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To the Graduate Council:

I am submitting herewith a dissertation written by John Reid Evans entitled "Response of established alfalfa to herbicides applied in the winter." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Larry S. Jeffery, Major Professor

We have read this dissertation and recommend its acceptance:

W.L. Parks, J.E. Reynolds, O.J. Schwarz

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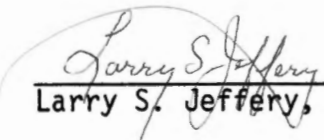
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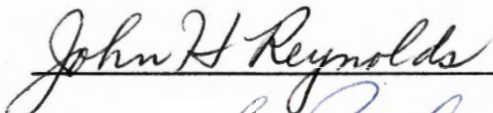

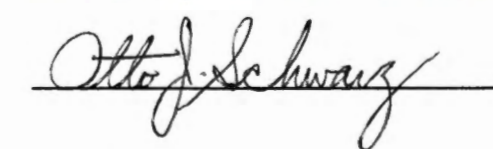
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and recommend its acceptance:

Accepted for the Council:


The Graduate School

RESPONSE OF ESTABLISHED ALFALFA TO
HERBICIDES APPLIED IN THE WINTER

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

John Reid Evans

March 1984

Ag-VetMed
Thesis
84b
.E925

ACKNOWLEDGMENTS

The author expresses his sincere thanks to Dr. Larry S. Jeffery, Chairman of his Graduate Committee, for his guidance and support during the course of this study. Appreciation is expressed to Drs. W. L. Parks, J. E. Reynolds, and O. J. Schwarz, members of the Graduate Committee, for their assistance in the planning of this research and in reviewing the manuscript.

Special appreciation is extended to Dr. J. W. High, Jr. of the Middle Tennessee Experiment Station for his generosity in providing support and facilities for this research. A thank you is expressed to the personnel of the Middle Tennessee Experiment Station for their assistance, especially to Louis Nichols, Scot Helgren, and Roy Thompson.

The author would like to thank his family and friends for their encouragement and support that he has been so fortunate to receive.

ABSTRACT

Herbicide injury to established alfalfa (Medicago sativa L.) was studied to determine the effects of single and repeated herbicide applications, dates of herbicide application, alfalfa dormancy, and alfalfa cultivars.

Metribuzin [4-amino-6-tert-butyl-3(methylthio)-s-triazin-5(4H)-one] at 0.56 and 1.12 kg/ha, simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] at 1.12 and 2.24 kg/ha, and terbacil (3-tert-butyl-5-chloro-6-methyluracil) at 0.84 and 1.68 kg/ha were applied to dormant alfalfa for three years. The low rate of each herbicide did not cause alfalfa injury. At high rates for a single season, alfalfa injury was greatest from terbacil and less from simazine. Metribuzin caused the least injury. After three annual applications simazine at 2.24 kg/ha caused the most alfalfa injury. A bioassay to detect herbicide residues revealed that alfalfa injury on simazine treated plots resulted from toxic soil residue.

Date of herbicide application was studied in two experiments. High rates of metribuzin, simazine, and terbacil were applied in December, January, and February to dormant alfalfa. Alfalfa was injured more at the later application dates. In a second experiment simazine at 2.24 kg/ha and terbacil at 1.68 kg/ha were applied to dormant alfalfa at six winter dates for three years. The trend of more alfalfa injury with later applications was not apparent in this experiment. Injury from terbacil was more dependent on climatological

conditions than injury from simazine. Precipitation for ten days following terbacil application correlated best with alfalfa injury.

The effect of alfalfa dormancy on herbicide injury was studied by stimulating alfalfa to break dormancy by using plastic covered frames. Terbacil was applied at 1.68 kg/ha to alfalfa plots both inside and outside the frames when 10% of the alfalfa plants inside the frames appeared to have broken dormancy. Terbacil injury on dormant and non-dormant alfalfa was not significantly different in 62% of the comparisons.

Thirty alfalfa cultivars were screened against several herbicides for injury as measured by alfalfa height. Herbicides included: chlorpropham (isopropyl m-chloro-carbanilate) at 3.4 kg/ha, metribuzin at 0.84 kg/ha, pronamide [3,5-dichloro (N-1,1-dimethyl-2-propynyl) benzamide] at 2.5 kg/ha, simazine at 1.7 kg/ha, terbacil at 1.7 kg/ha, and hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] at 3.4 kg/ha. Alfalfa cultivars did not vary significantly in susceptibility to herbicide treatments.

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CHAPTER I

INTRODUCTION

This series of studies was conducted to investigate the effect of several factors on herbicide injury to established alfalfa. These factors were: the susceptibility of different alfalfa cultivars to selected herbicides; the effect of varying the date of applying herbicides to established, dormant alfalfa on alfalfa injury; the effect of repeating herbicide applications annually on alfalfa injury and possible injury to subsequent crops; and the influence of alfalfa dormancy or non-dormancy at the time of herbicide application on alfalfa injury.

Originally these factors were investigated together in a single experiment conducted for three years. Later several factors were studied independently in separate experiments. Each study will be reported as a separate chapter.

CHAPTER II

REVIEW OF LITERATURE

Weeds compete with alfalfa for water, nutrients, space, and light. Weeds may not always reduce the total forage production, but will generally decrease the nutritional value of the forage. Additionally, livestock may reject forage if it contains coarse stems, spines, thorns or awns of certain weeds. The presence of weed seeds in forage provides a mechanism for their spread to non-infested areas (46).

Weed control in alfalfa can be divided into two distinct areas; weed control at the time of alfalfa establishment, and weed control in established alfalfa stands.

During alfalfa establishment, any method that promotes seedling vigor will make the alfalfa more competitive with weeds. Many components such as clean seed, time of planting, cropping systems, soil fertilization, and companion crops are necessary for successful establishment. These practices do vary among locations. For example, companion crops serve to reduce weed competition in the northern Corn Belt, but the companion crop competes too severely with the alfalfa to be used in the southern Corn Belt (46). Herbicides are often used in lieu of companion crops, especially for spring seedings.

Grasses can compete vigorously with seedling alfalfa. Pre-plant incorporated herbicides such as EPTC (S-ethyl

dipropylthiocarbamate), profluralin [N-(cyclopropylmethyl)-a,a,a-trifluoro-2,6-dinitro-N-propyl-p-toluidine], and trifluralin (a,a,a-trifluoro-2,6-dinitro-N-N-dipropyl-p-toluidine) give excellent control of most seedling grasses without causing serious injury to seedling alfalfa (13). Broadleaf weeds in seedling alfalfa are commonly controlled by post-emergence herbicides such as dinoseb (2-sec-butyl-4,6-dichlorophenoxy) (36) and 2,4-DB [4-(2,4-dichlorophenoxy) butyric acid] (40). A review of weed control practices that can be used when seeding alfalfa is given by Peters and Peters (46).

Several different methods can be used for weed control in established alfalfa. The primary means of weed control is proper management of the alfalfa stand. A dense, strong, well-managed alfalfa stand can compete successfully with most weeds. Conversely, with poor forage management even the best weed control practices will give only temporary results (55).

Cultural practices normally associated with alfalfa production may reduce weed competition. For example, repeated mowing or harvesting of the alfalfa over a growing season may reduce weed stands. A biennial mowing program on alfalfa pastures followed for several years can successfully control Canada thistle (Cirsium arvense (L.) Scop.) (54). However, other weeds such as quackgrass (Agropyron repens (L.) Beauv.), and leafy spurge (Euphorbia esula L.) are not adequately controlled by mowing (46).

Direct tillage can be used in alfalfa seed production when the proper implement is employed, but not in hay or pasture fields.

Tillage should be avoided in humid areas where disease organisms such as bacterial wilt (Corynebacterium insidiosum) are likely to invade the wounded alfalfa crowns (46).

Fire can also control weeds. Flaming of alfalfa with propane or butane burners has been used to control dodder (Cuscuta campestris Yunck), a parasitic weed (46). Flaming was once used to control early spring infestations of the alfalfa weevil (Hypera postica Gyllenhal) before the advent of effective insecticides. Flaming for insect control had the added benefit of controlling of many winter annual weeds (43).

The advent of selective herbicides in the 1940's offered alfalfa producers a new method of weed control. Little use was made of the early materials such as potassium cyanate (6), sodium isopropylxanthate and endothal [7-oxabicyclo(2,2,1)heptane-2,3-dicarboxylic acid] (2). Generally these materials had the disadvantages of high expense, poor efficiency, and risk of crop injury (46).

As new herbicides were developed in the 1950's, interest in chemical weed control in established alfalfa increased. Chlorpropham (isopropyl m-carbanilate) and several formulations of dinoseb were reported to be effective in reducing chickweed (Stellaria media (L.) Cyrillo) competition (6) (42). Of the phenoxy herbicides, 2,4-DB was shown to be less toxic to alfalfa than 2,4-D [2,4-dichlorophenoxy) acetic acid]. Broadleaf control with 2,4-DB was similar to that of 2,4-D, but 2,4-DB was safe to use at rates up

to 3.3 kg/ha on alfalfa (34). Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] provided broad spectrum weed control but was weak on Bromus species (39). Simazine offered weed control similar to that provided by diuron but also controlled the Bromus species. Early research with simazine revealed that alfalfa injury would occur with improper usage (35) (59).

Over the succeeding years several other herbicides have been found to be effective when applied to dormant, established alfalfa. Dormant alfalfa showed good crop tolerance to metribuzin (56) (57) (63). Pronamide offered good quackgrass control with minimum alfalfa injury (19). Terbacil effectively controls many weeds in alfalfa but is ineffective against quackgrass (4) (27).

Chemically controlling weeds can benefit alfalfa production. Increased alfalfa yields have been reported by some authors following successful weed control programs (8) (44) (45) (52). Stewart (61) found a significant correlation coefficient of 0.555 between decreased weed populations and increased alfalfa yields. Generally, when a yield increase occurred, it was most noticeable in the first alfalfa cutting of the year (45). Many authors did not measure alfalfa yield increases after successfully controlling the weeds (31) (32) (41) (48) (59). Several researchers argue that a weed control program in alfalfa should not be expected to produce greater forage yields; rather the benefit of weed control in alfalfa is in higher forage quality.

Crude protein content of forage generally improves with weed control (6) (71). Chase (7) found that the crude protein of alfalfa was 27.5%; while the crude protein of the weeds in the forage was only 17.2%. Thus, the presence of weeds in alfalfa forage will generally decrease the nutritional value of the forage. Several authors found high negative correlations between the weed content of alfalfa forage and the protein percentage of the forage (7) (11) (20). In a few cases, researchers have reported an increase in the crude protein content of the alfalfa after using triazine herbicides such as cyanazine [2-chloro-4-(1-cyano-1-methylethyl-amino)-6-ethylamin-s-triazine] and simazine (33) (71).

The digestibility of alfalfa forage is generally decreased by the presence of weeds (7). Fawcett et al. (20) found the in vitro digestible dry matter of weedy alfalfa to be 51.4%; while the digestibility of alfalfa forage when weeds were controlled with promanide was 61.3%.

Not all weed infestations will reduce alfalfa forage quality. Sheaffer and Wyse (58) found that controlling common dandelion (Taraxacum officinale Weber) infestations in alfalfa did not improve forage quality or yield. Thus, they could not recommend herbicide use for control of dandelion.

Despite the benefit of chemically controlling weeds in alfalfa, herbicide use can pose some problems. Herbicide use is disadvantageous if herbicides injure the crop or do not adequately control the weeds. Crop injury can be seen as malformed plants,

chlorosis, stunted plants, reduced plant yield, or plant death.

Injury that is apparent visually may not be measured quantitatively in plant yield. At the first cutting, Wilson (71) observed alfalfa stem and leaf chlorosis with metribuzin at 1.1 kg/ha and terbacil at 0.8 kg/ha, and rated this injury at 28% and 10%, respectively. This injury was not evident at the second cutting and did not significantly reduce alfalfa yields of either the first or second cutting.

Several factors can affect crop injury and weed control resulting from herbicide use. These factors include: the amount of herbicide applied to an area, the characteristics of the soil in which the alfalfa is grown, the date of herbicide application, the stage of alfalfa growth at the time of herbicide application, and the characteristics of the alfalfa cultivar.

The rate of herbicide application affects injury. Generally, a higher herbicide rate will mean an increased amount of crop injury. Seven percent more alfalfa injury was observed when simazine rates were increased from 1.3 to 1.7 kg/ha and when metribuzin rates were increased from 0.6 to 0.8 kg/ha (62). Similarly, an increase in terbacil rates from 1.1 to 1.7 kg/ha caused a significant reduction in alfalfa yield (25).

The soil type on which a crop is grown affects the activity of many herbicides. For example, alfalfa was injured on a Shano sandy loam when 0.45 kg/ha of terbacil was used (65); while on an Erie channery silt loam, neither 0.45 nor 0.90 kg/ha of terbacil injured alfalfa (60). Observations of alfalfa injury associated

with sandy soil types have been made by several authors (10) (24).

Differences in alfalfa injury and weed control due to the date of herbicide application have been well documented in the literature. Early investigations revealed that potassium cyanate killed established chickweed when applied in December but only stunted the chickweed when applied in March or April (6). Chlorpropham gave good chickweed control in October but caused more injury in October than when applied in December or February. Endothal, potassium cyanate, and sodium isopropylxanthate injured alfalfa in October but not at later dates. However, these chemicals did not control chickweed at later dates (2).

Michigan researchers found that spring applications of simazine and terbacil controlled white cockle (Lychnis alba Mill.) in alfalfa better than fall applications. Spring applications of terbacil gave better control of quackgrass than fall applications. Alfalfa injury was more pronounced with spring applications of these herbicides when they were applied at high rates (44).

Workers in New Jersey found that terbacil controlled yellow rocket (Barbarea vulgaris R. Br.) when applied in either fall or spring. However, simazine was only effective on yellow rocket when applied in the autumn. Fall applications of either herbicides gave better control of chickweed and corn chamonile (Anthemis arvensis L.) than spring applications. In this research alfalfa injury was not mentioned with either application date (28).

Researchers in Texas found that chlorpropham and simazine controlled annual rescuegrass (Bromus willenowii Kunth) and yellow foxtail (Setaria glauca (L.) Beauv.) in alfalfa when applied in September. The same herbicides were not effective when applied in February (8). In Oklahoma, simazine controlled henbit (Lamium amplexicaule L.) and maretail (Conyza canadensis (L.) Croq) in established alfalfa when applied in December or January, but gave poor control when applied in late February (4). In Connecticut, metribuzin was applied to established alfalfa in October, December, and April. Dandelions were controlled effectively only with December or April applications. Quackgrass was affected by the April application only (27).

Not all studies report differences due to date of herbicide application. Five dinitro herbicides were applied at each of four dates in Maryland. Dates of application included a pre-dormant application in October, two dormant applications, and a post-dormant application in April. Alfalfa yields did not reflect significant differences among treatment dates. However, some visual differences were noted between application dates. Differences among application dates were difficult to establish because the experimental design placed low precision on this comparison (42).

Conditions affecting herbicide performance will vary between different dates of herbicide application. Weeds and crop may change in size and stage of growth, and state of dormancy. Also, environmental conditions differ between application dates. Alfalfa

injury and weed control are affected by these changes.

Duke and Spear (16) applied simazine, terbacil, and bromocil (5-bromo-3-sec-butyl-6-methyluracil) to established alfalfa in the fall and spring. All herbicide treatments gave excellent weed control, but spring applications of uracil herbicides significantly reduced alfalfa yields. Simazine did not reduce yields. The spring applications were made two weeks prior to the initiation of spring growth. While lateral buds were not visibly growing, they were active at that time and susceptible to injury from the uracil herbicides.

The amount of foliage present can affect alfalfa injury. Simazine caused chlorosis when applied nine days after the last fall cutting. The following year the same treatment produced no chlorosis when applied the day after harvest. The alfalfa chlorosis produced the first year was judged to be due to excessive foliar absorption of simazine by the tender regrowth that had occurred nine days after the last harvest (26).

Differences in herbicide activity between dates of application can also be attributed to differences in environmental conditions. Bromoxynil (3,5-dibromo-4-hydroxy-benzonitrile) was applied to alfalfa in California on five separate dates. Alfalfa injury was found to be less severe when daylight temperatures were below 18.3° C., when humidity was high, and when the sky was hazy (51). Similarly, the occurrence of frost may affect herbicide performance. Glyphosate [N-(phosphonomethyl) glycine] was more effective on quackgrass the day after the first frost of the season than either five

days before or after the frost. Unfortunately, frost did not lessen the toxicity of glyphosate to alfalfa (12).

Repeated annual use of herbicides on alfalfa can cause crop injury. Swan (65) applied six herbicides annually for four years to alfalfa grown on a coarse textured soil. Only simazine and terbacil treatments reduced the total alfalfa yield. Terbacil reduced alfalfa yields significantly in only one year; but that injury was of such a magnitude as to be reflected in the four year average. Simazine at 0.45 kg/ha reduced yields in three of the four years and was the most phytotoxic of the herbicides tested on this coarse textured soil. Alfalfa injury did not appear to increase with successive years of simazine treatment.

In a separate experiment, Swan (64) applied simazine and terbacil at rates of 0.45, 0.90, and 1.80 kg/ha for three consecutive years. Only the high rate of each chemical reduced alfalfa yields. Alfalfa injury from the high rate of simazine appeared after the second application and increased with each additional application. Injury from the 1.80 kg/ha rate of terbacil remained at about the same level each year. A field bioassay using oats (Avena sativa L.) as an indicator plant showed stunting and leaf margin necrosis in treated plots 18 months after the last application of simazine or terbacil at 1.8 kg/ha.

Robison et al. (48) applied eight herbicides including metribuzin, simazine and terbacil to alfalfa plots in Utah and then retreated one half of the plot the following year. Herbicide residues

were not large enough to control weeds during the second year but did tend to reduce yields when combined with a second application. Simazine at 1.68 kg/ha was the most injurious treatment with a 57% reduction in alfalfa yield from the second application. However, a lower rate of simazine was effective in weed control.

In contrast, Waddington (66) in Saskatchewan, Canada, applied simazine at 0.8 and 1.7 kg/ha and terbacil at 0.6 and 1.1 kg/ha to alfalfa annually for four years. Alfalfa populations and seed yield were not affected by these treatments. No forage yields were recorded.

Varieties or cultivars of many crops vary in their susceptibility to certain herbicides. Differences in susceptibility have been reported in barley (Hordeum vulgare L.) (21), bermudagrass (Cynodon dactylon (L.) Pers.) (29), corn (Zea mays L.) (17), creeping bentgrass (Agrostis palustris Huds.) (1) cucumbers (Cucumis sativus L.) (70), peaches (Prunus persica L.) (38), soybeans (Glycine max Merrill) (22), and spring wheat (Triticum aestivum L.) (21). Weed biotypes may vary in herbicide susceptibility as shown with weeds such as common groundsel (Senecio vulgaris L.) (50), redroot pigweed (Amaranthus retroflexus L.) and lambsquarter (Chenopodium album L.) (47).

In many cases biotype resistance is due to the rate at which some plants are able to metabolize the active ingredient of the herbicide to a non-toxic form. Eastin et al. (17) showed differential susceptibility of corn lines to triazine herbicides such as atrazine

[2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and simazine. In tolerant corn the herbicides are metabolized to a hydroxy-form and amino acid conjugates much faster than in non-tolerant corn lines. Hardcastle (22) reported that metribuzin caused differences between soybean cultivars in plant stand, plant height, and yield. Later work revealed that soybean cultivars differ considerably in their rate of detoxifying metribuzin (37). However, the expression of metribuzin injury in the field will vary with such factors as herbicide rate, soil type, and rainfall after treatment (9).

Only limited work has been done on the response of various alfalfa cultivars to herbicides. McCarty and Sand (40) used several rates of 2,4-DB and dalapon (2,2-dichloropropionic acid) on seedling stands of five alfalfa cultivars. When injury appeared it was fairly uniform across alfalfa cultivars. Harvey et al. (24) studied the effect of four triazine herbicides on five alfalfa cultivars. In this Wisconsin study, herbicides were applied after the first cutting of the year. Yields were recorded in the autumn and the following spring. The DuPuits variety showed injury similar to the other varieties in the fall, but showed greater injury the following spring. DuPuits is a Flemish type alfalfa with less winter hardiness than the hardy American types (Vernal and Iroquois) or the modified Flemish types (Saranac and Tempo). The authors reasoned that the combined stress of the winter and the herbicide may be responsible for the greater yield reduction of the DuPuits cultivar in the following spring (24).

CHAPTER III

ALFALFA INJURY FROM ANNUAL AND REPEATED HERBICIDE APPLICATIONS

1. INTRODUCTION

Established alfalfa treated with herbicides for winter weed control may suffer herbicide injury. In the case of applying herbicides to a dormant, perennial crop such as alfalfa the occurrence of herbicide injury may be related to the stage of dormancy of the crop. This would be especially true in a location such as Tennessee where the relatively mild winters are quite variable. During a mild Tennessee winter, alfalfa may never become totally dormant. Periods of growth may occur during a warm interval in the winter.

Cultivars of several crops vary in their susceptibility to certain herbicides (17 (22) (70). Generally, differences in cultivar susceptibility are related to metabolic differences. However, if herbicide injury is related to crop dormancy, then alfalfa cultivars which genetically exhibit different degrees of dormancy would be expected to respond differently to winter herbicide treatments.

Alfalfa dormancy will vary over time and generally will decrease as the winter progresses (30). If herbicide injury is related to crop dormancy, then different dates of herbicide application should produce varying amounts of alfalfa injury. Also, since alfalfa is a perennial crop, several herbicide applications may be made to the same crop. Repeated annual herbicide applications may

result in crop injury not observed in single applications (65).

This research will be divided into two main parts. The purpose of experiment one is to study the relationship between alfalfa cultivars and herbicide injury and between date of application and herbicide injury. In turn, these relationships may give some indication of the relationship between herbicide injury and alfalfa dormancy. The purpose of the second experiment is to study the effect of repeated, annual herbicide applications on alfalfa injury.

2. MATERIALS AND METHODS

Two experiments were conducted on Maury silt loam soil at the Middle Tennessee Experiment Station near Spring Hill on three alfalfa cultivars. Cultivars were: Weevlc hek--a winterhardy, fairly vigorous alfalfa cultivar; Gladiator--a moderately winterhardy cultivar, which exhibits less fall dormancy than Weevlc hek; and Moapa--a non-winterhardy, nondormant cultivar. Weevlc hek and Gladiator were fall seeded in 1977 at site A and in 1978 in site B. Moapa was spring seeded in 1978 and 1979 at each site. Non-dormant Moapa cannot be fall seeded successfully in Tennessee due to severe winter injury. Each variety was established in blocks of 11.5 m by 26 m in a randomized complete block design with four replications. Two different sets of treatments were imposed on these alfalfa varieties.

In experiment one, crop injury resulting from different application dates of herbicide treatments was studied as a function of

the alfalfa varieties. Herbicide treatments were applied at monthly intervals in December, January, and February. Four herbicide treatments were used each month. Treatments were metribuzin at 1.12 kg/ha, simazine at 2.24 kg/ha, and terbacil at 1.68 kg/ha plus an untreated check. These herbicide rates were twice the normal use rates for the soil type and hereafter will be referred to as 2x rates. The 2x rates were used to increase the probability of herbicide injury.

Experiment one was arranged as a split, split pilot design. Alfalfa varieties were main treatments; monthly dates of application were split treatments; and herbicide treatments were split, split treatments. Experiment one was conducted initially in 1979 at site A and in 1980 on site B. Additionally, herbicide treatments were reapplied to the same plots of site A in 1980 and 1981.

In experiment one-laboratory test, the electrical conductance of soluble root contents was determined to detect plant injury. Plant cells lose their ability to regulate their soluble contents upon severe injury or death (15). The percent of soluble electrolytes leached from whole root samples should give a quantitative measure of injury. A modification of the electrical conductance method described by Dexter et al. (14) (15) was performed on alfalfa root samples collected from each treatment in 1979. Root samples were collected at intervals of 10, 16, 26, and 32 days after herbicide application. All branch roots were removed and root samples were trimmed to equal size. Washed alfalfa root samples were stored

for 20 hours at 2° C in separate test tubes containing 50 ml of distilled water. Electrical conductance was measured on the water after 20 hours. The water was returned to the sample and the sample was macerated in a blender. Solid matter was removed by filtering. The electrical conductance of the filtrate was then measured. All electrical conductances were corrected to 25° C. The percentage of total electrolytes leached following storage was calculated by dividing the specific conductance of the solution following storage by the specific conductance of the filtrate containing total electrolytes.

Experiment two compared the alfalfa injury from two rates of each herbicide on the three alfalfa cultivars at a single date. The seven herbicide treatments were metribuzin at 0.56 and at 1.12 kg/ha, simazine at 1.12 and at 2.24 kg/ha, and terbacil at 0.84 and at 1.68 kg/ha, plus an untreated check. These herbicide rates will be expressed at the 1x and 2x rates hereafter. Experiment two had a split plot design. Alfalfa cultivars were main treatments and herbicide treatments were split treatments. Herbicide treatments were applied in December for three years to the alfalfa cultivars at site A.

A bioassay to detect carry-over of toxic herbicide residue (53) was conducted on soil samples taken from experiment two after the third year of herbicide applications. Six months after the last herbicide application, soil was collected from the surface three inches of each treatment of one alfalfa cultivar and placed

in eight inch pots. Pots were moved to an area of partial shade where the soil was kept moist. Indicator plants, cucumbers and mustard (Brassica oleracea L.), were sown in each pot. Indicator plants were grown for four weeks. Herbicide injury to the indicator plants was measured by recording the plant height and dry matter yield of the cucumbers. Additionally, indicator plants were visually rated for herbicide injury as evidenced by chlorosis, stunting and stand reduction. The bioassay was analyzed as a randomized complete block.

In both experiments herbicides were applied through a CO₂ powered, hand held boom delivering 187 l/ha. Data collected included alfalfa plant height before the first harvest and forage yield of the first alfalfa cutting. Data from each year were analyzed separately. Means were separated by Duncan's Multiple Range Test at the 5% level of probability. Environmental conditions at the time of herbicide applications are given in the Appendix (Table A-1).

3. RESULTS AND DISCUSSION

Of the three cultivars tested, Gladiator tended to be the highest yielding cultivar followed closely by Weevilchek (Table 1). Yield of the non-dormant Moapa cultivar was less than half the yield of the other two cultivars. The stand of Moapa was reduced by severe winter injury during the first season. Forage yields of Moapa were not taken during the second and third years at site A; however,

Table 1. First cutting yields of three alfalfa cultivars following herbicide applications in the winter.

Cultivars	Locations and Years			
	Site A			Site B
	1979	1980	1981	1980
	-----Dry matter (kg/ha) ^z -----			
Gladiator	2704a	3111a	2009a	4409a
Weevilchck	2518b	3019a	1539a	4171b
Moapa	1071c	-----	-----	977c

^zMeans within a column sharing the same letter are not significantly different at the 5% level.

plant heights were recorded for all cultivars. Interactions between alfalfa cultivars and dates of application or between alfalfa cultivars cultivars and herbicide treatments were not significant. The alfalfa cultivars did not vary in their response to treatments.

In experiment one the date of herbicide application appeared to have an effect on alfalfa injury. Unfortunately, the experimental design did not provide enough precision at the first split level to separate means in most cases. Only during the first year at site A was the alfalfa yield significantly reduced by the later application dates (Table 2). Differences in plant height were not statistically different. Generally, differences between treatments were more pronounced in forage yields than in plant height. During the second and third years at site A alfalfa height and yield tended to be reduced by January and February herbicide applications. This trend was not statistically significant and was not evident at site B.

Individual herbicides varied in the amount of crop injury they produced. The 2x rate of metribuzin was the least injurious herbicide treatment to alfalfa height and yield (Table 3). Conversely, the 2x rate of terbacil tended to be the most injurious except in 1981. In 1981, after three years of annual herbicide application, the 2x rate of simazine was the most injurious.

Although metribuzin produced the least yield reduction, it showed a consistent pattern of more yield reduction with later application dates (Table 4). The most injurious treatment, terbacil,

Table 2. Alfalfa plant heights and yields of first cutting following herbicide applications in December, January, and February.

Month of Herbicide Application	Location and Year			
	Site A			Site B
	78-79 ^W	79-80 ^X	80-81 ^X	79-80 ^W
	-----Plant height (cm) ^{YZ} -----			
December	57.0a	29.8a	46.6a	26.9a
January	51.1a	28.1a	42.9a	26.4a
February	50.0a	27.2a	42.2a	27.1a
	-----Dry matter (kg/ha) ^{YZ} -----			
December	2315a	3225a	1832a	3113a
January	1770b	2786a	1496a	3204a
February	1734b	2665a	1275a	3239a

^WAverage of three cultivars.

^XAverage of two cultivars.

^YMeans within a column sharing the same letter are not significantly different at the 5% level.

^ZData collected at first cutting.

Table 3. Alfalfa plant heights and yields of first cutting following winter applications of metribuzin, simazine, and terbacil.

	Herbicide Rate (kg/ha)	Location and Year			
		Site A		Site B	
		1979 ^x	1980 ^y	1981 ^y	1980 ^x
-----Plant height (cm) ^z -----					
Untreated check		58.3a	30.2a	52.1a	27.7a
Metribuzin	1.12	56.8ab	29.2a	47.4b	27.0a
Simazine	2.24	51.2ab	28.7a	41.0c	27.0a
Terbacil	1.68	50.1b	28.6a	43.5bc	26.4a
-----Dry matter yield (kg/ha) ^z -----					
Untreated check		2440a	3561a	2495a	3349b
Metribuzin	1.12	2286a	3362a	1987b	3432a
Simazine	2.24	1815b	2788b	1187c	3121c
Terbacil	1.68	1717b	2513b	1403c	2841d

^xAverage of three cultivars.

^yAverage of two cultivars.

^zMeans within a column sharing the same letter are not significantly different at the 5% level.

Table 4. Yield reduction as a percent of untreated checks in first cutting of all alfalfa cultivars following winter herbicide applications in December, January, and February.

Month of Herbicide Application	Location and Year			
	Site A			Site B
	78-79	79-80	80-81	79-80
<u>Percent yield reduction from metribuzin at 1.12 kg/ha</u>				
December	0	1	12	0
January	12	2	10	0
February	7	14	35	4
<u>Percent yield reduction from simazine at 2.24 kg/ha</u>				
December	0	14	55	5
January	29	25	56	5
February	40	26	41	9
<u>Percent yield reduction from terbacil at 1.68 kg/ha</u>				
December	1	17	29	16
January	43	31	40	11
February	37	41	62	18

followed the same trend. Similarly, simazine showed more yield reduction with January and February applications except in 1981. After the third year of treatments on site A, the percent yield reduction with simazine increased dramatically regardless of the date of application. This suggests a possible build up of a herbicide residue.

In experiment one--laboratory test--the electrical conductance method was not an accurate way to detect early alfalfa injury. The method did not detect differences among the herbicide treatments and the untreated check even though these treatments did differ in both yield and height at the time of the first cutting. The electrical conductance method did detect the obvious varietal difference between the non-dormant Moapa variety and the other two varieties. This procedure was not repeated in the succeeding years of experiment one.

Experiment two compared the 1x and 2x rates of three herbicides applied for three consecutive years on the same plots at site A. In the first year, no significant differences were found between the 1x and 2x rates (Table 5). After the second year of application, the 2x terbacil treatment significantly reduced the alfalfa yield. The 1x metribuzin treatment appeared to be the least injurious to alfalfa. After three years of herbicide applications, the 2x rate of terbacil significantly reduced yields again. However, the 2x rate of simazine caused a drastic drop in alfalfa yield. Even after

Table 5. Alfalfa plant heights and yields of first cutting for three years following December applications of metribuzin, simazine, and terbacil.

Treatments	Rate (kg/ha)	Location and Years		
		Site A		
		1979	1980	1981
-----Plant height (cm) ^z -----				
Untreated check		43.3a	29.9a	53.1a
Metribuzin	0.56	43.4a	29.4a	51.3a
Metribuzin	1.12	43.1a	29.6a	50.4a
Simazine	1.12	43.4a	30.3a	47.3ab
Simazine	2.24	41.5a	28.8a	41.3b
Terbacil	0.84	43.0a	29.7a	49.7ab
Terbacil	1.68	42.0a	30.1a	47.9ab
-----Dry matter (kg/ha) ^z -----				
Untreated check		2445a	3606a	2622a
Metribuzin	0.56	2468a	3591a	2441a
Metribuzin	1.12	2452a	3553ab	2315ab
Simazine	1.12	2443a	3369ab	2019ab
Simazine	2.24	2259a	3103ab	1176c
Terbacil	0.84	2423a	3280ab	2187ab
Terbacil	1.68	2233a	2978b	1868b

^zMeans within a column sharing the same letter are not significantly different at the 5% level.

three annual applications, none of the 1x treatments significantly reduced yields.

The bioassay in experiment two showed that soil treated for three years with the 1x rates of the herbicides carried little or no toxic residues (Table 6). Soil treated with the 1x rate of simazine did give some early injury seen as leaf margin chlorosis, but the amount was not significant. No indication of residue build up from the 2x rates of metribuzin and terbacil was observed. However, the cucumbers and mustards grown in soil treated for three years with the 2x rate of simazine showed severe chlorosis and stunting. Some cucumber plants did not survive the third week of the bioassay. Injury was measured quantitatively as a significant decrease in cucumber height and dry matter (Table 6). The results of the bioassay indicate that the sharp increase in alfalfa injury after three years of 2x simazine treatments was due to a build up of one or more toxic residues in the soil.

Table 6. Results of bioassay on a sample of soil treated for three consecutive years with herbicides and planted six months after the last herbicide treatment with mustard and cucumbers.

Treatments	Herbicide Rate (kg/ha)	Mustard Injury ^{yz} (%)	Cucumbers Injury ^{yz} (%)	Cucumbers Plant Height ^y (cm)	Cucumbers Dry Matter ^y (grams)
Untreated check	---	0b	0b	10.2a	10.0a
Metribuzin	0.56	1b	0b	8.1ab	9.4a
Metribuzin	1.12	6b	8b	7.4ab	7.9a
Simazine	1.12	1b	19b	6.8ab	9.7a
Simazine	2.24	65a	84a	1.3c	0.2b
Terbacil	0.84	0b	0b	7.6ab	8.9a
Terbacil	1.68	1b	0b	6.2b	9.8a

^yMeans within a column sharing the same letter are not significantly different at the 5% level.

^zInjury included chlorosis, stunting and stand reduction with 0 as no visible injury and 100 as dead.

CHAPTER IV

RESPONSE OF ESTABLISHED ALFALFA CULTIVARS TO WINTER HERBICIDE APPLICATIONS

1. INTRODUCTION

Cultivars of many crops vary in their susceptibility to certain herbicides. Differences in susceptibility have been reported in barley (21), bermudagrass (29), corn (17), cucumbers (70) creeping bentgrass (1), peaches (38), soybeans (22) and spring wheat (21). Additionally, biotypes of weeds such as common groundsel (50), lambsquarter and redroot pigweed (47) vary in herbicide susceptibility.

Only limited work has been done on the response of various alfalfa cultivars to herbicides. McCarty and Sand (40) used several rates of 2,4-DB and dalapon on seedling stands of five alfalfa cultivars. When injury did appear, it occurred uniformly across alfalfa cultivars. Harvey et al. (24) studied the effect of four triazine herbicides including atrazine and simazine on five alfalfa cultivars. In this Wisconsin study, herbicides were applied after the first spring cutting. Yields were recorded in the autumn and the following spring. In the autumn the DuPuits cultivar showed injury similar to the other cultivars, but in the spring it showed greater injury. Dupuits is a Flemish type alfalfa cultivar with less winter hardiness

than the American cultivars (Vernal and Iroquois) or the modified Flemish types (Saranac and Tempo). The authors reasoned that the combined stress of the winter and the herbicide may be responsible for the greater yield reduction of the DuPuits cultivar.

The purpose of this study was to screen established alfalfa cultivars for injury from late winter herbicide applications.

2. MATERIALS AND METHODS

The response of various alfalfa cultivars to herbicides application was studied at four locations across Tennessee. The locations used in 1979 were Highland Rim Experiment Station (HRES) near Springfield, Middle Tennessee Experiment Station (MTES) near Spring Hill, and Plateau Experiment Station (PES) near Crossville. In 1980 the single location was the Tobacco Experiment Station (TES) near Greeneville.

An alfalfa cultivar yield test was at each location and had been established three to five years previously. Alfalfa yields were no longer recorded on these tests due to reduced stands of some cultivars. However, the alfalfa cultivars could be screened for herbicide susceptibility. Alfalfa cultivars were arranged in a randomized complete block design with four replications. Each plot was 1.8 to 2.1 meters by 6.1 to 7.6 meters.

Alfalfa cultivars in these tests had various sources of germplasm and associated degrees of winter hardiness. The most winter hardy cultivars were from the Medicago falcata line such as Titan,

less winter hardy were the Flemish cultivars such as Europa. The Kansas Common lines such as Cody and Williamsburg also had moderate winter hardiness. Most cultivars were combinations of these lines (5).

Herbicides were applied in two meter bands across each replication of the cultivar yield tests. This created a split block experimental design with alfalfa cultivars as main blocks and herbicides as split treatments. Herbicides rates were 50% above their normal use rate (1.5x) to increase the chance of herbicide injury (Table 7). Normal use rates of most herbicides produce little or no crop injury. However, under field conditions, higher than normal rates are often applied due to calibration error, or overlapping of the spray pattern, or other mischance.

Herbicides were applied to dormant alfalfa in late spring. The environmental conditions at the time of application are listed in the Appendix (Table A-2). Herbicides were sprayed in 187 liters of water per hectare through a CO₂ powered hand held boom with 2.1 kg/cm² pressure. A section of each cultivar was left untreated to serve as a check. Approximately two months after herbicide application, plant heights were obtained by measuring five randomly selected alfalfa plants in each sub plot and averaging these heights. Measurements of alfalfa yield were impractical due to the small plot size and low plant densities of some cultivars. Data from each location were analyzed separately at the 5% level of probability.

Table 7. Herbicides and rates used on alfalfa cultivars at each location.

Common Name	Commercial Name	Herbicide Rate (kg/ha)	Locations Where Applied
Chlorpropham	Furloe	3.4	HRES ^W
Metribuzin	Sencor	0.84	MTES ^X , TES ^Y
Pronamide	Kerb	2.5	HRES, PES ^Z
Simazine	Princep	1.7	MTES, TES
Terbacil	Sinbar	1.7	MTES, TES
Hexazinone	Velpar	3.4	HRES, PES

^WHighland Rim Experiment Station.

^XMiddle Tennessee Experiment Station.

^YTobacco Experiment Station.

^ZPlateau Experiment Station.

3. RESULTS AND DISCUSSION

Herbicides applied at 1.5x rates apparently injured the alfalfa as evident by the average height of the treated alfalfa cultivars compared to the average height of the non-treated plants (Table 8). Only at the Highland Rim Experiment Station did herbicide treatments not significantly reduce alfalfa height. Height measurements at Highland Rim Experiment Station were taken earlier in the season than at other locations, and the measurements of alfalfa height were less than half the measurements of alfalfa height at other locations. Because alfalfa growth was less advanced at the time of measurement at Highland Rim Experiment Station than at other locations, possible differences among treatments in plant height did not have the opportunity to manifest themselves as at other locations.

Metribuzin caused no height reduction at either Middle Tennessee Experiment Station or Tobacco Experiment Station. Simazine and terbacil showed a moderate amount of height reduction at two locations. Chlorpropham tended to reduce the early alfalfa growth less than either promanide or hexazinone at Highland Rim Experiment Station. However, at the Plateau Experiment Station chlorpropham reduced alfalfa height more than pronamide.

Most importantly, the interaction of alfalfa cultivars and herbicides was not significant at the 5% level of probability.

Table 8. Average alfalfa heights of all cultivars at four Tennessee locations following herbicide application in the late winter.

Herbicide Treatments	Herbicide Rate (kg/ha)	Locations			
		HRES ^W	MTES ^X	PES ^Y	TES ^Z
-----cm.-----					
Chloropropham	3.4	22.6	--	43.5	--
Metribuzin	0.84	--	53.6	--	45.2
Pronamide	2.5	21.4	--	45.8	--
Simazine	1.7	--	47.0	--	44.1
Terbacil	1.7	--	50.3	--	41.0
Hexazinone	3.4	20.8	--	--	--
Untreated check		23.3	52.5	46.7	45.2

^WHighland Rim Experiment Station in 1979.

^XMiddle Tennessee Experiment Station in 1979.

^YPlateau Experiment Station in 1979.

^ZTobacco Experiment Station in 1980.

The interaction was significant at the 10% level at the Tobacco Experiment Station only. Individual alfalfa cultivars did not show extremes of herbicide tolerance or susceptibility (Tables 9 and 10).

Table 9. Percent alfalfa height reduction after application of chlorpropham at 3.4 kg/ha, pronamide at 2.5 kg/ha, and hexazinone at 3.4 kg/ha at two Tennessee locations.

Varieties	Herbicides				
	Chlorpropham		Pronamide		Hexazinone
	HRES ^y	PES ^z	HRES	PES	HRES
-----Percent height reduction-----					
Anchor	3	8	18	7	16
Appollo	-	7	-	2	-
Atlas	5	-	7	-	0
Europa	8	8	10	1	15
Gladiator	0	12	7	4	18
K1-10	-	10	-	9	-
K0-612	0	-	12	-	11
Lancer	8	2	17	0	15
Olympic	-	0	-	0	-
Pioneer 520	0	6	0	0	0
Pioneer 530	0	6	5	3	3
Team	5	4	11	0	14
Tempo	10	2	10	0	19
Titan	0	10	8	7	11
Victor	0	-	1	-	0
Weevlchek	4	7	11	4	11
Williamsburg	5	4	7	0	14

^yHighland Rim Experiment Station in 1979.

^zPlateau Experiment Station in 1979.

Table 10. Percent alfalfa height reduction after applications of metribuzin at 0.84 kg/ha, simazine at 1.7 kg/ha, and terbacil at 1.7 kg/ha at two Tennessee locations.

Varieties	Herbicides					
	Metribuzin		Simazine		Terbacil	
	MTES ^y	TES ^z	MTES	TES	MTES	TES
-----Percent height reduction-----						
Apalachee	0	-	3	-	0	-
Apollo	0	0	3	1	4	14
Arc	0	5	16	3	7	9
Buffalo	0	0	8	0	0	3
Cimmaron	-	0	-	6	-	14
Cody	0	-	6	-	8	-
CW 2	-	7	-	15	-	24
CW 9	-	0	-	0	-	0
CW 27	-	0	-	0	-	5
Fame	-	0	-	0	-	13
Gladiator	9	2	23	3	12	8
K3-10	0	-	6	-	0	-
KO-612	0	-	6	-	0	-
Lancer	0	-	11	-	4	-
Liberty	-	1	-	0	-	0
Olympic	-	0	-	0	-	16
Pioneer 520	5	0	16	1	8	2
Pioneer 521	0	0	5	0	6	5
Saranac AR	3	9	12	13	6	14
Team	0	-	15	-	4	-
Tempo	-	0	-	2	-	3
Victor	0	8	10	0	0	10
Weevlchek	0	2	14	5	17	15
Williamsburg	0	0	9	0	4	8

^yMiddle Tennessee Experiment Station in 1979.

^zTobacco Experiment Station in 1980.

CHAPTER V

INFLUENCE OF DATE OF WINTER HERBICIDE APPLICATION ON INJURY TO ESTABLISHED ALFALFA

1. INTRODUCTION

Proper timing of herbicide application is often critical for a successful weed control program in alfalfa. The date of application can influence herbicide effectiveness for weed control in alfalfa (2), (4), (6), (8), (44). Equally important but less understood is that the date of herbicide application may affect crop injury (6), (42), (44). Proper timing of herbicide application in Tennessee is complicated by mild winters and alternating periods of alfalfa dormancy and non-dormancy. Previous work in Tennessee reported a trend toward more alfalfa injury with later application dates when comparing application dates in December, January, and February (18).

The purpose of this research is to study the effect of the date of winter herbicide application and the environmental conditions surrounding that application date on alfalfa injury.

2. MATERIALS AND METHODS

Research on the effect of date of herbicide application on injury to established Gladiator alfalfa was conducted at the Middle Tennessee Experiment Station near Spring Hill, Tennessee. The

experiment was conducted on separate alfalfa stands each winter of 1980, 1981, and 1982. Alfalfa stands were essentially free of weeds. Herbicides were applied at six different times each winter from December to March. The herbicide treatments were simazine at 2.24 kg/ha and terbacil at 1.68 kg/ha, plus an untreated check. These herbicide rates are twice their normal use rate and will be referred to as 2x rates. Herbicides were applied to alfalfa plots measuring 1.8 meters x 7.6 meters. These plots were arranged in a randomized complete block design with four replications. Herbicides were applied through a CO₂ powered, hand held boom delivering 187 l/ha.

Measured responses were alfalfa plant height and forage yield of the first alfalfa cutting. Data from each year were analyzed separately. Means were separated by Duncan's Multiple Range Test at 5% level of probability.

Environmental conditions on the days of herbicide application are given in the Appendix (Table A-3). Environmental conditions were recorded daily during the experimental period. Data collected included maximum and minimum air temperature measured one meter above sod, maximum and minimum soil temperature measured 2.5 cm below bare ground, and precipitation. Environmental conditions were compiled for periods of four days before application, ten days before application, four days after application, ten days after application, and twenty days after application. Time periods were combined to produce all possible combinations of before-application time intervals with after-application time intervals. Calculated

measurements included the mean daily soil and air temperatures accumulated for each time interval. The daily range of soil and air temperatures and the difference between the range in air temperature and the range in soil temperature were calculated. These ranges were averaged for each time interval. Additionally, daily precipitation amounts were totaled for each time interval. To measure the progressive change in the season, the days of the year were numbered sequentially with the fall equinox of each year being day one.

The independent variables mentioned above were correlated with the percent yield reduction from the untreated check and the percent height reduction from the untreated check for each date of herbicide application. The correlation coefficient (r) of each variable was calculated independently of other variables.

3. RESULTS AND DISCUSSION

Both simazine and terbacil applied at 2x rates injured the alfalfa crop. This injury can be seen in reduced plant height (Table 11) and in reduced dry matter yields (Table 12).

Simazine was the least injurious of the two herbicides. Simazine did not reduce the plant height of the alfalfa in 1980 and only reduced the yield for the last three application dates. In 1981, the 2x simazine treatments reduced plant height on four of the six application dates and reduced yields in every case. In 1982, simazine treatments reduced alfalfa height on three of

Table 11. Alfalfa heights following 2x applications of simazine and terbacil at different dates.

Application Date ^x	1980			1981			1982		
	Alfalfa Height (cm) ^y	Percent Reduction ^z		Alfalfa Height (cm)	Application Date	Percent Reduction	Alfalfa Height (cm)	Application Date	Percent Reduction
-----Simazine applications at 2.24 kg/ha -----									
12-21 (90)	30.5a	0		1-9 (103)		18.1	12-7 (76)		9.6
12-29 (98)	32.3a	0		1-19 (113)		17.0	1-5 (105)		15.8
1-25 (125)	30.3a	0		1-26 (120)		20.9	1-20 (120)		30.6
2-14 (145)	29.0abc	0.8		2-19 (144)		9.4	2-5 (136)		24.9
2-22 (153)	32.0a	0		3-6 (159)		8.4	3-1 (160)		20.7
3-27 (187)	30.0ab	0		3-17 (170)		12.5	3-18 (177)		3.1
-----Terbacil applications at 1.68 kg/ha -----									
12-21 (90)	29.8ab	0		1-9 (103)		26.3	12-7 (76)		14.1
12-29 (98)	29.5abc	0		1-19 (113)		17.2	1-5 (105)		15.2
1-25 (125)	26.0c	11.1		1-26 (120)		21.4	1-20 (120)		1.2
2-14 (145)	31.0a	0		2-19 (144)		19.7	2-5 (136)		16.0
2-22 (153)	26.5bc	9.4		3-6 (159)		30.4	3-1 (160)		35.9
3-27 (187)	29.3abc	0		3-17 (170)		25.0	3-18 (177)		27.6
-----Untreated check-----									
	29.3abc								
							38.6a		

^xNumerical month and date are followed by the consecutive date in parentheses with the fall equinox being day one.

^yMeans followed by the same letter are not significantly different at the 0.05 level of probability.

^zPercent height reduction from the untreated check.

Table 12. Dry matter yield of alfalfa following 2x applications of simazine and terbacil at different dates.

Application Date ^x	1980			1981			1982		
	Alfalfa Yield (kg/ha) ^y	Percent Reduction ^z	Application Date	Alfalfa Yield (kg/ha)	Percent Reduction	Application Date	Alfalfa Yield (kg/ha)	Percent Reduction	Application Date
-----Simazine applications at 2.24 kg/ha-----									
12-21 (90)	1861bcd	12.1	1-9 (103)	841bc	52.5	12-7 (76)	2027b	33.3	
12-29 (98)	2570a	0	1-19 (113)	630cd	64.4	1-5 (105)	1342bcde	55.8	
1-25 (125)	2092b	1.2	1-26 (120)	678cd	61.7	1-20 (120)	772ef	74.6	
2-14 (145)	1580cde	25.3	2-19 (144)	1162b	34.3	2-5 (136)	821ef	73.0	
2-22 (153)	1465cdef	30.8	3-6 (159)	1191b	32.7	3-1 (160)	921def	69.7	
3-27 (187)	1383def	34.7	3-17 (170)	927bc	47.6	3-18 (177)	1886bc	37.9	
-----Terbacil applications at 1.68 kg/ha-----									
12-21 (90)	1895bc	10.5	1-9 (103)	411d	76.7	12-7 (76)	1687bcde	44.5	
12-29 (98)	1474cdef	30.4	1-19 (113)	929bc	47.5	1-5 (105)	2059b	32.2	
1-25 (125)	1271ef	40.0	1-26 (120)	597cd	66.3	1-20 (120)	1831bcd	39.7	
2-14 (145)	1854bcd	12.4	2-19 (144)	927bc	47.6	2-5 (136)	1073cdef	64.7	
2-22 (153)	999f	52.8	3-6 (159)	403d	77.2	3-1 (160)	628f	79.3	
3-27 (187)	1218ef	42.4	3-17 (170)	704cd	60.2	3-18 (177)	491f	83.8	
-----Untreated check-----									
	2118b			1769a			3037a		

^xNumerical month and date are followed by the consecutive date in parentheses with the fall equinox being day one.^yMeans followed by the same letter are not significantly different at the 0.05 level of probability.^zPercent yield reduction from the untreated check.

six dates and reduced yields at every date. Generally, the alfalfa dry matter yield showed more differences among treatments than the alfalfa plant height. Simazine treatments did not show a trend toward more herbicide injury with later applications.

The 2x rate of terbacil was more injurious to alfalfa than the 2x rate of simazine. Terbacil treatments reduced plant height at two dates and reduced yields at four of the dates in 1980. Both alfalfa height and yield were reduced for every date in 1981. In 1982 only the last two applications reduced alfalfa height, but some reduction in yield occurred at all application dates. Generally, terbacil showed a trend toward more injury with later application dates. However, this trend was not always clear.

The variation in the alfalfa injury between dates can be partially explained by the climatological conditions surrounding each application date. However, before studying the relationship between weather conditions and alfalfa response to herbicides an analysis of variance of the alfalfa responses over a three year period was performed. A significant F test at $P = .05$ showed a definite year effect. This year effect could possibly be confounded in the correlation process, and thus will limit the interpretation of the correlations.

Climatological conditions were related to yield reduction and height reduction more for terbacil treatments than for simazine treatments. Hardly any of the variables gave significant correlations for the injury caused by simazine treatments. Generally,

climatological measurements accounted for less than 10% of the variation in alfalfa injury caused by simazine.

Significant correlations were found between four climatological measurements and the injury caused by terbacil. Total precipitation, the average ranges in soil and air temperature and the difference between the ranges in air and soil temperature correlated to terbacil injury (Tables 13 and 14). Precipitation was the highest correlated climatological measurement with both reduction in yield and height. The period of ten days after herbicide application was the most critical period for precipitation. The daily range in soil temperature was more highly correlated with the reduction in alfalfa yield and height than the range in air temperature. The differences between these ranges gave the least significant correlations.

The mean daily soil and air temperatures were not significantly correlated to alfalfa injury when they were accumulated for any time interval. Also, the relationship between numerical date of application and the alfalfa response to terbacil was not significant. The application date explained 18% of the variation in alfalfa yield reduction and 8% of the variation in plant height reduction. This indicated that the alfalfa did not become more susceptible to terbacil injury over time. However, application date was often included in the best correlated two and three variable models, indicating that date of application was explaining part of the variation in alfalfa injury not explained by the climatological measurements.

Table 13. Correlations of several climatological measurements compiled over different time intervals with the percent alfalfa height reduction from terbacil application at 1.68 kg/ha at Spring Hill, Tennessee.

Time Intervals		Total Precipitation	Average Daily Range in Air Temperature	Average Daily Range in Soil Temperature	Difference in Ranges of Air and Soil Temperatures
Days Before Application	Days After Application				
<u>Significant correlation coefficients with height reduction^z</u>					
4	0				.467
10	0		.507	.593	
0	4	.629		.592	
0	10	.708	.493	.507	
0	20	.555	.483		
4	4				.468
4	10				.467
4	20				.467
10	4	.573	.543	.613	
10	10	.607	.524	.602	
10	20	.531	.513	.597	

^z Five percent level of probability.

Table 14. Correlations of several climatological measurements compiled over different time intervals with the percent alfalfa yield reduction from terbacil application at 1.68 kg/ha at Spring Hill, Tennessee.

Time Intervals		Total Precipitation	Average Daily Range in Air Temperature	Average Daily Range in Soil Temperature	Difference in Ranges in Air and Soil Temperatures
Days Before Application	Days After Application				
4	0				
10	0			.555	.510
0	4	.509		.542	
0	10	.621	.508	.508	
0	20		.482	.517	
4	4				.507
4	10				.506
4	20				.509
10	4		.479	.573	
10	10		.471	.566	
10	20			.562	

Significant correlation coefficients with yield reduction^z

^z Five percent level of probability.

The best four variable model could explain only 54% of the of the variation in alfalfa yield reduction and 63% of the variation in alfalfa height reduction. Thus, a portion of the variation is left unexplained by the procedures used in this test.

Each of the time periods surrounding the date of application was significantly correlated with alfalfa injury for at least one climatological measurement. However, the time period of ten days after herbicide application was significantly correlated with more variables than any other time interval.

Finally, heavy precipitation within ten days after terbacil application will enhance the chance of alfalfa injury. Terbacil acts as a photosynthetic inhibitor that enters a plant primarily through the root system (67). Apparently, heavy rainfall after application leaches the herbicide into the root zone of the alfalfa. This should concern alfalfa producers with light textured soils where water permeability is greater.

CHAPTER VI

EFFECT OF ALFALFA DORMANCY ON TERBACIL CROP INJURY

1. INTRODUCTION

During the relatively mild Tennessee winters, alfalfa may become active during extended periods of warm weather. This lack of dormancy may render the alfalfa more susceptible to herbicide injury.

Previous research has not given a clear picture of the interaction between alfalfa dormancy and herbicide injury. Alfalfa cultivars with varying degrees of winter hardiness did not respond differently to terbacil treatments. However, alfalfa injury was greater with February applications of terbacil than with December applications (18). Was the alfalfa more susceptible to terbacil injury at one date because of a change in dormancy?

The purpose of this research is to artificially stimulate established alfalfa in the field to break dormancy in order to compare terbacil injury on dormant and non-dormant alfalfa.

2. MATERIALS AND METHODS

During the winters of 1981 and 1982, tests were conducted at the Middle Tennessee Experiment Station near Spring Hill to study the effect of terbacil on actively growing and non-actively growing alfalfa. Portable wooden frames covered with clear plastic

were used to provide conditions that would induce alfalfa growth. Frames were constructed of plywood and measured 2.4 by 2.4 by 0.46 meters. When 10% of the alfalfa plants under the frames showed new growth, terbacil was applied to plots both inside and outside the frames. The frames were left in place from one to eleven days after herbicide application. Afterwards, the frames were moved to new alfalfa plots and the procedure started again (Figure 1). This procedure was repeated four times each year with each repetition series comprising an independent set of observations.

Each experimental series was arranged in a split-plot design. Main treatments were the presence or absence of the plastic covered frames used to initiate alfalfa growth. Split treatments were the application or not of terbacil on both growing and non-growing alfalfa. Terbacil was applied at a 2x rate of 1.7 kg/ha through a CO₂ powered, hand held boom delivering 187 l/ha. Plot size was 1.2 by 2.4 meters. Each treatment was replicated five times per series.

Metal coil thermometers were used to measure daily maximum and minimum air temperatures both inside and outside the frames. The thermometers were concealed in ventilated, white wooden boxes resting on the ground. These metal coil thermometers were later compared to standard mercury thermometers, and appropriate adjustments were made in the temperature data.

Each year alfalfa injury was visually rated on a scale of 0 to 100 with 0 as no injury and 100 as dead. Injury ratings were



Figure 1. Alfalfa plots in foreground showing increased plant height where plastic covered wooden frames had stimulated new growth. Plastic covered wooden frames are in background.

based on the amount of chlorosis, alfalfa stunting, and stand reduction. Alfalfa height measurements were taken before the first harvest by measuring five randomly selected plants within each plot. Yield was recorded for the first cutting of alfalfa from an area of 2.6 m² and was reported as green weight.

Very little weed growth was present in any plots in 1981. However, in 1982, common chickweed was abundant in some plots. A botanical sample was taken from a 0.09 square meter area in each treatment of one replication and separated into alfalfa and weed samples. Yields were corrected for the percent weeds in each treatment in 1982.

Each experimental series was analyzed independently. Means were separated by Duncan's Multiple Range test at the 0.05 level of probability.

3. RESULTS AND DISCUSSION

Mean temperatures inside the plastic covered frames were raised an average of 5.9°C in 1981 and 4.1°C in 1982. Also, frames served to protect alfalfa plants from light frost. During severe cold weather frost did appear inside the frames.

The young alfalfa growth induced by the frames was often damaged by heavy frost when the frames were removed. In some cases visual ratings of injury describe the alfalfa under the frames as being more injured than the alfalfa outside the frames (Table 15). However, this injury did not translate into significant yield

Table 15. Effect of stimulated alfalfa growth and terbacil application on alfalfa injury as rated by visual estimate at Spring Hill, Tennessee.

	1981				1982			
	First Series	Second Series	Third Series	Fourth Series	First Series	Second Series	Third Series	Fourth Series
-----Percent Injury ^z -----								
Main treatments								
Frames present to induce alfalfa growth	8a	27a	33a	20a	14a	17a	13a	27a
Frames absent; alfalfa dormant	7a	12a	22a	7a	16a	14b	6b	14a
Split treatments								
Terbacil applied at 1.7 kg/ha	15a	35a	52a	27a	30a	30a	18a	41a
No terbacil applied	0b	4b	4b	1b	0b	0b	0b	0b
Interaction of treatments								
Frames present; terbacil applied	15	46	59	39a	27	33a	25a	53
Frames present; no terbacil	0	7	7	1b	0	0c	0c	0
Frames absent; terbacil applied	14	23	45	14b	32	27b	11b	28
Frames absent; no terbacil	0	1	0	0b	0	0c	0c	0
(Non significant interaction as NSI)	NSI	NSI	NSI		NSI			NSI

^z Alfalfa injury was rated on a scale of 0 to 100 with 0 as no injury and 100 as plant death. Means within a column and within a treatment group sharing the same letter are not significantly different at the 5% level of probability.

reductions or height reductions at the time of the first harvest. Only in the fourth and last series of 1982 were both alfalfa height and yield reduced by the presence of the frames. In this case the maximum temperature under the frames rose above 37.7°C for three consecutive days. Thus the alfalfa was probably injured by the excessive heat. Also, this series was unusual in that the alfalfa outside the frames started new growth soon after the alfalfa inside the frames did.

The application of 1.7 kg/ha of terbacil at the start of new growth did injure the alfalfa. Terbacil injury first appeared on plots where alfalfa growth had been stimulated. Initial injury appeared on new growth as burning of the whole leaf. Later, leaf chlorosis appeared in terbacil treated plots both where growth had been stimulated and where it had not (Figure 2). Leaf chlorosis would begin on the leaf margin and progress inward. Chlorosis had disappeared by the time of the first harvest except for the most recently treated series. At the time of the first harvest visible injury included stunted plants and reduced alfalfa stands. Terbacil treatments significantly reduced alfalfa height (Table 16) and alfalfa yield (Table 17). No significant correlation existed between the amount of herbicide injury and the time interval the frame remained in place after spraying. No general trend concerning alfalfa injury was discerned.

In only 38% of the experimental series did the interaction of the main and split treatments combine to increase the alfalfa

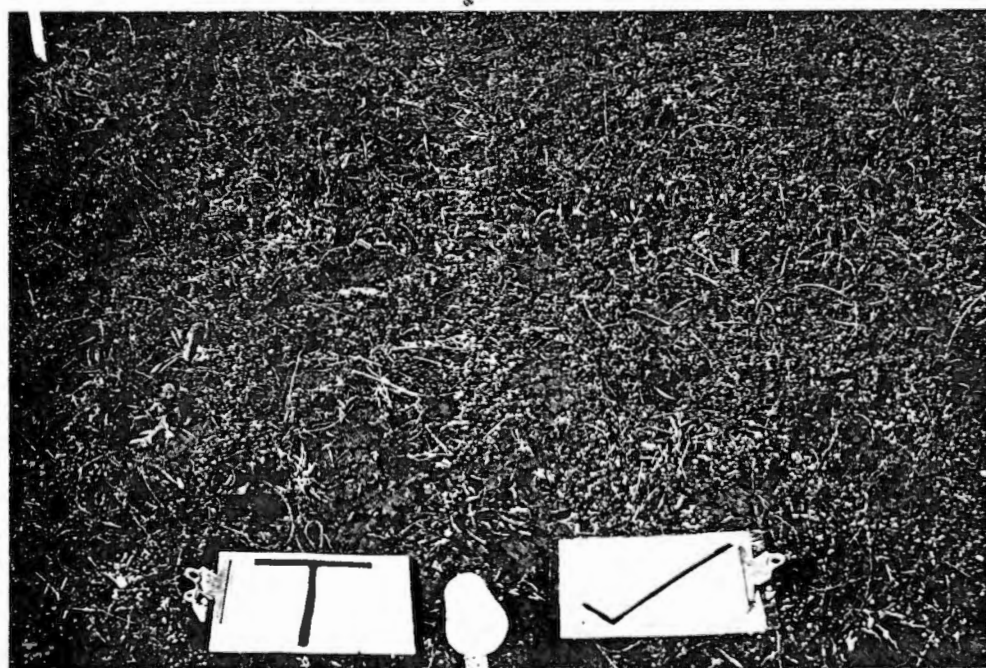


Figure 2. Alfalfa leaf chlorosis and dead grass plants resulting from an application of 1.7 kg/ha of terbacil is on left. An untreated plot is on right.

Table 16. Effect of stimulated alfalfa growth and terbacil application on alfalfa plant height measured before the first cutting at Spring Hill, Tennessee.

	1981				1982			
	First Series	Second Series	Third Series	Fourth Series	First Series	Second Series	Third Series	Fourth Series
-----cm ² -----								
<u>Main treatments</u>								
Frames present to induce alfalfa growth	78a	76a	73a	71a	33b	35a	32a	24b
Frames absent; alfalfa dormant	78a	80a	68a	71a	36a	34a	33a	33a
<u>Split treatments</u>								
Terbacil applied at 1.7 kg/ha	75b	75b	66a	65b	31b	29b	30a	23b
No terbacil applied	82a	81a	74a	78a	39a	40a	35a	34a
<u>Interaction of treatments</u>								
Frames present; terbacil applied	73	74	66	65	31c	29	27	19
Frames present; no terbacil	83	78	80	78	35b	40	36	30
Frames absent; terbacil applied	76	77	67	64	31c	29	32	28
Frames absent; no terbacil	81	83	69	78	42a	39	34	39
(Non significant interaction as NSI)	NSI	NSI	NSI	NSI	NSI	NSI	NSI	NSI

^zMeans within a column and within a treatment grouping sharing the same letter are not significantly different at the 5% level of probability.

Table 17. Effect of stimulated alfalfa growth and terbacil application on alfalfa yield measured as green weight at the first cutting at Spring Hill, Tennessee.

	1981				1982			
	First Series	Second Series	Third Series	Fourth Series	First Series	Second Series	Third Series	Fourth Series
	-----kg/2.6 m ² z-----							
Main treatments								
Frames present to induce alfalfa growth	3.7a	4.0a	3.5a	3.6a	1.3a	1.2a	1.3a	0.6b
Frames absent; alfalfa dormant	3.6a	4.3a	3.9a	3.7a	1.4a	1.3a	1.5a	1.7a
Split treatments								
Terbacil applied at 1.7 kg/ha	3.6b	3.5b	2.8b	2.8b	1.0b	0.8b	1.0b	0.6b
No terbacil applied	4.0a	4.7a	4.6a	4.4a	1.7a	1.7a	1.8a	1.7a
Interaction of treatments								
Frames present; terbacil applied	3.5	3.3	2.7c	2.7	1.2bc	0.7	0.9	0.2c
Frames present; no terbacil	3.9	4.6	4.2b	4.4	1.4b	1.6	1.8	1.0b
Frames absent; terbacil applied	3.2	3.8	2.8c	2.9	0.8c	0.8	1.1	1.0b
Frames absent; no terbacil	4.0	4.8	5.1a	4.4	2.0a	1.8	1.9	2.4a
(Non significant interaction as NSI)	NSI	NSI	NSI	NSI		NSI	NSI	

^zMeans within a column and within a treatment grouping sharing the same letter are not significantly different at the 5% level of probability.

injury measured at the first harvest. Surprisingly, the stage of activity of the alfalfa did not have a great bearing on the long-term injury incurred from terbacil applications.

CHAPTER VII

SUMMARY

Established alfalfa cultivars did not vary in their susceptibility to various herbicides. Three cultivars with varying degrees of dormancy did not respond differently to applications of metribuzin, simazine, and terbacil at various winter application dates. Many commercial cultivars were screened against six herbicides at different locations in Tennessee. Again no significant interaction occurred between herbicides and alfalfa cultivars.

Herbicide injury to established alfalfa was affected by the date of herbicide application. High rates of metribuzin, simazine, and terbacil were applied in December, January, and February. More injury was observed on alfalfa with the later application dates. Of the three herbicides, metribuzin was the least injurious and terbacil was the most injurious. Another experiment compared high rates of simazine and terbacil at six different application dates throughout the winter. The amount of injury did vary between dates, but the trend toward more alfalfa injury with later application dates was not evident. Terbacil was slightly more injurious than simazine. Also, injury from terbacil was more dependent on the climatological conditions surrounding the date of application. Of the climatological measurements taken, the precipitation for ten days following terbacil application had the highest correlation

with alfalfa injury. The ranges in soil and air temperatures were also correlated with terbacil injury.

The above experimentation gave varying results as to the effect of crop dormancy on herbicide injury to alfalfa. It could be assumed that if herbicide injury was related to alfalfa dormancy, then alfalfa cultivars with different degrees of dormancy would respond differently to herbicide applications. This was not the case as the cultivar interaction was not significant. However, alfalfa dormancy should vary over time and herbicide injury did vary with different dates of herbicide application. To resolve the problem, alfalfa was artificially stimulated to break dormancy and terbacil was applied to both dormant and non-dormant alfalfa on the same date. When injury was measured at the time of the first alfalfa cutting, it was determined that terbacil injury on non-dormant alfalfa was not significantly greater than terbacil injury on dormant alfalfa in 62% of the cases.

The effect of repeated herbicide application was studied by applying standard and high rates of metribuzin, simazine, and terbacil annually to alfalfa over three years. The standard rates of all three herbicides did not cause injury to alfalfa whether applied for a single season or applied annually to the same plots for three years. The high rate of terbacil caused injury each year, but the injury did not increase over time. The high rate of simazine produced dramatically more injury after the third annual application. A bioassay with indicator crops planted six months

after the last herbicide application revealed that this injury was the result of the accumulation of toxic residue in the soil.

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APPENDIX

Table A-1. Environmental conditions at the time of herbicide applications to established alfalfa as presented in Chapter III.

	Site A 1978-1979				Site A 1979-80				Site B 1979-1980				Site A 1980-1981			
	12-16-78	1-29-79	2-21-79	12-10-79	1-21-80	3-14-80	12-6-79	1-9-80	3-6-80	12-23-80	1-29-81	2-25-81	12-23-80	1-29-81	2-25-81	2-25-81
Date	12-16-78	1-29-79	2-21-79	12-10-79	1-21-80	3-14-80	12-6-79	1-9-80	3-6-80	12-23-80	1-29-81	2-25-81	12-23-80	1-29-81	2-25-81	2-25-81
Time	2:00 PM	1:30 PM	1:00 PM	9:00 AM	10:30 AM	2:00 PM	8:00 AM	9:00 AM	10:00 AM	10:00 AM	10:00 AM	8:00 AM	10:00 AM	10:00 AM	8:00 AM	8:00 AM
Air temperature (°C)	8.3	2.8	17.2	13.9	5.6	12.2	12.2	5.6	7.2	6.7	6.7	8.3	6.7	6.7	8.3	8.3
Soil temperature (°C) ^z	5.0	7.2	13.9	8.3	7.2	17.2	8.3	7.7	12.8	2.2	6.1	7.2	2.2	6.1	7.2	7.2
Relative humidity	53%	63%	78%	53%	81%	65%	89%	72%	72%	67%	58%	66%	67%	58%	66%	66%
Wind (estimated km/hr)	6-8	8-10	3-6	3-5	0-3	5-6	2-3	6-10	0-8	6-13	6-13	0-3	6-13	6-13	0-3	0-3
Sky	Clear	Clear	Clear	Clear	Overcast	Clear	Overcast	Clear	Clear	Overcast	Clear	Clear	Overcast	Clear	Clear	Clear
Soil	Moist	Wet	Moist	Dry	Moist	Dry	Dry	Moist	Wet	Dry	Moist	Moist	Dry	Moist	Moist	Moist

^zSoil temperature measured 2.5 cm below bare ground.

Table A-2. Environmental conditions at the time of herbicide application to alfalfa cultivars listed in Chapter IV and dates of alfalfa height measurements.

Conditions	Locations			
	HRESV	MTESW	PESX	TESY
Date of application	3-3-79	2-21-79	3-7-79	2-29-80
Time of day	1:00 PM	3:30 PM	11:30 AM	8:30 AM
Air temperature (°C)	18.3	16.7	12.8	-1
Soil temperature (°C) ^z	11.1	13.8	12.2	3.9
Wind (estimated km/hr)	3-8	5-10	16-18	0-5
Sky	Clear	Clear	Partly cloudy	Overcast
Soil moisture	Moist	Moist	Moist	Dry
Date of alfalfa height measurements	4-17-79	5-14-79	4-24-79	4-24-80

^VHighland Rim Experiment Station.

^WMiddle Tennessee Experiment Station.

^XPlateau Experiment Station.

^YTobacco Experiment Station.

^ZSoil temperature measured 2.5 cm below bare ground.

Table A-3. Environmental conditions at the time of herbicide applications at several dates in the winters of 1980, 1981, and 1982, as referred to in Chapter V.

Date	12-21-79	12-29-79	1-25-80	2-14-80	2-22-80	3-27-80
Consecutive date ^y	(90)	(98)	(125)	(145)	(153)	(187)
Air temperature (°C)	8.9	12.8	10.6	15.0	23.9	17.2
Soil temperature (°C) ^z	6.1	12.2	7.8	7.2	20.0	16.1
Relative humidity	56%	57%	59%	48%	50%	50%
Wind (estimated km/hr)	10-11	0-2	6-8	2-3	5-6	3-6
Sky	Overcast	Clear	Overcast	Clear	Clear	Overcast
Soil moisture	Moist	Moist	Moist	Moist	Moist	Moist
Date	1-9-81	1-19-81	1-26-81	2-19-81	3-6-81	3-17-81
Consecutive date	(103)	(113)	(120)	(144)	(159)	(170)
Air temperature (°C)	2.8	11.7	17.8	20.0	10.0	22.8
Soil temperature (°C)	0.6	8.3	14.4	20.0	16.1	23.3
Relative humidity	58%	45%	58%	60%	55%	37%
Wind (estimated km/hr)	8-13	0-8	3-6	10-13	10-13	13-16
Sky	Partly cloudy	Partly cloudy	Overcast	Partly cloudy	Clear	Clear
Soil moisture	Wet	Dry	Moist	Wet	Moist	Dry
Date	12-7-81	1-5-82	1-20-82	2-5-82	3-1-82	3-18-82
Consecutive date	(76)	(105)	(120)	(136)	(160)	(177)
Air temperature (°C)	8.9	10.6	5.0	10.0	15.0	21.1
Soil temperature (°C)	6.1	8.9	2.2	11.7	19.4	18.9
Relative humidity	63%	50%	95%	74%	48%	77%
Wind (estimated km/hr)	0-3	0-8	0-2	5-8	0-3	0-3
Sky	Overcast	Sunny	Cloudy	Overcast	Clear	Clear
Soil moisture	Dry	Wet	Wet	Wet	Wet	Moist

^yConsecutive date numbered with the fall equinox being day one.

^zSoil temperature measured 2.5 cm below bare ground.

VITA

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In 1974, he completed his Bachelor of Arts in Cell Biology at The University of Tennessee, Knoxville. After working at the Middle Tennessee Experiment Station he decided to enter a graduate program in agriculture. In 1976, he completed the Master of Science degree in Agricultural Biology with an emphasis in Entomology. The same year he began work toward a Doctor of Philosophy degree with a major in Plant and Soil Science. In October of 1978, he accepted a full-time position at the Middle Tennessee Experiment Station as a Research Assistant in Plant and Soil Science.

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