THE EFFECTS OF TERBACIL ON CO_2 EXCHANGE, TRANSPIRATION, DIFFUSIVE RESISTANCE, AND YIELD OF ALFALFA

By

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Bachelor of Science in Arts and Sciences

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Stillwater, Oklahoma

1980

Submitted to the faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1983

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ACKNOWLEDGMENTS

I wish to express my appreciation to my major adviser, Dr. Jimmy F. Stritzke for his guidance, training, and patience throughout the course of this study. Gratitude is also extended to Dr. Lavoy Croy and Dr. Eddie Basler for their assistance and advice while serving as members of my graduate committee.

My sincere thanks is offered to my parents Bob and Shirley Winton for their love and encouragement through all the years. I am grateful to those special friends who provided so much support.

I extend appreciation to the Department of Agronomy at Oklahoma State University for use of their facilities and financial support. I also extend thanks to Melanie Bayles for her typing assistance.

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CHAPTER I

INTRODUCTION

Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) is a herbicide with selective photosynthetic inhibition used for control of both annual and some perennial weeds in alfalfa (*Medicago sativa* L.). Use rates vary from 0.55 to 1.0 kg/ha. The lower rate is effective for control of cool season weeds if applied during the dormant season and the higher rates can be effective for control of summer grasses.

There have been some alfalfa yield increases associated with the lower rates even when weeds were not a problem. These increases may be associated with effects on transpiration similar to those reported for some of the triazine herbicides. The purpose of this research was to evaluate the effects of terbacil rates on alfalfa yield as well as to evaluate its effects on some of these physiological processes such as CO_2 exchange, transpiration, and diffusive resistance.

CHAPTER II

REVIEW OF LITERATURE

Herbicide effects on alfalfa. Alfalfa (Medicago sativa L.) is a very high quality legume forage that originated in Southwest Asia. It was believed first cultivated in Iran. The first state recorded to grow alfalfa in the USA was Georgia in 1736. Alfalfa is often referred to as the "Queen of Forages" because it is the highest quality of all the common hay crops (4). It serves mainly the dairy and horsemen due to its high protein content.

Weeds are a problem in alfalfa hay production. Animal production losses result from lower quality weed infested hay (9). Weeds also may increase hay curing time, reduce stands, and serve as alternate hosts for insect and disease pests (22). Cords (9) reported a decrease in forage quality because percent protein of alfalfa forage was negatively correlated to weed content. Dutt et al. (10) reported a 20% increase in milk production as well as an increase in efficiency in dry matter conversion into milk when quackgrass (Agropyron repens (L.) Beauv.) was controlled in alfalfa with pronamide [3,5-dichloro-N-(1,1-dimethyl-2-propynyl) benzamide]. Waddington (44) reported an increase in alfalfa seed production when dandelion (Taraxacum

officianale Wiggers) and perennial sowthistle (Sonchus arvensis L.) were controlled. Weed control in older alfalfa stands may also increase stand longevity. Alley and Lee (1) reported that alfalfa yields were doubled in weed controlled plots versus untreated check three growing seasons after herbicide application.

Physiological effects have been reported for crop plants even though crop plants are resistant to particular herbicides (31). Alfalfa injury has been reported with higher rates of the triazine and substituted uracil herbicides, especially simazine, metribuzin and terbacil (2, 20, 33, 40, 46, 47). Alfalfa cultivars vary in their susceptibility to triazine herbicides as demonstrated by Harvey et al. (14). Wilson (47) reported that increasing the rates of metribuzin and simazine above the amounts needed for effective weed control caused an increase in the protein content of alfalfa. This may be due to an increase in nitrogen content as was found in corn (Zea mays L.) by Fink and Fletchall (12) and Ries and Gast (32). Cords (9) found an increase in protein content of alfalfa with application of simazine and terbacil. Shabir and Fletcher (35) reported that diuron decreased transpiration and increased water use efficiency in corn. Van Oorschot (43) also discussed the role of certain herbicide groups such as phenoxys, triazines, and ureas on water uptake, stomatal aperature, transpiration, and photosynthesis. Urea and triazine herbicides have been reported to reduce

transpiration of excised bean (Phaseolus vulgaris L.) leaves and cotton (Gossypium hirsutum L.), corn, and soybeans (Glycine max (L.) Merr.) leaves (29, 41, 46).

The biological activity of terbacil was first reported by Bucha et al. (7). It is a selective herbicide which is absorbed through the roots and translocated apoplastically to the site of action in the mesophyll cells (3, 5). Terbacil is a photosynthetic inhibitor which inhibits the Hill reaction (15). The terbacil tolerance mechanism in orange (Citrus sinensis L. 'Koethen Sweet Orange') (18) and alfalfa is its metabolism to non-toxic derivatives. Rhodes (31) reported on the different metabolites formed by alfalfa.

Terbacil is currently listed as a recommended alfalfa herbicide in Oklahoma and often is included as a standard herbicide treatment. Some forage yield increases have been noted when terbacil was applied and these increases were not necessarily correlated to weed control (39). Increased production might be due to some physiological changes. For example, Tucker and Mansfield (41) hypothesized that transpiration could be reduced without a comparable reduction in photosynthesis since diffusive resistance constitutes a greater part of the whole diffusion pathway for water than for carbon dioxide. This might result in water being used more efficiently without a serious suppression of crop growth. Similar results were noted by

Shabir and Fletcher (35). These differences in production appeared more prominent when plants were subjected to drought conditions.

Photosynthetic measurements. Many methods have been described as means of measuring photosynthesis. One is a semi-closed compensating system for the control of CO_2 and water vapor concentrations and the calculation of their exchange rates described by Samish and Pallas (34). Another method described by Naylor and Teare (30) involves the use of $^{14}CO_2$ labeling. One method commonly used involves measuring the amount of carbon dioxide injection necessary to keep the carbon dioxide level at 310 ul/l in a sealed growth chamber. Wolf et al. (48) describes the construction and operation of chambers which measure net carbon exchange of intact leaves in which a leaf is sealed into the chamber by positive air pressure. Probably the simplest and fastest method of measuring carbon dioxide exchange is one described by Clegg et al. (8). This method involves inserting the intact leaf into a closed plexiglass chamber and drawing out two air samples. One sample is taken from ambient air conditions, and the other is taken after the leaf has been exposed to light. The air samples are then injected into a infrared gas analyzer.

Transpiration measurements. Transpiration, the physiologic process by which a plant exchanges water with its environment, is affected by many environmental factors such as water stress, illuminance, carbon dioxide

concentrations, and diffusive (or stomatal, or leaf) resistance. For example, as illuminance increases, stomatal opening will cause a progressive lowering of stomatal resistance, hence an increase in transpiration (11). In some plants stomatal resistance is similar in both their upper and lower epidermal surfaces. Alfalfa is one of these species since it has a similar number and size of stomates on both surfaces (11).

Diffusive resistance measurements. There has been a discrepancy over methodology of measuring the stomatal diffusive resistance directly (42, 45). Slatyer (37) and Barrs (6) described methods to indirectly measure diffusive resistance. Other discrepancies have been reported in the method of calibration of stomatal diffusion porometers (19, 42). A new instrument, a steady state porometer[1], has recently become available, which rapidly and precisely measures both water loss and diffusive resistance. The advantage of the steady state porometer is that it takes into account many of the environmental factors and either holds constant or does not disturb such factors as light level, CO_2, relative humidity, ambient temperature, leaf temperature, wind, and leaf water potential (25).

Water stress. In some physiologic studies it is crucial to maintain a particular level of water stress for

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[1]Li-Cor Inc., Lincoln, NE.

extended lengths of time. This is accomplished by using osmotic agents in hydroponic solutions. When plants are grown in soil, the physiological study must be very short so the water potential of the soil will not change much, or they must involve repeated drying cycles.

Methods of producing water stress by an artificial osmoticum has also been an issue of controversy (27, 28, 38). Lawlor (23) concluded that polyethylene glycols (PEG) with a molecular weight of 1000 or greater were not absorbed by plants with undamaged roots. He also concluded that other osmoticums such as manitol were absorbed. Kaufmann et al. (21) concluded also that larger polyethylene glycol molecules are more useful in simulation of water stress than small molecular weights. Toxic properties of polyethylene glycol have been suspected by Leshem (24) but Lawlor (23) dismissed this. Jackson (17) disclaims the use of polyethylene glycol on the grounds that it inhibits the elongation of root hairs. The van't Hoff equation which is used to determine the amount of polyethylene glycol necessary to create a particular water potential is also subject to criticism (27, 36, 38).

CHAPTER III

METHODS AND MATERIAL

Field experiments. The field experiments were located at two experiment stations in Oklahoma. One experiment was located at the Oklahoma State University Eastern Research Station at Haskell and will hereafter be referred to as Experiment I. Two experiments were located at the Agronomy Research Station at Perkins. The March applied experiment will be referred to as Experiment II and the June applied experiment as Experiment III.

The experimental design of all field experiments was a randomized complete block arrangement of treatments. Herbicide treatments varied from 0.14 to 1.2 kg/ha of terbacil. All treatments were replicated four times. Herbicide applications were made with a hand-held carbon dioxide sprayer equipped with 8003 flat fan nozzles. The carrier volume was 187 l/ha and the pressure was 1.5 kg/ha. All alfalfa stands were planted using a Brillion seeder. Plots were harvested by means of a Carter flail type harvester when the plants reached 10% bloom.

Experiment I was harvested on the following dates: May 4, June 17, July 21, and August 19, 1982. Experiment II was harvested on the following dates: May 3, June 16, July 16,

and August 16, 1982. Experiment III was harvested on June 16, July 16, and August 16, 1982. Weeds, when present, were determined by visually estimating composition (26). See Table 1 for additional details for the individual experiments.

Physiological data (photosynthesis, transpiration, and diffusive resistance) were taken from Experiments II and III. Photosynthesis was measured in the field by monitoring the rate of carbon dioxide exchange. This was accomplished by using the closed chamber method described by Wolf, Pearse, Carlson and Lee (48) and Clegg et al. (8). Transpiration and diffusive resistance of leaves were measured with a steady state porometer[1] equipped with a special apperature (0.6 cm^2) designed especially for alfalfa leaves. Measurements were confined to the uppermost, first fully expanded leaf.

Controlled environment experiments. All plants in the controlled environment experiments were clones from a single group of parent plants ('Kanza' variety) that were taken from the field August 10, 1981. Plants were vegetatively propagated by cutting the parent plants into two-node sections and dipping the basal 2 cm segment into a root stimulator (0.067% naphthaleneacetamide/0.033% 2-methyl-1-naphthaleneacetic acid/ 0.013% 2-methyl-1-naphthaleneacetamide/0.057% indole-3-buteric acid) with a

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[1]Li-Cor Model 1600, Li-Cor Inc., Lincoln, NE.

Table 1. Seeding and spraying information, measurements taken, and other general information for field experiments.

	Experiment		
	I	II	III
Seeding information			
Alfalfa variety	Kanza	Riley	Riley
Seeding data	Apr 1, 1981	Mar 13, 1981	June 21, 1981
Spray information			
Date of application	Mar 18, 1982	Mar 16, 1982	June 21, 1982
Relative humidity	76%	78%	81%
Soil temperature (10 cm)	16 C	11 C	22 C
Soil moisture	excellent	excellent	excellent
Wind speed (km/hr)	16	0-3	2
Growth stage at spraying	18 cm	8 cm	post-first harvest
Measurements			
Forage production	yes	yes	yes
Photosynthesis	no	yes	yes
Transpiration	no	yes	yes
Field information			
Irrigation	no	July 21, 1982	July 21, 1982
Soil type	taloka silt	sandy loam	sandy loam
Plot dimensions	1.83 X 4.58	1.83 X 3.06	2.14 X 3.90

fungicide (4% tetramethylthiuramdisulfide). All leaves except the terminal trifoliolate (used as an indicator of water relations) were excised before placing onto a mist bench. The basal 2.5 cm section of the stem was then placed in sterile sand. Sand filled flats were maintained on a mist bench (12 sec spray/12 min) until the stems rooted. A solution of granular 15-30-15 plant food[2] made into a 3 g/l (w/v) solution served as nutrient media. Greenhouse temperature was maintained at approximately 27 C. When plants had established a root system, they were then transferred to 25 cm by 4 cm plastic containers filled with 50% perlite/50% sand (v/v). Malathion (diethylmercapto-succinate, 5-ester with 0,0-dimethyl phosphorodiothioate) was used to control insects. Plants were cut to a 2 cm stubble height each time they reached 10% bloom. Light intensities in the greenhouse varied from 400 to 600 uE/m^2/sec on a cloudy day to 900-1700 uE/m^2/sec on sunny days.

Plants were transferred to the hydroponic conditions and allowed to acclimate for 1 week before treatments were applied. This involved placing plants in 500 ml amber jars filled with one-half strength modified Hoagland's solution (Appendix A, Table 17). The system solutions were aerated by plastic tubing connected to a 4 W aquarium pump. Plants were supported by wax coated corks.

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[2]Miracle Gro, Sterns Nurseries Inc.

Herbicide treatments were applied via the nutrient solution. Terbacil rates varied from 0 to 10 ppm depending on the experiment. See Table 2 for lists of individual treatments. The terbacil used in the experiments was an 80% wettable powder formulation.

Water stress effects were attained by addition of polyethylene glycol (PEG) as an osmoticum. A 400 MW PEG and a 1000 MW PEG were used in the Greenhouse experiment I. In successive experiments only PEG 1000 was used. Water potentials range from 0 to -8 bars (Table 2). Water potentials were calculated by the van't Hoff equation.

Transpiration in the greenhouse experiments was monitored by weighing the plants in the jars at timed intervals and determining the amount of water loss per leaf area. Leaf area was determined by means of a leaf area meter.[3] Photosynthesis was measured at approximately 1:00 PM on bright, sunny days. The closed chamber method was used to measure photosynthesis (8). Greenhouse experiment I was a completely randomized design and Greenhouse experiment II was a randomized complete block design.

The growth chamber dimensions were 0.79 by 1.82 by 1.22 m. The light intensity inside the growth chamber was approximately 300 uE/m^2/s (measured by a quantum sensor[4]

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[3]Li-Cor Model 1600, Li-Cor Inc., Lincoln, NE.

[4]Li-Cor Model Li-1905, Li-Cor Inc., Lincoln, NE.

Table 2. Listing of treatments applied during controlled environment experiments.

Greenhouse I[a]		Greenhouse II[b]		Growth Chamber[c]	
Terbacil	Water Stress	Terbacil	Water Stress	Terbacil	Water Stress
(ppm)	(bars)	(ppm)	(bars)	(ppm)	(bars)
0.00	0	0.0	0	0.0	0
0.001	0	0.0	-4	0.01	0
0.01	0	0.5	-4	0.10	0
0.10	0	5.0	-4	1.0	0
1.00	0	0	-8	0.0	-6
10.00	0	0.5	-8	0.01	-6
0.00	-2 PEG 400	5.0	-8	0.10	-6
0.00	-4 PEG 400	5.0	0	1.0	-6
0.00	-8 PEG 400	-	-	-	-
0.00	-2 PEG 1000	-	-	-	-
0.00	-4 PEG 1000	-	-	-	-
0.00	-8 PEG 1000	-	-	-	-

[a]Treatments were applied February 18, 1982.

[b]Treatments were applied April 6, 1982.

[c]Treatments were applied May 24, 1982.

attached to the sensor head of the steady state porometer). The quantum sensor measures the photosynthetically active radiation in the 400-700 nm wave band. Other conditions were 12 hr light, 31 C temperature, and 50% humidity. Night conditions were maintained at 12 hr darkness, 21 C temperature, and 95% humidity.

In the growth chamber experiment, measurements of transpiration and diffusive resistance were taken with the steady state porometer. Measurements were confined to the uppermost first fully expanded leaves to decrease variation in the stomatal aperatures (13). The measurements were taken each day 2 to 4 hours after the plants had been exposed to the lights.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I. Significant differences in alfalfa forage yield resulted at the May 4 and the July 21 harvests (Table 3). At the May 4 harvest there was some decreased production with all rates of terbacil with the production from the three highest rates being significantly lower than the untreated plots (Table 3). This reduction might be attributed to failure of the alfalfa plants to be completely dormant at application (18 cm tall). There were no significant differences in forage yields among treatments at the June harvest. By the July 21 harvest, the lowest alfalfa yield resulted in the untreated plots and it was significantly lower than the yields from three of the terbacil treatments (0.28, 0.56, and 1.12 kg/ha). Weed control would not appear to be a major factor with these yield increases since weed infestations were low and not different among treatments. Even though differences were not great at the August 19 harvest, trends were similar to the July 21 Harvest. That is, alfalfa yields from all terbacil treatments were higher than yields from the untreated plots.

Increases in alfalfa yield of the terbacil treated

Table 3. The effects of various rates of terbacil[a] on forage production in Experiment I at Haskell.

	Harvest dates							
	May 4	June 17	July 21		August 19		Season total	
Terbacil rate	Alfalfa	Alfalfa	Alfalfa	Weed[b]	Alfalfa	Weed[c]	Alfalfa	Forage
(kg/ha)	(kg/ha)							
0.00	5194	4741	3438	57	1276	69	14651	14776
0.14	4600	4781	3906	66	1399	87	14686	14841
0.28	4698	4941	4257	75	1590	102	15488	15665
0.56	4222	4863	4421	45	1719	85	15225	15355
0.84	3815	4717	3655	163	1465	128	13652	13943
1.12	3329	4944	4656	102	1641	95	14570	14767
LSD(0.05)	611	NS	786	NS	NS	NS	1066	NS
LSD(0.10)	503	NS	645	NS	272	NS	877	877

[a]Treatments were applied March 18, 1982.

[b]Weeds in the plots consisted of crabgrass (Digitaria sanguinalis (L.) Scoop) and other grasses.

[c]Weeds in the plots consisted of crabgrass and carpetweed (Mollugo verticillata L.).

plots at the July and August harvests may be a result of physiological changes which occur when plants are both water stressed and terbacil treated. The Haskell area received only 8.15 cm and 6.02 cm of rain in July and August respectively, (Appendix B, Table 18). No terbacil treatments resulted in season total yields which were significantly different from the untreated plots. Since the untreated plots yielded higher than terbacil treated plots at the May harvest and lower than the terbacil treated plots at subsequent harvests, the effects cancel themselves with respect to the season totals. The terbacil may be affecting a basic function of the alfalfa plants such as transpiration or photosynthesis. This effect of the terbacil may keep the alfalfa from going dormant when it is drought stressed, hence the increase in alfalfa yield is only seen in times of drought stress. Another possibility is the decrease in water used by the alfalfa in treated plots early in the season and this simply left more water in the soil profile for later in the season.

Experiment II. No significant differences in forage yield of alfalfa and weeds were observed among treatments at any harvest (Table 4). Weeds were not a major problem in this experiment and only at the August 16 harvest were there enough to record. Weeds were pigweed (*Amaranthus* spp) and crabgrass (*Digitaria sanguinalis* L. (Scoop)) with yields being less than 650 kg/ha.

Photosynthesis as measured by CO_2 exchange rate (mg

Table 4. The effects of various rates of terbacil[a] on forage production in Experiment II at Perkins.

	Harvest dates						
	May 3	June 16	July 16	August 16		Season total	
Terbacil rate	Alfalfa	Alfalfa	Alfalfa	Alfalfa	Weed[b]	Alfalfa	Forage
	(kg/ha)						
0.00	4347	4436	3625	2880	306	15291	15597
0.14	4402	4859	3790	3307	224	16358	16602
0.28	4195	4959	3317	2418	570	14893	15462
0.56	4208	4222	3488	2790	419	14708	15127
0.84	4324	5043	3304	2753	296	15425	15720
1.12	4832	4524	3209	2774	623	15338	15961
LSD(0.05)	NS	NS	NS	NS	NS	NS	NS

[a]Treatments were applied March 16, 1982.

[b]Weeds in the plots consisted of pigweed (Amaranthus spp) and crabgrass (Digitaria sanguinalis (L.) Scoop).

$CO_2/dm^2/hr$) was unaffected by any of the terbacil treatments. There were no significant differences among treatments at either date that measurements were taken (July 14 and July 15) (Table 5). There are two possible explanations for the lack of significant differences of CO_2 exchange among the terbacil treatments. One, terbacil does not affect photosynthesis of alfalfa when applied at the given rates in the field, or two, terbacil does not affect photosynthesis of the plants when the plants are not water stressed.

No significant differences in transpiration were measured. All transpiration measurements in Experiment II July 15 were within one $ug/cm^2/s$ on (Table 5). This may be because the plants were not water stressed. Transpiration rate measurements were more variable and lower on August 18. The lowest transpiration rate resulted in the untreated and lowest rate of terbacil plots, and the highest resulted in plots with the highest rate of terbacil; however, none of these differences were significant. These plants may have been starting to be mildly water stressed since the last water they received was the irrigation on July 21. Even though the differences were not significant the mild water stress in addition to the hot August weather may have induced the trends seen in the August 18 transpiration measurements.

The only real difference observed in Experiment II

Table 5. The effect of various rates of terbacil[a] on physiological responses of alfalfa in Experiment II at Perkins.

Treatment	CO_2 exchange		Transpiration		Diffusive resistance	
Terbacil	July 14	July 15	July 15	August 18	July 15	August 18
(kg/ha)	(mg $CO_2/dm^2/hr$)		(ug/cm^2/s)		(s/cm)	
0.00	12.39	12.35	31.98	16.30	0.810	1.120
0.14	10.68	14.89	32.05	15.85	0.639	1.262
0.28	12.17	12.59	31.74	17.30	0.486	1.120
0.56	10.48	14.40	32.00	16.45	0.660	1.400
0.84	9.20	14.38	31.95	19.97	0.604	0.902
1.12	11.97	12.90	31.15	20.06	0.678	0.855
LSD (0.10)	NS	NS	NS	NS	NS	0.318

[a]Treatments were applied March 16, 1982.

physiological measurements were in diffusive resistance on August 18. These differences were significant at the 10% level. The lowest diffusive resistance measurements were observed in plots treated with 0.56 kg/ha terbacil.

Differences in alfalfa yield responses resulted between Experiments I and II. Two possible explanations might be differences in conditions of plants at spraying and of differences in growing conditions during the growing season. The terbacil was applied in Experiment II on March 16 when the plants were only approximately 8 cm tall compared to a March 18 application date in Experiment I with plants already 18 cm tall. Decreased moisture stress may also have been a factor since this site received 36.47 cm of rain in May, 13.2 cm of rain in June, 9.27 cm of rain in July plus an additional 7.5 cm of water applied by overhead irrigation (Appendix B, Table 18). The increased forage yield observed in Experiment I may be due to some changes in physiological process induced by water stress and may involve plant water maintenance. No production increase or differences in photosynthesis or transpiration were observed in Experiment II when plants were not considered to be water stressed.

Experiment III. There were no yield increases in this experiment that could be attributed to terbacil. Forage yields on June 16 were taken before the plots were treated with various rates of terbacil (Table 6). There were significant decreases in alfalfa production at the July 16 harvest associated with the two highest rates of terbacil

Table 6. The effects of various rates of terbacil[a] on forage production when applied post-first harvest on alfalfa in Experiment III at Perkins.

Terbacil rate	Harvest Dates: June 16 Alfalfa	July 16 Alfalfa	August 16 Alfalfa	Season total
(kg/ha)	(kg/ha)			
0.00	4016	4111	2643	10771
0.14	3799	4077	2790	10667
0.28	3853	3936	2769	10558
0.56	3976	3743	2710	10429
0.84	4004	3338	2396	9738
1.12	3768	2972	2250	8990
LSD(0.05)	NS	438	NS	912

[a]Herbicide applied June 21, 1982.

(Table 6). The same trends of decrease in production at higher terbacil rates occurred at the August 16 harvest, however the differences were not significant. There was a significant reduction in season total alfalfa production at the two highest rates of terbacil. The decrease in forage yields at these two highest rates was primarily attributed to herbicide injury.

The 1.12 kg/ha rate of terbacil significantly decreased transpiration compared to other treatments at the time that the July 2 measurements were taken (Table 7). However, effects on transpiration were short lived since no trends were noticeable and no significant differences occurred by the July 14 measurements. The untreated check had the lowest transpiration rate at the time the August 18 measurements were taken, however this difference was not significant.

The 1.12 kg/ha rate of terbacil also significantly increased the diffusive resistance at the time the July 2 measurements were taken (Table 7). The untreated check plots also had the highest diffusive resistance at both the July 14 and August 18 measuring dates, however these differences were not significant at the 5% level.

Greenhouse experiment I. No significant differences in the rate of CO_2 exchange were observed among plants before the treatments were applied (Table 8). Greenhouse environmental conditions for the various times are listed in Appendix B, Table 19. The 1 ppm and 10 ppm terbacil

Table 7. The effects of various rates of terbacil[a] on physiological responses of alfalfa when applied post-first harvest in Experiment III at Perkins.

Treatment	Transpiration			Diffusive Resistance		
Terbacil	July 2	July 14	August 18	July 2	July 14	August 18
	($ug/cm^2/s$)			(s/cm)		
0.00	23.03	14.97	16.56	0.458	0.911	0.752
0.14	23.47	14.10	24.38	0.472	0.906	0.506
0.28	23.49	15.08	21.35	0.452	0.826	0.581
0.56	24.28	16.22	19.43	0.434	0.754	0.571
0.84	20.76	16.05	21.24	0.536	0.763	0.511
1.12	16.70	16.14	21.71	0.801	0.747	0.533
LSD(0.05)	3.50	NS	NS	0.191	NS	NS

[a]Treatments were applied June 21, 1982.

Table 8. CO_2 exchange rates measured during Greenhouse I.

Treatment			CO_2 exchange							
			Before treatment				After treatment[a]			
Terbacil		PEG	Feb 6	Feb 9	Feb 11	Feb 16	Feb 21 (3 days)	Feb 24 (6 days)	Feb 26 (8 days)	Feb 28 (10 days)
(ppm)	(bars)	(mw)	(mg $CO_2/dm^2/hr$)							
0			13.4	14.1	13.5	6.4	20.1	19.6	14.2	12.2
0.001			10.5	15.4	14.8	5.8	15.1	15.0	21.5	20.0
0.010			9.5	13.3	16.6	5.3	18.7	24.4	19.3	19.4
0.100			13.2	16.3	16.0	4.4	13.0	17.1	20.9	15.3
1.000			14.1	17.3	17.8	8.5	-1.4	-0.9	0.7	0.6
10.000			7.2	15.3	12.8	5.0	-1.8	-2.4	-4.6	-1.5
0	-2	400	17.1	17.3	16.7	4.9	10.9	9.5	8.3	13.6
0	-4	400	12.5	13.3	17.0	2.3	13.6	8.4	11.9	5.9
0	-8	400	20.2	21.5	18.0	7.8	10.6	8.7	6.4	1.3
0	-2	1000	12.2	19.4	18.7	6.7	12.6	14.1	17.9	12.1
0	-4	1000	8.9	12.3	11.2	8.5	8.0	12.0	8.8	10.9
0	-8	1000	10.0	16.6	19.3	4.1	4.8	10.5	8.4	4.7
LSD(0.05)			NS	NS	NS	NS	8.2	8.0	14.3	7.3
LSD(0.10)							6.7	6.5	11.7	6.0

[a]Treatments were applied February 18, 1982.

treatments significantly decreased CO_2 exchange as compared to all other treatments on all days after the plants were treated (Table 8). The 10 ppm terbacil resulted in severe leaf chlorosis within 3 days after treatment. The 1 ppm terbacil treatment resulted in yellowing of new leaves and slight loss of turgidity 6 days after treatment. Since the three lowest rates of terbacil (0.1, 0.01, and 0.001 ppm) did not decrease CO_2 exchange rate this indicated that alfalfa could metabolize the three lower terbacil rates sufficiently to maintain full photosynthetic capacity. There was also a significant increase in CO_2 exchange 10 days after treatment with 0.001 ppm terbacil. No injury symptoms occurred with the three lower rates.

A decrease in CO_2 exchange occurred after 3 and 6 days of exposure to the PEG (Table 8). This decrease in CO_2 exchange would be expected since plant stomates close when the plant is water stressed (36). This would decrease the amount of CO_2 which could enter. None of the water stress conditions significantly reduced CO_2 exchange by 8 days after treatment and only water stress of -8 bars significantly reduced CO_2 exchange 10 days after treatment. It appears the alfalfa plants adjusted to the water stress over time and this agrees with what Hsiao (16) reported for bean plants.

No significant differences in transpiration rate were observed among plants before they were treated with terbacil (Table 9). Environmental conditions varied daily in the

Table 9. Transpiration rates measured during Greenhouse I before treatments were applied.

Treatment			Transpiration[a]							
Terbacil		PEG	Day[b] Feb 2	Day Feb 4	Night[c] Feb 5	Night Feb 6	Day Feb 9	Night Feb 9	Day Feb 11	Day Feb 15
(ppm)	(bars)	(MW)	($mg/cm^2/hr$)							
0			17.0	44.3	38.3	35.9	119.6	24.4	71.7	40.3
0.001			19.8	52.1	34.9	32.4	109.0	25.9	76.1	36.7
0.010			18.4	43.3	29.1	26.2	91.9	19.8	64.5	33.3
0.100			19.8	46.2	34.5	36.2	115.3	23.8	72.0	38.5
1.000			20.0	44.2	34.4	31.7	105.1	23.9	72.1	32.4
10.000			21.2	53.9	36.5	34.7	106.3	27.4	76.5	31.3
0	-2	400	19.0	25.6	35.0	34.5	94.0	17.3	56.7	21.9
0	-4	400	19.5	52.0	39.1	36.3	119.5	23.9	71.3	43.5
0	-8	400	16.6	33.2	28.8	23.7	93.3	17.8	59.9	37.8
0	-2	1000	17.4	41.5	38.8	34.1	130.5	24.2	82.2	40.3
0	-4	1000	20.8	52.1	39.3	35.4	122.4	25.6	74.9	34.7
0	-8	1000	17.4	40.3	27.8	29.2	97.5	21.5	66.1	34.0
LSD(0.05)			NS	NS	NS	NS	NS	NS	NS	NS

[a]Values given are an average of two measurements.

[b]Values represent measurements taken while plants were exposed to only day conditions (see Appendix B, Table 19 for times measurements were taken).

[c]Values represent measurements taken after plants were exposed to both day and night conditions.

greenhouse (Appendix B, Table 19). The 1.0 and 10.0 ppm terbacil treatments resulted in a significant decrease in transpiration 1, 4, 5 and 8 days after treatment (Table 10). Only the 10 ppm terbacil treatment resulted in a significant decrease in transpiration 2, 3, and 6 days after treatment. The 1 ppm treatment did not result in a significant decrease of transpiration 3 and 6 days after treatment and this was attributed to those days being overcast.

All levels of water stress with both 400 and 1000 mw PEG resulted in significantly reduced transpiration 1 day after treatment (Table 10). All water stress levels except the -2 bars using 1000 mw PEG, significantly decreased transpiration 3, 4, and 8 days after treatment. The 400 mw PEG was probably absorbed, hence the decrease in transpiration of plants treated with 400 mw PEG may be due to tissue damage as well as water stress (23). The 400 mw PEG resulted in brown tips and dark veins and the leaves, indicating that it was being absorbed. This is consistent with the findings of others (23). Symptoms of water stress appeared before signs of PEG absorption when plants were treated with 1000 mw PEG. The 1000 mw PEG gave more consistent results than the 400 mw PEG. That is, the -2 bar stress level consistently resulted in the highest transpiration rate and the -8 bar level of water stress consistently resulted in the lowest transpiration rate. The symptoms of PEG absorption were different with the 1000 mw

Table 10. Transpiration rates measured during Greenhouse I after treatments[a] were applied.

Treatment			Transpiration[b]					
Terbacil		PEG	Night[c] Feb 19 (1 days)	Night Feb 21 (2 days)	Night Feb 22 (3 days)	Night Feb 23 (4 days)	Night Feb 24 (5 days)	Night Feb 26 (6 days)
(ppm)	(bars)	(MW)			(mg/cm^2/hr)			
0			26.9	25.7	20.5	27.2	7.3	22.8
0.001			24.0	27.8	16.7	25.4	6.7	22.0
0.010			23.4	21.1	18.7	24.0	9.1	26.4
0.100			27.5	24.6	18.3	25.3	7.9	21.8
1.000			14.3	18.9	16.1	18.7	5.6	17.3
10.000			6.2	3.4	1.9	3.1	3.2	1.3
0	-2	400	14.6	13.4	12.0	13.8	3.6	13.7
0	-4	400	14.5	13.0	11.6	15.8	4.0	12.1
0	-8	400	13.7	13.5	8.5	9.3	4.3	7.8
0	-2	1000	19.3	21.2	19.0	18.3	4.6	19.8
0	-4	1000	13.0	13.7	13.6	17.9	6.5	13.3
0	-8	1000	5.8	6.5	5.5	8.4	3.9	7.3
LSD(0.05)			4.8	11.7	5.0	8.3	NS	6.6
LSD(0.10)			2.6	9.5	4.1	6.8	3.0	5.4

[a]Treatments were applied February 18, 1982.

[b]Values given are an average of two measurements.

[c]Values represent measurements made after plants were exposed to day and night conditions (see Appendix B, Table 19 for times measurements were taken).

PEG than with the 400 mw PEG. The only plants which showed signs of absorption were those treated with the -8 bar water stress level using 1000 mw PEG. Those plants treated with 1000 mw PEG had a grey cast to them rather than the leaf rolling and turning brown which occurred when the 400 mw PEG was used. The 1000 mw PEG was selected as the osmoticum for use in subsequent experiments, since it did not injure the alfalfa plants at low rates as did the 400 mw PEG.

Greenhouse experiment II. There were no significant differences in CO_2 exchange among plants before treatments were applied (Table 11). All rates of terbacil significantly (5% level) decreased CO_2 exchange 4 days after treatment except the treatment of 0.5 ppm terbacil at -4 bars (Table 11). The 5 ppm terbacil at all levels of water stress resulted in a significant decrease of CO_2 exchange 7 days after treatment. All treatments significantly (5% level) decreased CO_2 exchange compared to the untreated check 9 days after treatment except the -4 bars without terbacil and these differences were also significant at the 10% level. The terbacil and level of water stress effects were not additive on CO_2 exchange, for example, the 0.5 ppm terbacil in combination with a -4 bar level of water stress decreased CO_2 exchange more than 0.5 ppm terbacil at -8 bars.

No significant differences in transpiration occurred among plants before they were treated (Table 12). All rates of terbacil and water stress significantly

Table 11. CO_2 exchange rates measured during Greenhouse II.

Terbacil		CO_2 exchange rate[a]				
		Before treatment		After treatment[b]		
		Mar 24	Mar 25	Apr 10 (4 days)	Apr 13 (7days)	Apr 15 (9 days)
(ppm)	(bars)	(mg $CO_2/dm^2/hr$)				
0.0	0	5.8	10.2	9.3	5.7	7.1
0.0	-4	5.2	6.0	5.6	2.6	3.5
0.5	-4	5.3	7.9	6.3	-0.7	0.9
5.0	-4	7.2	8.5	-1.5	-1.3	-0.8
0.0	-8	6.0	9.2	4.3	1.8	0.4
0.5	-8	5.8	6.8	2.9	4.3	2.1
5.0	-8	5.0	12.7	1.5	1.7	-0.7
5.0	0	4.1	8.8	1.0	-1.3	-1.1
LSD(0.05)		NS	NS	3.1	4.8	NS
LSD(0.10)		NS	NS	2.5	3.9	4.7

[a]Values given are an average of three measurements.

[b]Treatments were applied April 6, 1982.

Table 12. Transpiration rates measured in Greenhouse II before treatments were applied.

Treatment		Transpiration[a]						
Terbacil		Mar 23	Mar 24	Mar 25	Mar 26	Mar 27	Mar 28	Apr 1
(ppm)	(bars)			($mg/cm^2/hr$)				
0.0	0	39.0	21.0	7.3	23.9	15.4	14.4	25.6
0.0	-4	34.2	18.5	5.7	18.9	11.9	12.8	22.5
0.5	-4	24.3	20.8	6.9	23.6	13.4	14.1	26.6
5.0	-4	39.2	23.1	6.2	25.3	13.1	13.1	23.0
0.0	-8	40.4	19.5	5.0	20.6	12.0	11.2	21.3
0.5	-8	36.2	20.2	6.6	21.6	13.4	13.0	26.3
5.0	-8	37.1	18.0	5.8	18.8	11.8	11.8	22.1
5.0	0	42.2	19.0	6.8	22.1	18.3	13.4	24.4
LSD(0.05)		NS	NS	NS	NS	NS	NS	NS

[a]Values listed are an average of three measurements.

decreased the transpiration rates on the day they were treated except the 0.5 ppm terbacil at -4 bars water stress (Table 13). All treatment combinations significantly decreased transpiration 3 days after treatments were applied except the -4 bar level of water stress alone and in combination with 0.5 ppm terbacil. All treatment combinations significantly reduced transpiration 7 days after treatments were applied. All treatments decreased transpiration 8 and 9 days after treatment except the -4 bar level of water stress. The effects of terbacil and water stress are not additive in regard to their effect on transpiration. Differences among treatments within levels of water stress may be occurring, but more replications would be necessary to determine these differences.

Growth chamber study. No significant differences in CO_2 exchange among plants were observed before the plants were treated (Table 14). No statistical differences in CO_2 exchange could be detected on any day after treatments were applied. All plants treated with PEG at the -6 bar water stress level were severely injured, with symptoms being leaf rolling, dark green veins, and within 3 days after treatment, death. Part of the problem in detecting differences in the growth chamber was that the system was small and the ambient air may be subject to CO_2 contamination from the person taking the measurements. Precautions should be taken to protect the growth chamber environment from the CO_2 of the experimentor's breath as

Table 13. Transpiration rates measured in Greenhouse II after treatments[a] were applied.

Treatment Terbacil		Transpiration[b]					
		Apr 6 (0 days)	Apr 7 (1 days)	Apr 9 (3 days)	Apr 13 (7days)	Apr 14 (8days)	Apr 15 (9 days)
(ppm)	(bars)			($mg/cm^2/hr$)			
0.0	0	17.9	11.8	12.7	21.6	11.3	8.7
0.0	-4	11.3	7.2	9.6	11.8	9.6	6.5
0.5	-4	13.2	8.7	11.3	9.7	5.4	4.1
5.0	-4	10.4	3.8	4.4	3.2	2.0	3.2
0.0	-8	6.9	4.2	5.2	6.2	2.6	3.5
0.5	-8	8.1	5.5	6.0	8.6	5.0	3.6
5.0	-8	5.7	2.8	4.4	4.7	1.4	2.5
5.0	0	9.2	4.6	4.6	9.2	3.1	2.7
LSD(0.01)		6.7	4.3	NS	11.4	6.6	NS
LSD(0.05)		4.8	3.1	5.2	8.2	4.8	3.4

[a]Treatments were applied April 6, 1982.

[b]Values listed are an average of three measurements.

Table 14. CO_2 exchange rates measured in Growth Chamber.

Treatment Terbacil		CO_2 exchange rate[a] Before treatment			After treatment[b]	
		May 20	May 21	Average	May 25 (1 days)	May 27 (3 days)
(ppm)	(bars)	(mg $CO_2/dm^2/hr$)				
0.00	-	4.5	5.9	5.2	4.8	1.0
0.01	-	7.7	1.7	4.7	6.0	6.3
0.10	-	5.7	4.9	5.3	10.3	7.7
1.00	-	4.7	4.2	4.5	5.4	-6.3
0.00	-6	4.0	2.5	3.3	7.2	0.8
0.01	-6	4.1	2.2	3.1	1.2	0.2
0.10	-6	2.5	3.4	2.9	4.3	-2.0
1.00	-6	6.6	1.4	4.0	2.5	4.9
LSD(0.05)		NS	NS		NS	NS

[a]Values listed are an average of three measurements.

[b]Treatments were applied on May 24, 1982.

this can cause variation in the measurements.

No differences in transpiration were observed between plants before treatment combinations of terbacil and water stress were applied (Table 15). Treatments which contained 0.01, 0.1, and 1.0 ppm terbacil in combination with -6 bars water stress significantly decreased transpiration 1 day after treatments were applied (these differences were significant at the 10% level). All plants which were water stressed had quit transpiring on subsequent days. The various rates of terbacil applied did not cause significant changes in transpiration.

No signficant differences among plants occurred in diffusive resistances before the plants were treated (Table 16). All plants treated with -6 bars water stress had a significant increase in diffusive resistance 1 day after the plants were treated (these differences were significant at the 10% level) regardless of the rate of terbacil applied. No effects on diffusive resistance were attributed to terbacil treatments. Plants treated with -6 bars water stress were damaged too severely to get accurate diffusive resistance measurements by 3 and 4 days after treatments were applied.

Table 15. Transpiration rates measured in Growth Chamber.

Terbacil		Transpiration[a]					
		Before treatment			After treatment[b]		
		May 20	May 21	Average	May 25 (1 days)	May 27 (3 days)	May 28 (4 days)
(ppm)	(bars)			(mg/cm^2/hr)			
0.00	-	17.6	15.5	16.6	16.6	14.0	15.5
0.01	-	13.3	12.2	13.0	13.0	12.2	13.7
0.10	-	18.4	15.8	17.3	13.3	13.3	15.5
1.00	-	23.0	18.7	20.9	21.6	13.3	5.4
0.00	-6	18.7	11.9	15.5	7.6	1.8	1.8
0.01	-6	13.7	15.8	14.8	4.0	1.4	1.4
0.10	-6	15.1	10.6	13.0	4.3	1.4	1.8
1.00	-6	14.8	9.7	12.2	4.3	1.8	1.4
LSD(0.10)		NS	NS		9.8	NS	NS

[a]Values listed are an average of three measurements.

[b]Treatments were applied on May 24, 1982.

Table 16. Diffusive resistance measured in Growth Chamber.

Treatment		Diffusive resistance[a]					
		Before treatment			After treatment[b]		
Terbacil		May 20	May 21	Average	May 25 (1 days)	May 27 (3 days)	May 28 (4 days)
(ppm)	(bars)			(s/cm)			
0.00	-	5.2	8.6	6.8	7.9	5.5	6.2
0.01	-	6.4	8.2	7.3	5.1	6.4	7.4
0.10	-	5.1	7.4	6.2	7.5	5.7	6.0
1.00	-	3.5	5.2	4.3	4.0	6.0	19.8
0.00	-6	4.9	8.0	6.4	23.3	70.2	-[c]
0.01	-6	8.1	7.4	7.7	25.1	76.4	-
0.10	-6	6.2	11.4	8.7	25.8	211.3	-
1.00	-6	6.1	10.1	8.1	23.3	193.1	-
LSD(0.10)		NS	NS		17.8	NS	NS

[a]Values listed are an average of three measurements.

[b]Treatments were applied May 24, 1982.

[c]Plants were dead.

CHAPTER V

CONCLUSIONS

Field research indicated that drought stress may be a factor involved in the production increases observed in terbacil treated alfalfa. Production increases were observed in the plots treated with terbacil in Experiment I (which received less water than Experiment II). These production increases occurred at the last two harvests in Experiment I when one would expect the area to be drought stressed. No production trends or significant differences in alfalfa production occurred in Experiment II which was irrigated. Terbacil should not be applied to alfalfa post-harvest at rates greater than 0.56 kg/ha since higher rates cause alfalfa injury symptoms and significant reductions of season total forage production.

Physiology measurements taken in the field also indicated that terbacil applied post-harvest can cause crop injury. Terbacil rates greater than 0.56 kg/ha reduced transpiration and increased diffusive resistance. Trends in transpiration and diffusive resistance were seen in Experiment II, but diffusive resistance was the only measurement significantly (10% level) affected. Increasing the number of replications for physiological measurements

would improve these experiments. Physiological measurements from Experiment I would have been valuable since comparison of dryland versus irrigated could have been analyzed.

Controlled environment research indicated that 5 and 10 ppm terbacil were high enough to severely injure or kill alfalfa when terbacil was applied via nutrient solution. The lower rates of terbacil (0.001, 0.01, 0.1 ppm) were effectively metabolized since they did not result in injury symptoms or effects on physiological processes. The effects of terbacil and water stress were not additive as seen with the 0.5 ppm terbacil at -4 and -8 bars in Experiment II. This may be an actual difference in response of terbacil treated plants to water stress, or it may be an interaction of the terbacil (or a metabolite) with the PEG which was absorbed. Increased replication of treatments would distinguish if the trends seen with the 0.5 ppm terbacil were actually significant differences. It is doubtful that even a PEG with a mw higher than 1000 would be satisfactory in use with established alfalfa since root damage is inevitable when transplanting occurs. The plants were more sensitive to all treatments when applied in the growth chamber. This may be due to differences in growth morphology (i.e. cuticle thickness) that occur in various environments.

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APPENDIXES

APPENDIX A

MODIFIED HOAGLAND'S NUTRIENT SOLUTION

Table 17. Modified Hoagland's nutrient solution.

Stock solutions	
	ml stock solution/l of nutrient solution
0.5 M $Ca(NO_3)_2 \cdot 4H_2O$ (236.16)	20.0
1.0 M KH_2PO_4 (163.13)	3.0
1.0 M $MgSO_4 \cdot 7H_2$) (246.49)	2.0
0.5 M $(NH_4)_2HPO_4$ (132.06)	1.0
1.0 M KNO_3 (101.10)	5.0

Micro nutrients[a]	
	compositon of stock solution gm/l
H_3BO_3 (61.84)	2.86
$MnCl_2 \cdot 4H_2O$ (197.91)	1.81
$ZNSO_4 \cdot 7H_2O$ (287.55)	0.22
$CuSO_4 \cdot 5H_2O$ (249.71)	0.08
$H_2MoO_4 \cdot H_2O$ (179.98)	0.02

Iron
Sequestrene 330 Fe Iron Chelate Stock Solutions: 10 g/l Use 1 ml stock solution for each ml nutrient solution. Sequestrene 330 Fe contains 10% iron as metallic thus, [Fe] in nutrient solution is 1 ppm.

[a]These micronutrients can be combined in a single solution or made in five separate solutions. In either case 1.0 ml of the micro nutrients stock solution or solutions is added per 1 of nutrient solution.

APPENDIX B

RAINFALL DATA AND ENVIRONMENTAL CONDITIONS

Table 18. Rainfall data for 1982 at Haskell and Perkins.

Month	Rainfall	
	Haskell	Perkins
	-------- cm ---------	
January	9.90	6.12
February	2.42	4.35
March	3.17	3.42
April	4.45	5.90
May	22.17	36.47
June	15.40	13.20
July	8.15	9.27
August	6.02	0.82
September	5.87	2.20
October	3.07	2.27
November	13.77	7.55
December	9.02	9.12

Table 19. Environmental conditions in Greenhouse I.

Date	Time 1	Time 2	Hours	C°	RH
Feb 2 d[a]	11:00 am	9:15 pm	10.00	21	22
Feb 4 d	9:15 am	3:00 pm	5.75	24	18
Feb 4 n[b]	3:00 pm	12:00 am	21.00	24	18
Feb 5 d	12:00 am	10:20 pm	22.50	29	18
Feb 6 d	10:20 am	5:15 pm	7.00	30	15
Feb 6 n	5:15 pm	5:00 pm	48.00	28	35
Feb 9 d	9:30 am	2:30 pm	5.00	29	29
Feb 9 n	2:30 pm	10:00 am	44.00	24	20
Feb 11 d	10:00 am	2:00 pm	4.00	24	20
Feb 15 d	12:00 pm	4:00 pm	3.50	26	50
Feb 18 n	4:00 pm	2:30 pm	22.50	25	35
Feb 19 n	2:30 pm	10:00 am	43.50	25	22
Feb 21 d	10:00 am	4:30 pm	6.50	30	25
Feb 21 n	4:30 pm	3:30 pm	23.00	30	25
Feb 22 n	4:00 pm	1:15 pm	21.00	30	25
Feb 23 n	1:15 pm	4:00 pm	27.00	21	23
Feb 24 n	4:00 pm	9:15 am	17.00	18	25
Feb 25 n	9:15 am	3:30 pm	30.00	24	35
Feb 26 n	4:15 pm	12:00 pm	44.00	22	35
Feb 28 n	2:00 pm	1:00 pm	23.00	23	40

[a]Transpiration measured after plants were exposed to only day conditions.

[b]Transpiration measurements were taken after plants were exposed to both day and night conditions.

Table 20. Environmental conditions in Greenhouse II.

Date	Time 1	Time 2	Hours	C°	RH
Mar 23	9:00 am	1:00 pm	16.0	29	40
Mar 24	1:00 pm	9:00 am	20.0	28	35
Mar 25	9:30 am	2:00 pm	28.0	28	30
Mar 26	11:45 pm	12:00 pm	48.0	18	30
Mar 28	12:00 pm	8:30 am	44.5	21	50
Apr 1	2:30 pm	3:30 pm	25.0	32	30
Apr 6	1:30 pm	3:45 pm	26.0	28	20
Apr 7	3:45 pm	3:45 pm	24.0	27	65
Apr 8	3:45 pm	3:45 pm	24.0	27	45
Apr 13	9:00 am	4:00 pm	7.0	32	35
Apr 14	4:15 pm	3:45 pm	23.5	31	40
Apr 15	3:45 pm	10:30 am	19.0	27	25

VITA

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