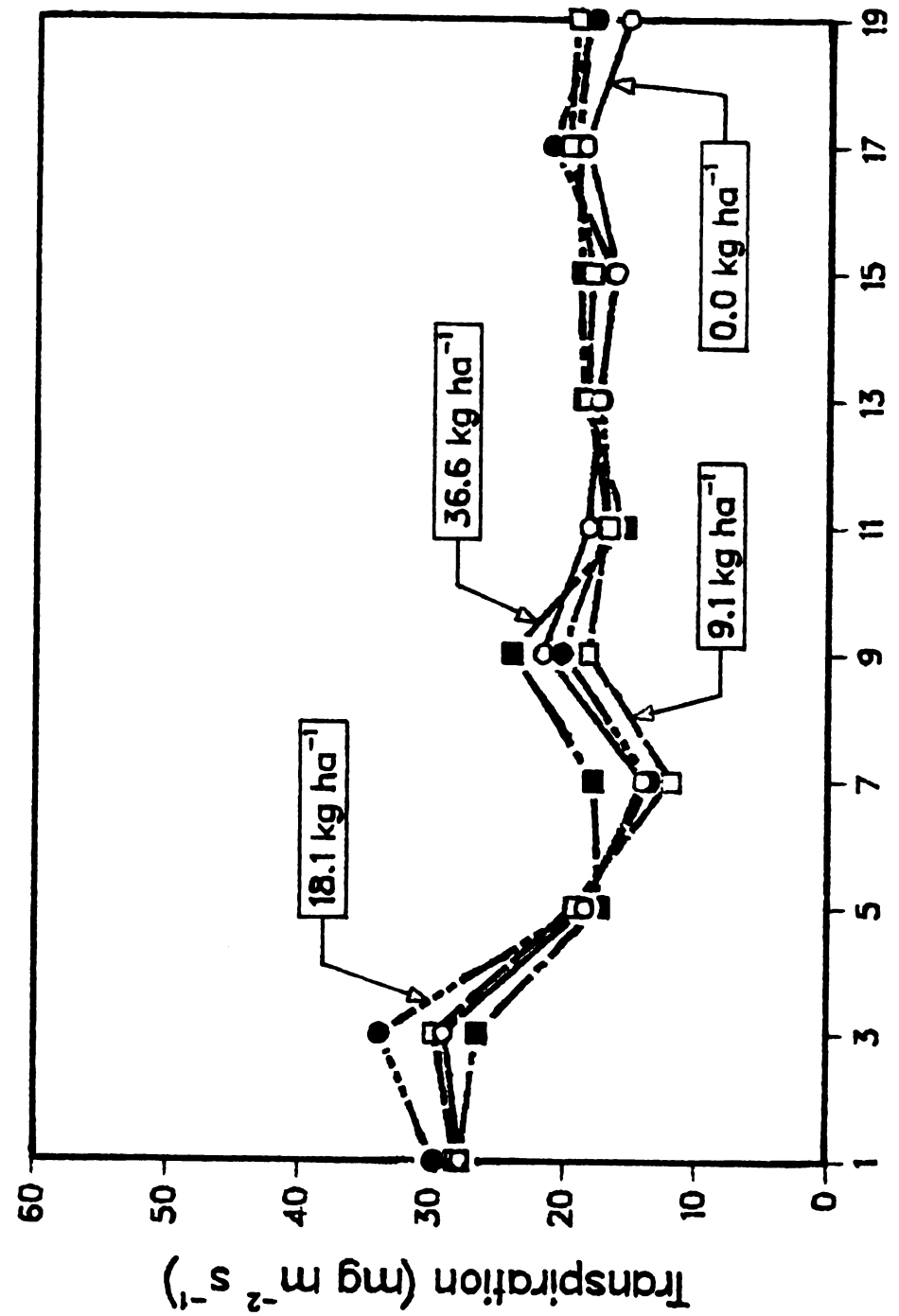

Transpiration (mg m^{-2} s^{-1})
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
Days After Treatment
18.1 kg ha^{-1}
36.6 kg ha^{-1}
9.1 kg ha^{-1}
0.0 kg ha^{-1}

Figure 2

Figure 3. The effects of benomyl on assimilation of Adelphi Kentucky bluegrass leaves maintained under a wet regime and measured at 85% relative humidity. Each point is an average of three replications.

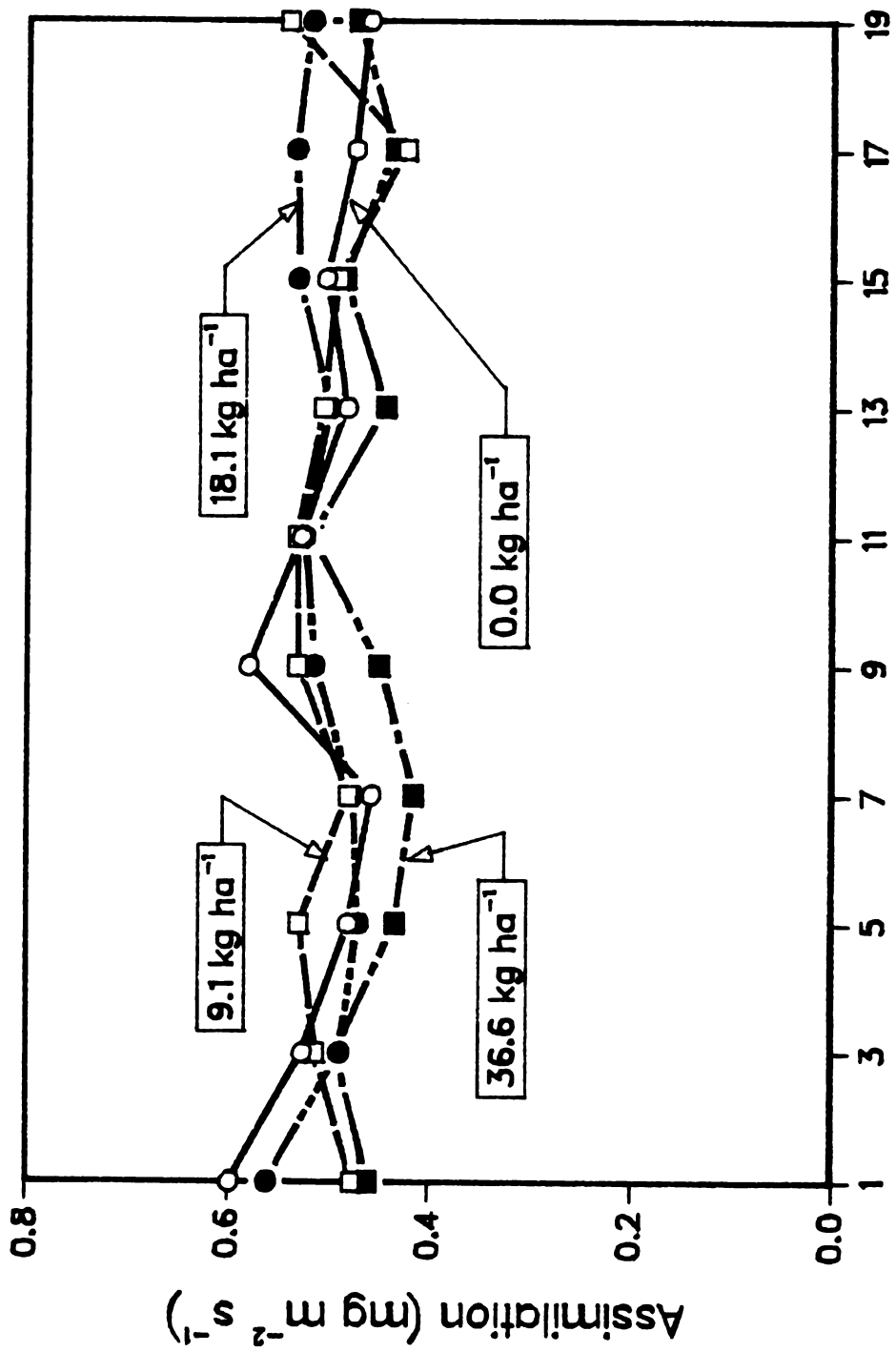

Assimilation (mg m⁻² s⁻¹)
0.0
0.2
0.4
0.6
0.8
1
3
5
7
9
11
13
15
17
19
Days After Treatment
0.0 kg ha⁻¹
9.1 kg ha⁻¹
18.1 kg ha⁻¹
36.6 kg ha⁻¹

Figure 3

Figure 4. The effects of benomyl on assimilation of Adelphi Kentucky bluegrass leaves maintained under a dry regime and measured at 85% relative humidity. Each point is an average of three replications.

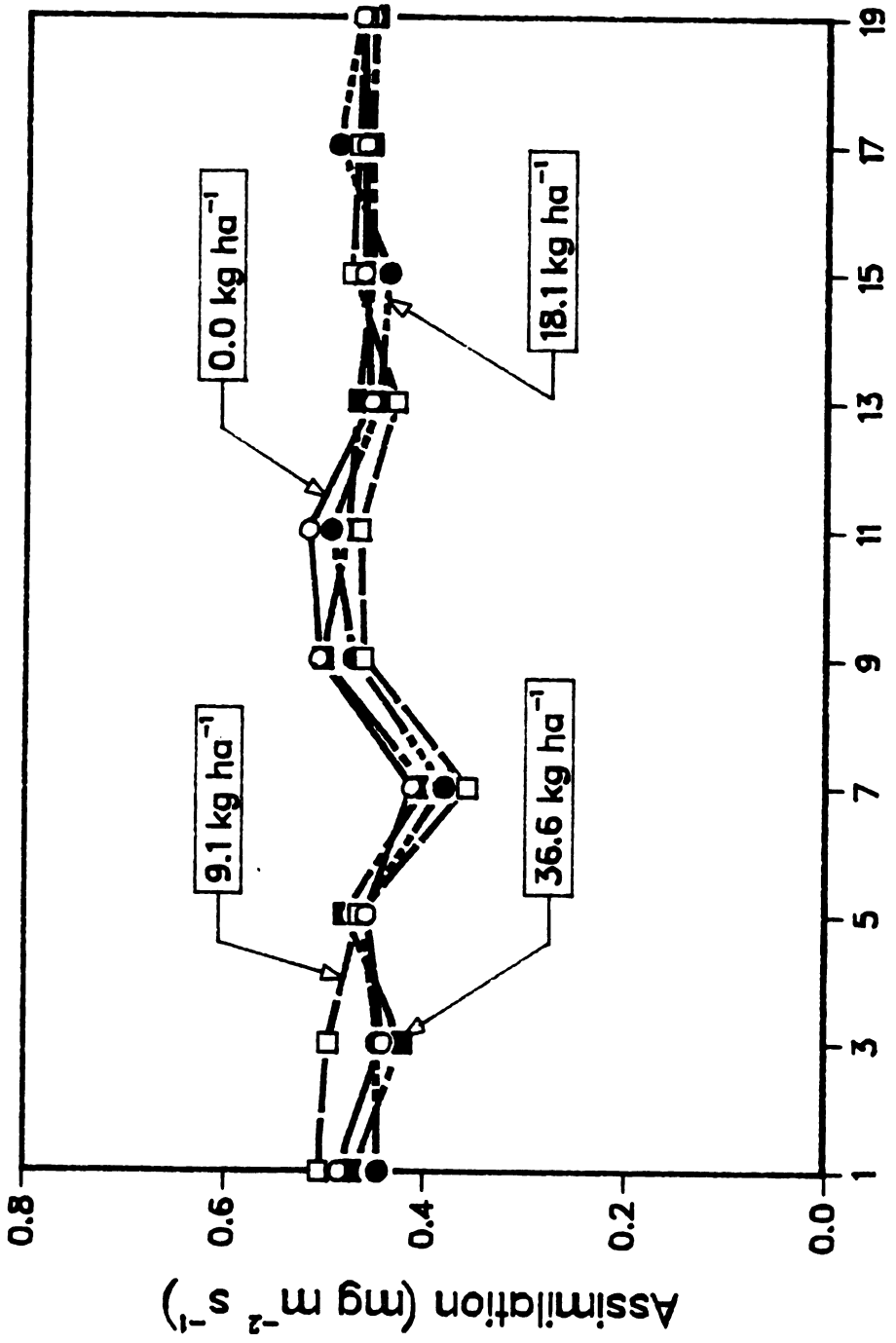

Assimilation (mg m^{-2} s^{-1})
0.0
0.2
0.4
0.6
0.8
1
3
5
7
9
11
13
15
17
19
Days After Treatment
0.0 kg ha^{-1}
9.1 kg ha^{-1}
36.6 kg ha^{-1}
18.1 kg ha^{-1}

Figure 4

Figure 5. The effects of benomyl on transpiration of Adelphi Kentucky bluegrass leaves maintained under a wet regime and measured at 50% relative humidity. Each point is an average of three replications.

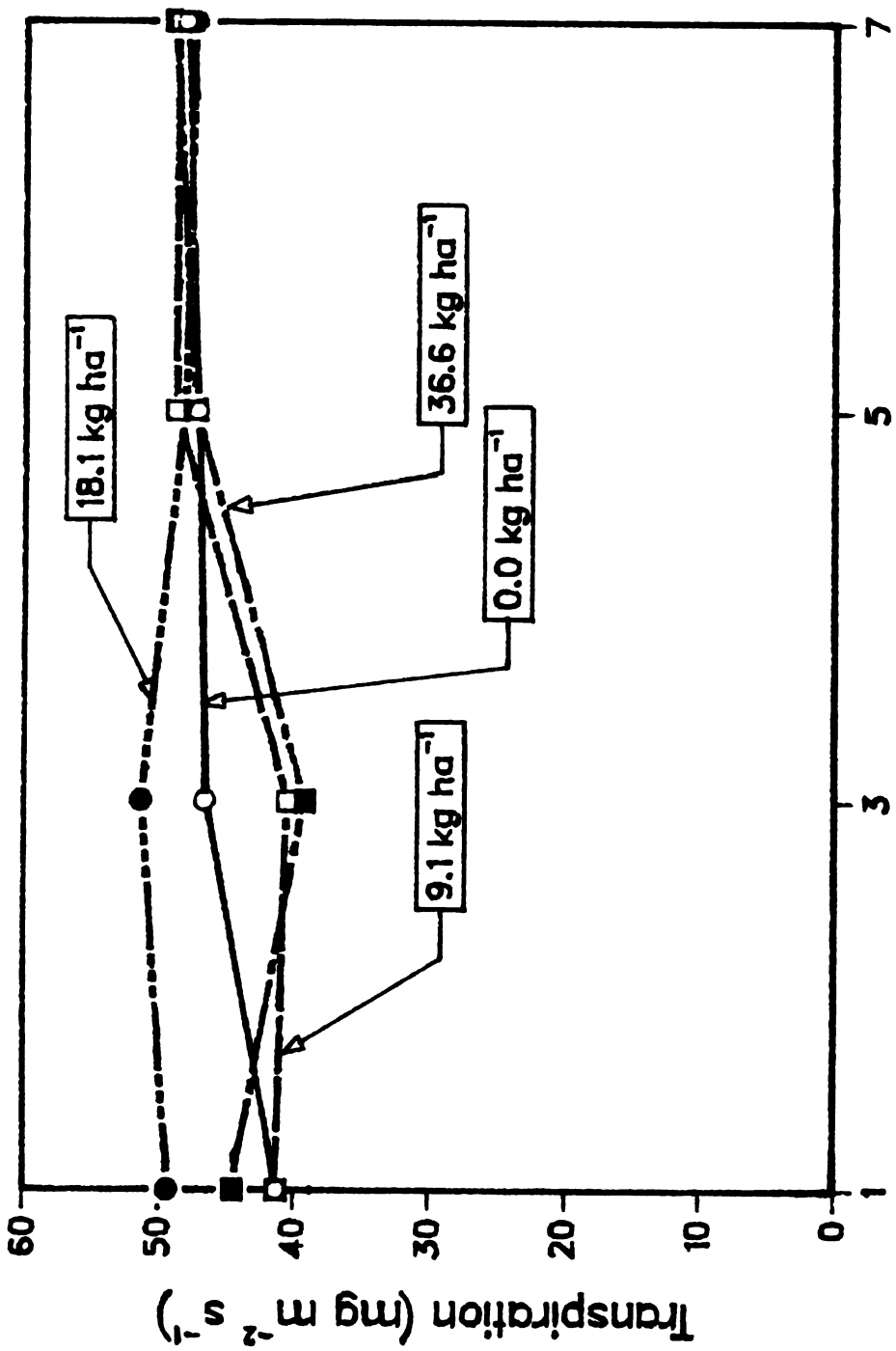

Transpiration (mg m⁻² s⁻¹)
60
50
40
30
20
10
0
1
3
5
7
Days After Treatment
18.1 kg ha⁻¹
36.6 kg ha⁻¹
0.0 kg ha⁻¹
9.1 kg ha⁻¹

Figure 5

Figure 6. The effects of benomyl on transpiration of Adelphi Kentucky bluegrass leaves maintained under a dry regime and measured at 50% relative humidity. Each point is an average of three replications.

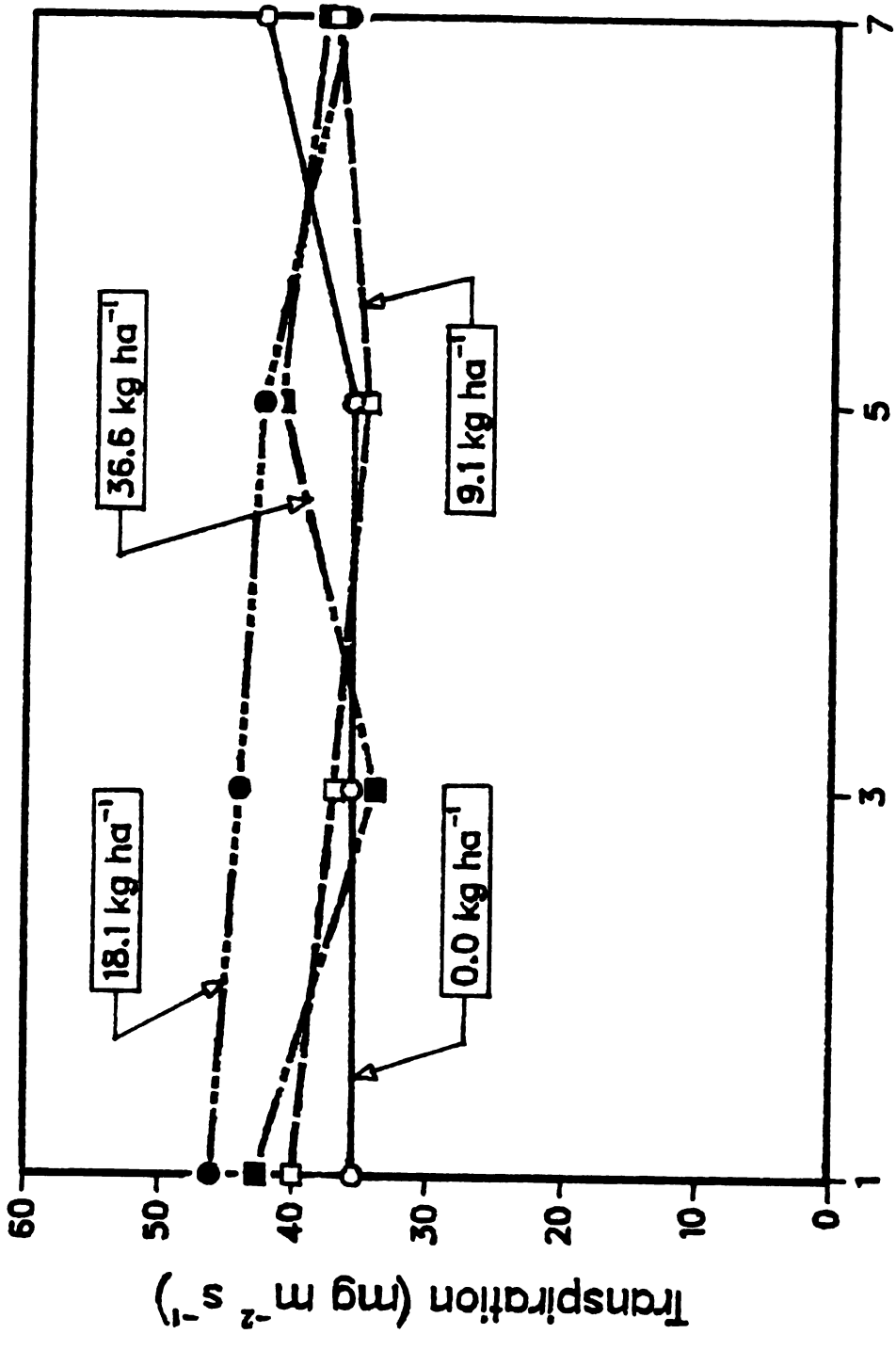

Transpiration (mg m^{-2} s^{-1})
60
50
40
30
20
10
0
1
3
5
7
Days After Treatment
36.6 kg ha^{-1}
18.1 kg ha^{-1}
9.1 kg ha^{-1}
0.0 kg ha^{-1}

Figure 6

Figure 7. The effect of benomyl on assimilation of Adelphi Kentucky bluegrass leaves maintained under a wet regime and measured at 50% relative humidity. Each point is an average of three replications.

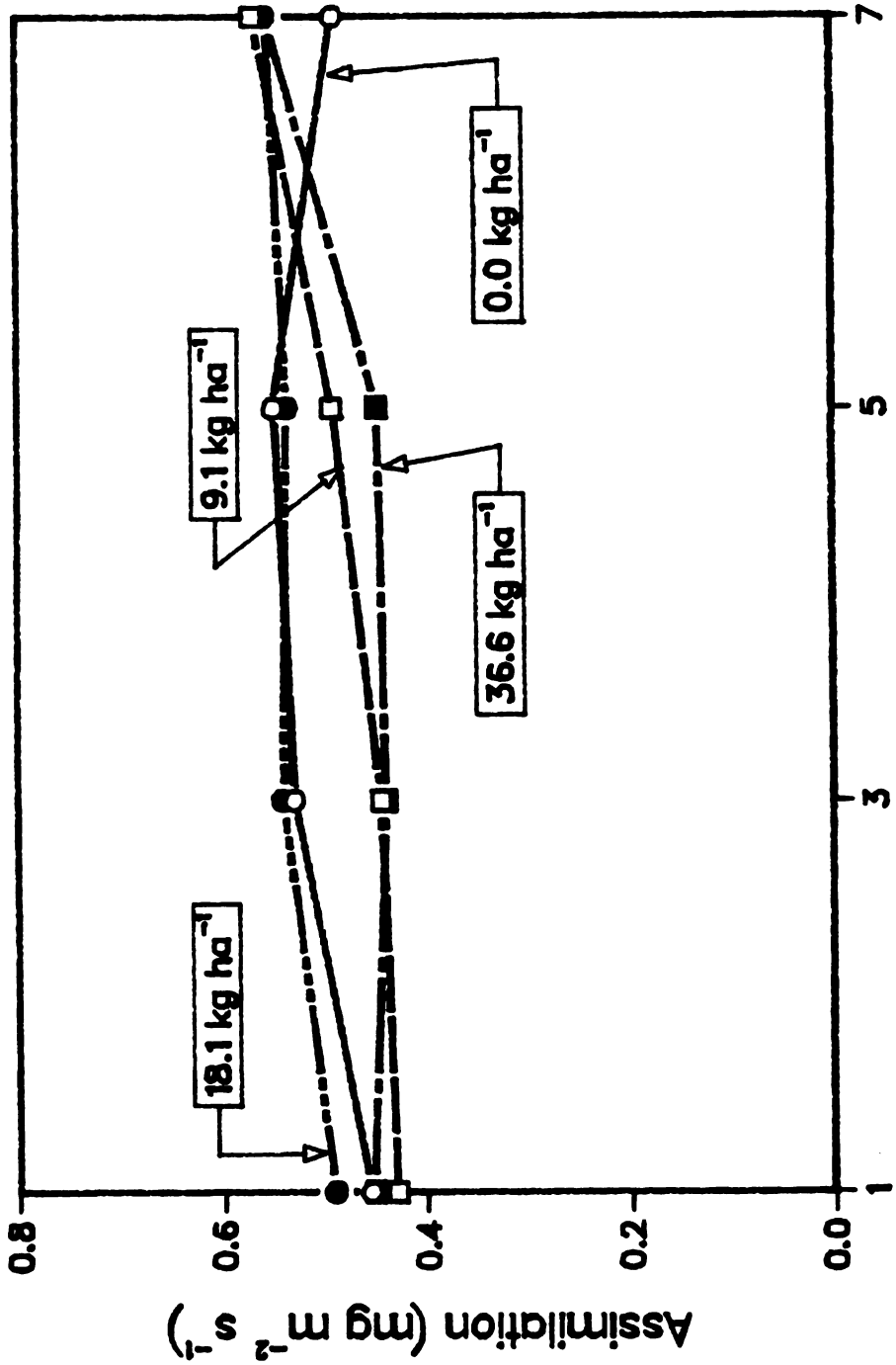

Assimilation (mg m⁻² s⁻¹)
0.0
0.2
0.4
0.6
0.8
1
3
5
7
Days After Treatment
0.0 kg ha⁻¹
9.1 kg ha⁻¹
18.1 kg ha⁻¹
36.6 kg ha⁻¹

Figure 7

Figure 8. The effects of benomyl on assimilation of Adelphi Kentucky bluegrass leaves maintained under a dry regime and measured at 50% relative humidity. Each point is an average of three replications.

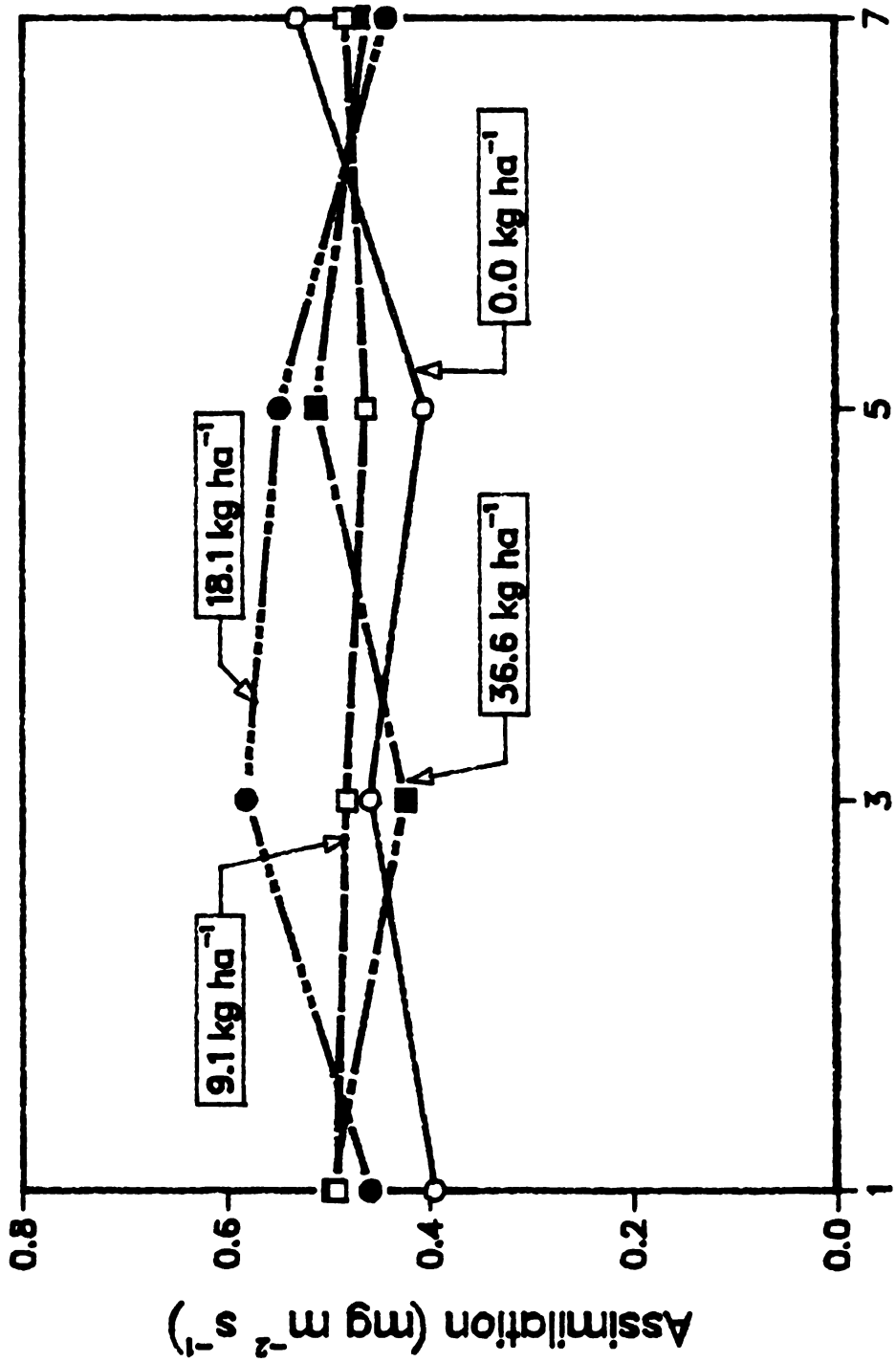

Assimilation (mg m⁻² s⁻¹)
0.0
0.2
0.4
0.6
0.8
1
3
5
7
Days After Treatment
0.0 kg ha⁻¹
18.1 kg ha⁻¹
36.6 kg ha⁻¹
9.1 kg ha⁻¹

Figure 8

weeks, it was concluded that the leaves being evaluated had been initiated and grew after one or more applications of the compound. The leaves tested in this study had not been initiated after the application of the fungicide. The leaf tested on the 8th day of this study was likely already initiated and in the sheath when the treatments were applied. Some cell division could still have taken place in the leaves tested on the 8th day after the initial application of the fungicide. The foliage of the plants treated with triadimefon and CGA 64251 were darker green than the control plants. This agrees with previous reports of darker green foliage on plants treated with these compounds (Buchenauer and Grossman, 1977; Hardison, 1974; Kelley and Jones, 1981; Sanders et al, 1978). The darker green color was in the entire plant and not just in the new foliage, so the effect of triadimefon and CGA 64251 affects existing as well as new and developing tissue.

Triadimefon did not affect the gas exchange rate of the turfgrass plant. Since triadimefon regulates growth by blocking GA biosynthesis somewhere after geranylgeranyl pyrophosphate (Buchenauer and Grossman, 1977), it can be speculated that the darker color exhibited in the leaves may be due to increased levels of carotenoids or chlorophyll. Several growth regulators that inhibit GA biosynthesis have been reported to result in darker green leaves (Buchenauer and Grossman, 1977). If it is a precursor of chlorophyll, it is not in an active form because assimilation rates were not

increased. Thus it was concluded from this study that if triadimefon is altering the physiology of the host plant to prevent Fusarium Blight, triadimefon is not accomplishing disease prevention by reducing the transpiration rates, to conserve water and reduce water stress.

The overall lower T/A ratio of the 500 ug ml^{-1} application of CGA 64251 compared to the control occurred through increased assimilation and not by reduced transpiration. The exact mode of action of CGA 64251 in the plant is not known. Increased assimilation suggests that the darker green color may be due to increased chlorophyll content. Application of CGA 64251 on apple trees to control apple scab resulted in shortened internodes and thicker, darker green leaves. The treated apple leaves had 3-5 layers of palisade cells while the control leaves had 2-3 layers of palisade cells. This would suggest the higher assimilation response to triadimefon treatment, the mode of action of these two compounds appears to be different.

It is unclear at this time if CGA 64251 could be used to reduce transpiration rates. It is possible that when the plant undergoes water stress and the stomata close, assimilation may continue at a higher rate than would normally occur under these conditions, thus allowing the plant to better survive the stress.

Significant stimulation of transpiration due to benomyl in the first experiment indicates alteration of some mechanism in the plant other than the micro-morphological

structure. The 50 ug ml^{-1} application is equal to a very high field rate. The T/A ratio was not different from the control which indicated a wider stomatal aperture and a higher rate of gas exchange.

Benomyl is relatively immobile in the soil (Helling et al, 1974). When applied as a soil drench at field rates uptake occurs for a few days, but benomyl is rapidly tied up in the soil eliminating further uptake (Peterson and Edgington, 1970). High levels of benomyl result in a longer period of availability. Benomyl transport occurs in the apoplast with none being transported to new foliage once root uptake terminates. In bean leaves five days after the supply of benomyl is removed from the roots, no benomyl is found in the stems or central areas, indicating it is translocated to the edges of the leaves in the transpiration stream (Peterson and Edgington, 1970). The 50 ug ml^{-1} treatment of benomyl one day after application was at a concentration in the plant to stimulate the transpiration (Table 3). The 500 ug ml^{-1} treatment was apparently too high to cause an initial increase in transpiration. However, as benomyl levels decreased in the plant by day 8, benomyl was at a concentration that stimulated transpiration.

Benomyl, a derivative of benzimidazole, and benzimidazole have been related to cytokinins by having similar activities of delayed protein breakdown, increased chlorophyll retention and increased transpiration in detached leaves (Person et al, 1957; Samborski et al, 1958).

Benomyl has shown similar effects as cytokinins on soybean callus (Skene, 1972) and celery seed germination (Thomas, 1974). Kinetin has also been shown to increase transpiration in excised leaves by opening the stomata of grasses (Incoll and Whitelam, 1977). The amount of stimulation of transpiration is concentration dependent. Thus, cytokinin-like properties of benomyl in the plant may explain why the stomata opened and increased gas exchange in the first experiment. In the second experiment at field rates the concentrations were not high enough to cause the stomata to open.

Benomyl at normally applied field rates will not alter the number of stomata as indicated by no changes in the gas exchange rates after 3 weeks (Figure 1 & 2). Apparently the levels of benomyl are not maintained high enough in the developing leaf tissue to cause micro-morphological changes.

Benzimidazole has also been shown to increase the cell size of pea epicotyls and increase uptake of water within the cell (Galston et al, 1953). Constant exposure of duckweed (Lemna minor) to benzimidazole increased the frond size (Hillman, 1955). The increase in frond size was due to an increase in the size of the cells and not more cells per frond. The larger epidermal cells reported in a previous paper (Kaufmann and Williamson, 1981) were apparently due to high rates of benomyl applied repetitively to the soil which maintained high levels in the developing tissues.

Since normal field rates of benomyl applied under both wet and dry regimes did not alter gas exchange rates, it is

concluded that the concentration of benomyl within the plant is too low to cause a significant stimulation of transpiration in a cytokinin-like response or an enlargement of cells. Thus, the application of benomyl as a plant growth regulator to alter the gas exchange rate is not feasible in the field because of the prohibitive costs of high rates and repeated applications.

LITERATURE CITED

1. Buchenauer, H. and F. Crossman. 1977 Triadimefon: mode of action in plants and fungi. Neth. J. Pl. Path. 83 (Suppl 1_:93-103.

2. Cale, J. and R.M. Hagan. 1966. Plant antitranspirants. Ann. Rev. Plant Phys. 17:269-282.

3. Galston, A.W., R.S. Baker, and J.W. King. 1953. Benzimidazole and the geometry of plant growth. Physiol. Plant. 6:863-872.

4. Gilpatrick, J. D. 1979. Control of apple and stone fruit diseases with CGA 64251. Phytopath. 69:1028.

5. Hardison, J.R. 1974. Control of stripe smut and flag smut in Kentucky bluegrass by a new systemic fungicide, BAY MEB 6447. Crop Science 14:769-770.

6. Helling, C.S., D.G. Dennison, and D.D. Kaufman. 1974. Fungicide movement in soils. Phytopath. 64:1091-1100.

7. Hillman, W.S. 1955. The action of benzimidazole on Lemna minor. Plant Phys. 30:535-542.

8. Incoll, L.D. and C.C. Whitelam. 1977. The effect of kinetin on stomata of the grass Anthephora pubescens Nees. Planta 137:243-245.

9. Kaufmann, J.E. and D.R. Williamson. 1981. Chemical manipulation of stomatal number and behavior in Merion Kentucky bluegrass (Poa pratensis L.). Fourth Int. Turf. Res. Proc. 501-508

10. Kelley, R.D. and A.L. Jones. 1981. Evaluation of two triazole fungicides for postinfection control of apple scab. Phytopath. 71:737-742.

11. Person, C., D.J. Samborski, and F.R. Forsyth. 1957. Effect of benzimidazole on detached wheat leaves. Nature 180:1294-1295.

12. Peterson, C.A. and L.V. Edgington. 1970. Transport of the systemic fungicide, benomyl, in bean plants. Phytopath. 60:475-478.

13. Samborski, D.J., F.R. Forsyth, and C. Person, 1958. Metabolic changes in detached wheat leaves floated on benzimidazole and the effect of these changes on rust reaction. Can. J. Bot. 36:591-601.

14. Sanders, P.L., L.L. Burpee, H. Cole, Jr., J.M. Duich. 1978. Uptake, translocation, and efficacy of triadimefon in control of turfgrass pathogens. Phytopath. 68:1482-1487.

15. Skene, K.G.M. 1972. Cytokinin-like properties of the systemic fungicide benomyl. J. Hort. Sc. 47:179-182.

16. Smiley, R.W. and M.M. Craven. 1977. Control of benzimidazole-tolerant Fusarium roseum on Kentucky bluegrass. Plant Dis. Rep. 61:484-488.

17. Staub, T., F. Schwinn, and P. Urech. 1979. CGA 64251, a new broad-spectrum fungicide. Phytopath. 69:1046.

18. Thomas, T.H. 1974. Investigations into the cytokinin-like properties of benzimidazole-derived fungicides. Ann. Appl. Biol. 76:237-241.

APPENDIX

APPENDIX TABLE 1. Composition of the nutrient solution.

Chemical	ml. stock solution per liter of nutrient solution	ug ml^{-1} of element in nutrient sol.
(1) M-KH_2PO_4	1	K: 39.1; P: 30.9
M-KNO_3	6	K: 234; N: 84
M-$Ca(NO_3)_2$	3	Ca: 120; N: 74
M-$MgSO_4$	2	Mg: 48; S: 64
(2) Micronutrients	gm L^{-1} of H_2O	ug ml^{-1}
H_3BO_3	2.86	B: 0.5
$Mn_2.4H_2O$	1.81	Mn: 0.5
$ZnSO_4.7H_2O$	0.22	Zn: 0.05
$CuSO_4.5H_2O$	0.08	Cu: 0.02
$H_2MoO_4.H_2O$	0.02	Mo: 0.01
Sequestrene 330Fe(10%Fe)*		Fe: 5

(1) M stands for Molar Concentration.

(2) Composition of stock solution. One milliliter per liter of nutrient solution was used.

*Iron was supplied with Sequestrene 330Fe, a chelate product containing 10%Fe. The iron chelate stock solution was prepared by dissolving 50 g sequestrene indistilled water brought to 1 liter volume. One ml of stock solution per liter of nutrient solution twice a week.