SELECTIVITY OF CYANAZINE (2-[[4-CHLORO-6-(ETHYLAMINO)-S-TRIAZIN-2-YL] AMINO]-2-METHYLPROPIONITRILE) ON FALL PANICUM (PANICUM DICHOTOMIFLORUM MICHX.), GREEN FOXTAIL (SETARIA VIRIDIS L.), AND CORN (ZEA MAYS L.)

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SELECTIVITY OF CYANAZINE (2-[[4-CHLORO-6-(ETHYLAMINO)-S-TRIAZIN-2-YL] AMINO]-2-METHYLPROPIONITRILE) ON FALL PANICUM (PANICUM DICHOTOMIFLORUM MICHX.), GREEN FOXTAIL (SETARIA VIRIDIS L.), AND CORN (ZEA MAYS L.)

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ABSTRACT

SELECTIVITY OF CYANAZINE (2-[[4-CHLORO-6-(ETHYLAMINO)-S-TRIAZIN-2-YL] AMINO]-2-METHYLPROPIONITRILE) ON FALL PANICUM (PANICUM DICHOTOMIFLORUM MICHX.), GREEN FOXTAIL (SETARIA VIRIDIS L.), AND CORN (ZEA MAYS L.)

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The response of corn (Zea mays L.) and fall panicum (Panicum dichotomiflorum Michx.) to postemergence herbicide applications at three stages of growth was examined. Early postemergence application of cyanazine (2-[[4-chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile) at 3.4 kg/ha provided the best control with minimum crop injury and greatest corn yield. Hand removal of fall panicum at the 7 to 8-leaf stage resulted in corn yield reductions of 25% as compared to plots which were weed-free from the 2-leaf stage. The addition of various adjuvants increased cyanazine activity on both fall panicum and corn in field and greenhouse experiments; however, field trials showed no differences in grain yield at harvest. In the greenhouse, less corn injury was observed with a vegetable oil additive than with other adjuvants.

Greenhouse studies indicated that root absorption after postemergence application of cyanazine enhanced phytotoxicity to fall panicum, green foxtail (Setaria viridis, L.), and corn. Less ^{14}C-cyanazine was taken up by the foliage of corn than by the weed species. A lower concentration of parent cyanazine in corn leaves was also evident. The addition of a phytobland oil to the treatment solution resulted in increased foliar cyanazine absorption 1 and 5 days following treatment. Although rapid metabolism occurred in corn roots, the large amounts of cyanazine absorbed via the root system resulted in internal concentrations of parent cyanazine similar to that observed in weed species. Cyanazine translocation was mainly acropetal from the point of application. Selectivity was not solely based on differential foliar uptake of cyanazine, but also on the proportion taken up by the foliage and roots. Under conditions favoring uptake by roots, the margin of selectivity may be reduced.

The metabolism of cyanazine by corn, fall panicum, and green foxtail was compared to determine the contribution of metabolism to selectivity. Cyanazine metabolism by plants with four leaves was examined following foliar or root treatments with ^{14}C-cyanazine. Parent material was separated from metabolites by thin-layer chromatography. Five days following foliar application, 32.5 and 41.1% of a water-soluble metabolite were found in fall panicum and green foxtail, respectively. In corn two metabolites with

19.1 and 25.7% of ^{14}C activity were found in the water-soluble fraction. Only 12.0 and 6.2% of the same breakdown products were found in corn after a single day of treatment. Small quantities of other metabolites were also found. Metabolism of root-applied cyanazine appears to differ from foliar treatments in the weedy grasses as more parent cyanazine was recovered. Green foxtail had equal amounts of unaltered cyanazine present in both shoot and root portions. The roots of corn and fall panicum contained less cyanazine than did the shoots. Rapid metabolism of cyanazine by corn roots provided evidence for an active detoxification mechanism.

Tolerance of greenhouse-grown corn to cyanazine and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-_s_-triazine] was compared when grown in Conover sandy loam soil. When preemergence and postemergence applications of cyanazine were allowed to contact the soil, reductions in dry weight were obtained under both low and high soil moisture conditions.

SELECTIVITY OF CYANAZINE (2-[[4-CHLORO-6-(ETHYLAMINO)-S-TRIAZIN-2-YL] AMINO]-2-METHYLPROPIONITRILE) ON FALL PANICUM (*PANICUM DICHOTOMIFLORUM* MICHX.), GREEN FOXTAIL (*SETARIA VIRIDIS* L.), AND CORN (*ZEA MAYS* L.)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The elimination of undesirable plants is a problem in crop production. The annual losses from weeds plus the cost of their control in agriculture are great. Reduced crop yield results from competition between crops and weeds for soil nutrients, space, water, CO_2, and light. Effective chemical weed control practices have aided farmers in increasing production. Of the many herbicides used, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] is one of the most widely used for selective weed control in corn (Zea mays L.).

Fall panicum (Panicum dichotomiflorum Michx.), an increasing annual grass weed problem, appears to be associated with the continuous use of atrazine. The resistance of fall panicum to atrazine and the elimination of competing weed species during the growing season have provided the avenue for fall panicum invasion. Green foxtail (Setaria viridis L.), another annual grass, is moderately resistant to postemergence applications of atrazine. Although green foxtail has not become as serious as fall panicum, the threat of Michigan cornfields becoming infested with another grass species should be avoided.

Failure of preemergence weed control in corn without effective postemergence control has resulted in reduced yields. Until recently, postemergence control of fall panicum and green foxtail was difficult. Cyanazine (2-[[4-chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile) offers an effective control measure for these grass weeds, although corn injury has been observed with cyanazine. Examinations of cyanazine application rates and timeliness are needed to determine optimum levels for full season weed control to maximize corn yield. Age of corn, type of adjuvant, and environmental conditions appear to influence corn phytotoxicity following cyanazine treatment in field trials.

Cyanazine has only recently been registered for postemergence weed control in corn. Many factors which influence crop phytotoxicity and annual grass control with cyanazine are not understood. The objectives of this study were to (1) evaluate the performance of cyanazine and cyanazine combinations as postemergence treatments for full-season fall panicum control, (2) determine the factors affecting corn tolerance and weed control at various stages of growth, (3) determine the extent of foliar penetration, translocation, and metabolism of cyanazine in corn and two weed species to determine the basis of selectivity.

CHAPTER 1

LITERATURE REVIEW

Fall Panicum and the Nature of the Problem

Fall panicum, a monocotyledonous plant, has recently become a serious weed problem in row crops (23, 24). It is an annual grass which can attain a height in excess of one meter, has an erect to decumbent or diffuse growth habit, and tillers profusely (20, 24, 56). It has glaborous sheaths and blades, with a ligule consisting of a dense ring of white hairs 1 to 2 mm long (78). The mature plant produces a panicle type inflorescence and is characterized by swollen nodes (20, 56, 79). The lower nodes of decumbent stems are often rooted (20, 46). This annual grass produces many seeds which can germinate throughout the growing season (24, 46, 56). A single fall panicum plant can produce up to 647 g of dry weight (56). Once established, fall panicum thrives well under a variety of soil conditions and reduces corn yield (24, 25, 32, 46, 47, 56, 80). Additionally, fall panicum is a nuisance at corn harvest. Recent reports and surveys have found fall panicum infestations in 23 Northeastern and corn belt states (2, 3, 21, 46, 52, 78, 79, 83).

Recent practices of reduced tillage, earlier planting dates, shorter cultivars, and the elimination of other

weed species have encouraged fall panicum infestations (24, 46, 56, 71). In addition, the widespread use of atrazine for weed control in corn has allowed the atrazine-tolerant fall panicum to spread and flourish after competing species have been eliminated (2, 3, 21, 22, 46, 70). Many investigators have shown the ineffectiveness of atrazine as a preemergence or postemergence treatment to combat fall panicum (22, 23, 32, 47, 53, 80, 81).

It has been hypothesized that the continuous use of atrazine killed susceptible strains of fall panicum, whereas, the population of tolerant strains survived and spread (23, 24, 71). Applications of hydroxy-atrazine, a breakdown product of atrazine, to fall panicum in the field and greenhouse raised speculation that repeated use of atrazine may have stimulated the growth of fall panicum (56). Other investigators suggested that the recent occurrence of fall panicum is due to the elimination of competing broadleaf and grass weeds over the last 10 to 15 years (23, 46, 56). Recently, atrazine-resistant redroot pigweed (Amaranthus retroflexus L.) and common groundsel (Senecio vulgaris L.) plant populations have been reported (49, 51, 72). Inherent physiological differences between two groundsel biotypes resulted in different responses to triazine herbicides (49, 51). The investigations were conducted on plants grown in nutrient solutions. The study suggested that resistance was not due to differences in plant exposure to the herbicides caused by variation in germination time,

rooting depth, or morphology. The existence of other tolerant species stimulated interest in the theory of survival of resistant strains to atrazine. However, fall panicum was the first weed species to emerge on soil treated with simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine] in 1957 and appeared tolerant to chloro-s-triazines (54). Fall panicum became the dominant species in cultivated fields following simazine and other isomers in other studies (6). These 1957 observations were made one year before the release of triazines for commercial use.

The concept of artificial selection to atrazine has been denied by a recent study on fall panicum plants taken from uncultivated fields (63). The researchers suggested that plants from populations never exposed to atrazine would be expected to be more susceptible to the herbicide than those from atrazine-treated fields. Results indicated that plants from both areas were equally tolerant to 4.5 kg/ha of atrazine.

Green Foxtail

Green foxtail is an annual grass that grows 30 to 90 cm tall. The growth habit is erect producing a dense panicle at maturity (20, 79). Green foxtail seedlings may be distinguished, although with difficulty, from other Setaria species by the glaborous upper leaf surface and the short hairs along the margin of the sheath. It differs anatomically from the closely related yellow foxtail

[Setaria glauca (L.) Beauv.] in the lack of the several long, curly hairs at the base of the green foxtail leaf. Green foxtail is one of the most serious and widespread grass weeds (68, 79).

Researchers have recommended the same herbicide control measure for both green and yellow foxtail (68, 83). While reports have shown that poor postemergence control of green foxtail was obtained with atrazine plus phytobland oil (42, 47, 68, 83), other studies revealed that green foxtail is more tolerant to atrazine than are some other Setaria species (33, 34, 35, 70). It has been the observation of the author that green foxtail is frequently associated with other annual grasses like witchgrass (Panicum capillare L.) or crabgrass [Digitaria sanguinalis (L.) Scop.] which are moderately tolerant to atrazine. In many cases, green foxtail has been the predominant weed present.

Fall Panicum and Green Foxtail Control

Preemergence treatments of cyanazine, simazine, butylate (s-ethyl diisobutylthiocarbamate), and alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] have been effective control measures for annual grass control in corn. Until recently, there has been no effective postemergence herbicide for fall panicum or green foxtail. Early postemergence treatments of atrazine plus alachlor resulted in a synergistic response on Japanese millet (Echinochloa crusgalli var. frumentacea) (1). These data suggested that early herbicide combination treatments may

be candidates for removal of emerged fall panicum.

Cyanazine, registered recently as a selective postemergence treatment in corn, has resulted in good to excellent control of a wide variety of annual grasses. Unlike atrazine, cyanazine has a short-term soil residue with no carry-over to successive crops (82) and controls the atrazine-tolerant species (26, 32, 33, 34, 76, 83). The short residual life in the soil has been measured under a number of artificial and field situations. Treatments of cyanazine at 2.2 kg/ha failed to show toxicity to oats (Avena sativa L.) seeded within 10 weeks after application (26). A similar study resulted in no soybean [Glycine max (L.) Merr.] injury or reduction in yield when planted 4 to 6 weeks after a 3.4 kg/ha cyanazine treatment (30). An Illinois study indicated that soybeans could be planted as a rescue crop following the failure of cyanazine-treated corn (76). Three soil residue studies using instrumental and bioassay analysis indicated that cyanazine was less persistant than atrazine or simazine in all tested soil types (19, 44, 69). Cyanazine degradation in soils resulted in the loss of one-half of the initial activity in 1.3 to 5 weeks (9). The rate was greater under moist conditions.

Although fall panicum and green foxtail are susceptible to postemergence treatments of cyanazine, little information is available concerning efficacy of the herbicide on various growth stages of the annual grasses. Corn injury has occasionally been observed following cyanazine treatments.

Cyanazine at 3.4 kg/ha resulted in slight injury to corn in the 3 and 4-leaf stage (29). Cyanazine plus phytobland oil resulted in additional crop injury in several trials, especially when applied to older corn (29, 32, 42).

Adjuvants for Herbicide Enhancement

Incomplete wetting and spreading of water-herbicide solutions on waxy leaves of weeds may result in poor control. Numerous studies have shown that the addition of a phytobland oil to atrazine resulted in greater weed control without losing selectivity to the crop (15, 18, 28, 39, 55, 67, 85). The purpose of adjuvants in a postemergence herbicidal spray is to provide a spreading action, solubilizing the cuticle, and to keep the leaf surface moist longer, allowing increased foliar penetration and absorption (4, 15, 62, 84). Studies using ^{14}C-atrazine revealed greater herbicide uptake when oil additives were used in the water solution (4, 55, 62). The increased activity of the foliar sprays may permit herbicide rates to be reduced. The effective rate of atrazine 4.2 kg/ha with water was comparable to 2.8 kg/ha when 9.4 L of phytobland oil was added (28, 39). Many other herbicides which are applied postemergence call for the use of a surfactant or oil adjuvant (82). Some of these herbicides demand special types of surfactants because of their composition (39, 84).

Recent interest in the use of phytobland oils from an agricultural origin has provided an alternative to the

petroleum oils. Nalewaja (43) suggested that "the use of replenishable crop origin oils in place of nonreplenishable petroleum oils should be encouraged to help preserve our fossil fuels for tasks in which they are essential". Linseed oil and sunflower oil were compared to various adjuvants for the effectiveness of atrazine on green and yellow foxtail control (41, 42, 67). The vegetable oils and petroleum oils were superior to the surfactants. Similar weed control results have been reported when phytobland vegetable oils were added to cyanazine (42, 43). The addition of linseed oil significantly enhanced ^{14}C-dicamba (3,6-dichloro-o-anisic acid) uptake by leafy spurge (Euphorbia esula L.) and resulted in greater control of yellow foxtail with dalapon (2,2-dichloropropionic acid). Although phytotoxicity data with cyanazine plus vegetable oil were not conclusive, another study showed less sugar beet (Beta vulgaris L.) injury with phenmedipham (methyl-m-hydroxycarbanilate-m-methylcarbanilate) plus linseed or sunflower oils (40).

Effect of Time of Annual Grass Removal on Corn Yield

Bell and Koeppe (7) demonstrated that allelopathy (noncompetitive influence) plus competition of giant foxtail (Setaria faberii Herm.) reduced corn growth in the greenhouse. The researchers suggested that the interference of crop growth resulting from the release of phytotoxic substances from the grass plants plays a similar role under field conditions.

Bunting and Ludwig (13) removed weeds from corn at 3, 4, and 6 weeks after emergence. Two to four-week periods of competition during the early growth stages of corn were sufficient to reduce grain yield. Similar studies revealed that sorghum [Sorghum bicolor (L.) Moench.] yields were significantly reduced when weeds were not removed for 4 to 8 weeks after planting (14). Knake and Slife (37) found that corn yield were reduced only slightly by early season competition over a 3-year study. Reductions in yield occurred when competition was not eliminated until the giant foxtail was greater than 23 cm in height. Giant foxtail left to maturity resulted in only a 12.5% average yield reduction. Others (18, 55) found that corn yields were reduced when atrazine plus oil treatments were delayed until the late growth stages (5 to 10 cm) of yellow foxtail and barnyardgrass [Echinochloa crusgalli (L.) Beauv.] growth.

Root and Foliar Absorption of Postemergence Herbicides

Several investigators have noted the influence of root uptake of atrazine for maximum weed kill following postemergence treatments (36, 42, 55, 73, 74, 75). Studies at Cornell University indicated that non-foliar uptake appears to play a major role in herbicide uptake of early postemergence treatments (1). Elimination of root uptake of atrazine by seedling Japanese millet reduced control by four to six-fold. Thompson and Slife (74) found that root absorption of atrazine applied postemergence to small

broadleaf weeds is not a requisite for their control. However, greater giant foxtail control was obtained when root uptake supplemented the foliar uptake from an over-the-top spray (73).

Phytotoxicity of atrazine on yellow and green foxtail was greater under high moisture conditions when spray treatments were applied to both soil and foliage (55). Atrazine treatments applied to giant foxtail on dry soil followed by no rainfall reduced dry weight, but did not reduce the stand (73). In another study, the researchers (75) reported that corn seedlings treated with atrazine and grown under cold, wet conditions were injured. They concluded that low temperatures decreased the rate of detoxification to peptide conjugates from foliarly-absorbed atrazine in the stressed plants. The data showed the presence of dihydroxybenzoxazin-3-one and hydroxy-atrazine in the roots which indicates root uptake. However, the researchers reported that the effect of the reduced amount of benzoxazinone under these conditions did not contribute to the death of the corn.

Timing the applications of postemergence treatments to young annual grass weeds was required for effective control (28, 36, 55, 85). Late season green foxtail control with 2.2 kg/ha of cyanazine was greater than with cyanazine at 0.6 kg/ha with oil (42). Nalewaja suggested that the residue in the soil resulted in effective kill of late germinating seeds. Excellent control was obtained when

applications were made to annual grasses 1.5 to 4.0 cm high. Later treatments to 5 to 10-cm grass resulted in poor control. Some short weeds escaped control when under the canopy of taller treated weeds (28). Additionally, the lack of residual cyanazine under dense weed conditions after treatment may allow new weeds to emerge.

Cyanazine Action and Metabolism

Cyanazine is readily absorbed by plant foliage; and when applied to the soil, it is absorbed by the roots and translocated to the leaves (82). It is a photosynthetic inhibitor which interfers with the "Hill reaction". The chemical moves acropetally to the site of action via the apoplast toward areas of highest transpiration. Cyanazine at 171 ppm is five times more soluble in water at 25 C than is atrazine. Availability of this herbicide for plant uptake via the soil depends upon the total amount of water present and the adsorption characteristics of the soil for cyanazine (26, 82). Cyanazine rate adjustments vary from 1.1 kg/ha on a sandy soil with little organic matter to 4.5 kg/ha on a clay soil with 4% organic matter to achieve satisfactory weed control from preemergence applications.

Cyanazine differs from atrazine by the presence of a nitrile group which appears to be more easily attacked than the chlorine atom (8, 9, 10, 12, 82). Degradation involves removal of the ethyl group, hydration of the cyano group, and the exchange of the chlorine with a hydroxyl

group (10, 11, 12, 16, 27). Evidence for conjugation of cyanazine with peptides has been reported as a possible detoxification mechanism (10, 11, 12, 76). Although conjugation detoxification mechanisms with cyanazine are not fully understood, glutathione conjugation of atrazine has been reported to be a major detoxification mechanism in corn (57, 59, 61, 70, 71).

In corn plants, hydrolytic and dealkylation reactions detoxify cyanazine; however, in soils, large amounts of dealkylated products are formed (10). While hydroxy-cyanazine was not detected in corn, a hydroxy-acid [2-hydroxy-4-(1-carboxy-1-methylethylamino)-6-(ethylamino)-1,3,5-triazine] was found in corn sap 22 hr after treatment. A dealkylated hydroxy-acid [2-hydroxy-4-(1-carboxy-1-methylethylamino)-6-amino-1,3,5-triazine] was also found in large quantities in corn grown in ^{14}C-cyanazine treated soils. Both of these hydroxy compounds were found to have non-herbicidal properties (12).

Degradation products found in wheat (Triticum vulgare L.) and potatoes (Solanum tubersum L.) were similar to those found in corn (11). However, wheat leaves contained significant amounts of the chloro-acids which only occurred in the roots of corn.

CHAPTER 2

INFLUENCE OF STAGE OF GROWTH AND ADJUVANTS ON THE CONTROL OF FALL PANICUM IN CORN WITH CYANAZINE

Abstract

The response of corn (Zea mays L.) and fall panicum (Panicum dichotomiflorum Michx.) to postemergence herbicide applications at three stages of growth was examined. Early postemergence application of cyanazine (2-[[4-chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile) at 3.4 kg/ha provided the best control with minimum crop injury and greatest corn yield. A reduction of 25% in corn yield resulted when fall panicum was removed by hand at the 7 to 8-leaf stage as compared to removal of plants with 2 leaves. The addition of various adjuvants increased cyanazine action on both fall panicum and corn in field and greenhouse experiments; however, field trials showed no differences in grain yield at harvest. In the greenhouse, less corn injury was observed with a vegetable oil additive than with other adjuvants.

Introduction

Fall panicum is increasing as a weed problem in many areas of the United States (11, 12, 13, 17). The occurrence of this annual grass appears to be associated with the continuous use of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine].

Fall panicum is tolerant to applications of atrazine, detoxifying it by conjugation with peptides (12, 13, 15, 16, 18). Tolerance of fall panicum to atrazine and the elimination of other competing weed species during the growing season has provided an avenue for ecological change in the corn field. When allowed to grow without competition, fall panicum plants tiller profusely producing large numbers of seed to infest the area.[1]

Insufficient preemergence control of fall panicum without effective postemergence control results in reduced corn yields. This late germinating grass must be controlled for the entire season to maximize corn yield. Corn yields are not reduced if weeds are removed during the early growth stage of the crop (1, 2, 6, 7, 14). Since fall panicum

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[1]Serdy, F. S. 1973. The effect of competition, herbicides, and ethylene on germination, growth, and development of fall panicum. Ph. D. Thesis. The Pennsylvania State University, University Park. 77 pp.

is capable of metabolizing cyanazine[2] or simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] (5, 15), control may be achieved using postemergence applications of these chemicals. Addition of nonphytotoxic petroleum oils or phytobland oils increased herbicidal activity of cyanazine (4, 8, 9, 10), but resulted in crop injury and at present are not recommended for use with cyanazine. Kapusta (4) suggested that cyanazine application made prior to the fifth leaf stage of corn provided maximum selectivity.

The purpose of this research was to examine various postemergence herbicide treatments for fall panicum control in corn. The study was designed to determine the tolerance of corn and the control of fall panicum when cyanazine was applied at various growth stages. In addition, the experiment was conducted to determine the competition between corn and fall panicum and the extent of yield losses it caused when left for various periods of time. The efficacy and selectivity of cyanazine with various adjuvants were examined.

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[2]Thompson, R. P. and F. W. Slife. 1973. Uptake and degradation of ^{14}C-Bladex and ^{14}C-atrazine by four crop and two weed species. Weed Sci. Soc. Abstr. No. 142.

Materials and Methods

Field studies during 1972 and 1973 were conducted on soils heavily infested with fall panicum. Plots were 3.1 by 12.2 m in 1972 and 3.1 by 15.2 m in 1973 and consisted of four corn rows per plot. The herbicides were applied with a tractor-mounted sprayer at 2.11 kg/cm^2 pressure delivering 215 L/ha. All treatments were replicated four times. Fall panicum control and corn injury were visually rated on a 0 to 10 scale, with 0 representing no control or injury and 10 representing complete kill. Corn injury was also measured by dry weight, plant height, and grain yields. Plant height measurements were taken 3 and 7 weeks following treatment by measuring the height of the tallest hanging leaf. The average of ten random measurements per plot determined the plot mean. Additionally, 3 m of the center two rows were harvested 3 weeks following treatment for dry weight determination in one study. The remaining center two rows were harvested at maturity and grain weights corrected to 15.5% moisture.

The first field experiment was designed to evaluate the performance of various herbicide treatments on fall panicum and corn over three stages of plant growth. After disking, the field was planted with a no-till planter with rows 1 m apart. The area was planted to 'Teweles 80' corn on May 19, 1972 and May 18, 1973. The plots were arranged in split-plot design. Herbicide applications were made on June 2, 13, and 22, 1972 and June 7, 14, and 22, 1973. At

treatment time fall panicum was at the 2 to 3, 4 to 5, and 7 to 8-leaf stage and corn at the 3 to 4, 4 to 5, and 6 to 7-leaf stage. The corn was sidedressed with 134 kg/ha N during the study. Soil was a sandy clay loam with 2.0% organic matter.

Another experiment was designed to evaluate the effect of various adjuvants on cyanazine toxicity to weeds and corn. Experimental procedures were similar to the preceding study. The sandy loam soil contained 2.4% organic matter. Early postemergence treatments were applied to fall panicum with three leaves and corn with four leaves.

Greenhouse experiments were used to supplement the adjuvant field study. Corn and fall panicum were grown in 946-ml containers filled with sandy clay loam soil containing 2.5% organic matter. Corn and fall panicum were treated in the 4 and 4 to 6-leaf stage, respectively. The plants were grown under 23 to 27 C with supplemental lighting of 12.9 klux during a 16 hr day. Pots were watered daily with Hoagland's no. 1 solution (3). A phytobland crop oil,[3]

[3]Sun 11E is a phytobland oil produced by Sun Oil Company, Philadelphia, Pennsylvania.

an oil concentrate[4], a surfactant[5], and a vegetable oil[6] were compared. The plants were harvested 9 days following treatment and the dry weights obtained. Data presented are the means for two experiments both containing five replications.

Results and Discussion

Response of fall panicum and corn following herbicide treatment at three stages of growth. Evaluation several weeks after application indicated that the herbicide applications gave the greatest fall panicum control when applied to the 2 to 3-leaf stage (Table 1). Variation in fall panicum control existed between years with the late treatment stage. Shorter and less dense fall panicum at the late stage treatment in 1973 could have resulted in greater plant coverage and soil interception of cyanazine.

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[4]Agri-oil plus is a blend of surfactant and paraffinic oil produced by Gordon's Chemicals, Kansas City, Kansas.

[5]Citowett is a surfactant containing alkylaryl polyglycol ether produced by BASF Wyandotte Corporation, Parsippany, New Jersey.

[6]Bio-Veg is an emulsified vegetable oil produced by Barzen of Minneapolis, Inc., Minneapolis, Minnesota.

Table 1: Fall panicum control 3 and 7 weeks following postemergence herbicide treatments to three stages of growth, 1972 and 1973.

Treatment	Rate (kg/ha)	Three week rating[a]						Seven week rating[a]					
		Leaf stage						Leaf stage					
		2 to 3		3 to 4		7 to 8		2 to 3		3 to 4		7 to 8	
		1972	1973	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973
Weedy control	--	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Weeded control	--	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cyanazine	2.2	8.8	6.9	4.0	6.8	1.2	2.9	7.2	3.1	1.3	6.0	0.0	2.8
Cyanazine	3.4	9.0	9.0	7.5	8.3	2.8	7.8	7.0	7.9	4.6	7.8	1.0	5.9
Cyanazine + oil	1.7+9.4L	8.5	8.8	8.0	7.6	1.8	--	5.5	5.6	4.8	6.4	0.0	--
Cyanazine + oil	2.2+9.4L	10.0	9.3	8.8	8.5	3.5	8.5	8.5	8.2	6.6	7.9	1.5	5.9
Cyanazine + alachlor + oil	1.1+2.2+9.4L	8.5	9.8	8.0	7.3	1.8	--	2.0	8.5	4.2	6.5	0.0	--
Atrazine + simazine + oil	1.1+2.2+9.4L	7.8	7.1	7.5	3.3	0.0	0.0	6.5	3.9	4.9	5.6	0.0	0.6

[a]0=no control; 10=complete control.

Cyanazine plus crop oil at 2.2 kg + 9.4 L/ha resulted in greater fall panicum control than did cyanazine alone at 3.4 kg/ha at all treatment dates. Although cyanazine plus oil at 1.7 kg + 9.4 L/ha gave similar control as cyanazine at 3.4 kg/ha, eventually the remaining herbicide in the soil was inadequate for control of late season germinating grass seeds. Greater control was obtained with early application of cyanazine plus alachlor during the 1973 season. The fall panicum was nearer to the 2-leaf stage in 1973 and nearer to the 3-leaf stage in 1972. Desired fall panicum control was not obtained with atrazine plus simazine plus oil.

Cyanazine alone did not injure corn with 3 to 5 leaves. Reduced grain yields in 1973 were the result of ineffective fall panicum control (Tables 2, 3, and 4). The addition of a nonphytotoxic crop oil or alachlor plus crop oil reduced crop vigor 3 weeks following treatment in 1972. A decrease in dry weight was not measured in 1973. While no rainfall accompanied the 2-day period following the treatments in 1973, rainfall amounting to 0.7 cm, 3.9 cm, and 0.5 cm fell during the 2-day period following the 1972 applications. The increased injury in the 1972 season may have resulted from additional wetting and a modified cuticle which aided in penetration.

Allowing fall panicum competition to persist until corn had 6 to 7 leaves reduced corn yields by 25% (Tables 2 and 4). Herbicide injury combined with weed competition

Table 2. Response of corn following postemergence herbicide treatments to plants with 3 to 4 leaves, 1972 and 1973.[a]

Treatment	Rate (kg/ha)	1972			1973		
		Injury rating[b]	Dry weight[c] (gm/plant)	Grain yield (kg/ha)	Injury rating[b]	Dry weight[c] (gm/plant)	Grain yield (kg/ha)
Weedy control	--	0.0	2.5 a	3613 a	0.0	4.0 ab	985 a
Weeded control	--	0.0	2.5 a	6956 d	0.0	4.5 ab	6034 d
Cyanazine	2.2	0.5	2.4 a	6510 c	0.0	5.1 a	4246 b
Cyanazine	3.4	0.5	2.0 ab	6642 cd	0.5	4.0 ab	5301 cd
Cyanazine + oil	1.7+9.4L	0.2	2.4 a	6912 d	0.5	4.0 ab	4566 bc
Cyanazine + oil	2.2+9.4L	4.2	1.5 c	5243 b	0.3	4.0 ab	5406 cd
Cyanazine + alachlor + oil	1.1+2.2+9.4L	0.8	1.8 bc	6291 c	0.4	3.4 bc	6065 d
Atrazine + simazine + oil	1.1+2.2+9.4L	0.0	1.7 bc	7188 d	0.4	3.0 bc	4917 cd

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]0 = no injury; 10 = complete kill.

[c]Dry weight of crop was harvested 3 weeks following treatment. The plants were harvested from 3 meters of the middle two rows.

Table 3. Response of corn following postemergence herbicide treatments to plant with 4 to 5 leaves, 1972 and 1973.[a]

Treatment	Rate (kg/ha)	1972			1973		
		Injury rating[b]	Dry weight[c] (gm/plant)	Grain yield (kg/ha)	Injury rating[b]	Dry weight[c] (gm/plant)	Grain yield (kg/ha)
Weedy control	--	0.0	6.4 ab	3054 a	0.0	6.9 bc	1192 a
Weeded control	--	0.0	6.8 a	6755 d	0.0	9.1 a	5871 d
Cyanazine	2.2	0.0	4.9 bc	6247 cd	0.0	8.5 ab	4673 bc
Cyanazine	3.4	0.0	5.2 abc	6216 cd	0.0	9.0 a	5946 d
Cyanazine + oil	1.7+9.4L	1.0	3.8 cd	5820 c	0.2	7.1 ab	4334 b
Cyanazine + oil	2.2+9.4L	2.5	2.8 d	6172 cd	1.6	6.9 ab	5275 cd
Cyanazine + alachlor + oil	1.1+2.2+9.4L	3.5	2.5 d	4516 b	0.5	8.8 a	4190 b
Atrazine + simazine + oil	1.1+2.2+9.4L	0.2	3.4 cd	6561 d	0.1	6.2 bc	4008 b

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]0 = no injury; 10 = complete kill.

[c]Dry weight of crop was harvested 3 weeks following treatment. The plants were harvested from 3 meters of the middle two rows.

Table 4. Response of corn following postemergence herbicide treatments to plants with 6 to 7 leaves, 1972 and 1973.[a]

Treatment	Rate (kg/ha)	1972 Injury rating[b]	1972 Dry weight[c] (gm/plant)	1972 Grain yield (kg/ha)	1973 Injury rating[b]	1973 Dry weight[c] (gm/plant)	1973 Grain yield (kg/ha)
Weedy control	--	0.0	15.0 a	3569 ab	0.0	12.0 abc	1323 a
Weeded control	--	0.0	15.2 a	4967 c	0.0	12.4 a	4648 d
Cyanazine	2.2	0.2	11.9 bc	4648 c	1.4	11.6 bcd	3381 bc
Cyanazine	3.4	1.0	10.8 cd	5432 c	0.9	12.2 ab	4848 d
Cyanazine + oil	1.7+9.4L	4.5	7.2 e	3581 ab	--	--	--
Cyanazine + oil	2.2+9.4L	5.5	7.7 de	3418 ab	4.9	11.0 d	3995 cd
Cyanazine + alachlor + oil	1.1+2.2+9.4L	5.0	7.8 de	2477 a	--	--	--
Atrazine + simazine + oil	1.1+2.2+9.4L	0.0	15.4 a	5087 c	1.0	11.5 bcd	2296 ab

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]0 = no injury; 10 = complete kill.

[c]Dry weight of crop was harvested 3 weeks following treatment. The plants were harvested from 3 meters of the middle two rows.

eliminates the feasibility of treating 6 to 7-leaf corn infested with 7 to 8-leaf fall panicum. Full season fall panicum control is required for maximum yields (Tables 1 and 2). This can be accomplished by applying cyanazine or cyanazine plus oil prior to the time fall panicum has 4 leaves and corn has 6 leaves.

Plant height was employed as a measure of corn response following treatments at the early stage of growth (Table 5). Measurements taken 3 and 7 weeks after treatment indicate the ability of corn to compensate for early injury. Comparing effective treatments, no reduction in corn height was observed following cyanazine at 3.4 kg/ha or cyanazine plus oil at 2.2 kg + 9.4 L/ha. However, crop damage was reflected in the 1972 grain yields from cyanazine plus oil at 2.2 kg + 9.4 L/ha. With the exception of the 3-week measurement in 1973, plant height differences were not observed with cyanazine plus alachlor plus oil or atrazine plus simazine plus oil. Some yield reduction was obtained in plots receiving these treatments due to either excessive crop injury or erratic long season fall panicum control.

Comparison of adjuvants on cyanazine action. The addition of several adjuvants to cyanazine improved fall panicum control (Table 6). Adjuvants added to 2.2 kg/ha of cyanazine resulted in similar or greater control than the 3.4 kg/ha rate alone. The nonphytotoxic crop oil and oil concentrate resulted in greater weed control and more

Table 5. The effect of cyanazine on corn with 3 to 4 leaves as measured by plants height 3 and 7 weeks following treatment.[a]

Treatment	Rate (kg/ha)	Plant height[b] 1972 3 weeks (cm)	1972 7 weeks (cm)	1973 3 weeks (cm)	1973 7 weeks (cm)
Weedy control	--	34 a	94 e	44 ab	97 d
Weeded control	--	31 ab	131 a	46 a	151 ab
Cyanazine	2.2	28 bc	101 de	46 a	150 ab
Cyanazine	3.4	28 bc	123 ab	44 ab	154 a
Cyanazine + oil	1.7+9.4L	30 ab	123 ab	41 bc	151 ab
Cyanazine + oil	2.2+9.4L	22 c	118 abc	37 cd	155 a
Cyanazine + alachlor + oil	1.1+2.2+9.4L	25 bc	111 bcd	39 cd	153 ab
Atrazine + simazine + oil	1.1+2.2+9.4L	27 bc	117 abc	39 cd	148 abc

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]Measurements were taken to the highest point of top hanging leaf on the plants. Treatment mean represents the average of 10 measurements per plot.

Table 6. The effect of cyanazine and adjuvant additives on fall panicum control and corn injury, 1972 and 1973.[a]

Treatment	Rate (kg/ha)	Fall panicum control[b] 3 week 1972	Fall panicum control[b] 3 week 1973	Fall panicum control[b] 7 week 1972	Fall panicum control[b] 7 week 1973	Corn injury[b] 1972	Corn injury[b] 1973	Grain yield 1972 (kg/ha)	Grain yield 1973 (kg/ha)
Weedy control	--	0.0	0.0	0.0	0.0	0.0	0.0	3657 a	978 a
Weeded control	--	10.0	10.0	10.0	10.0	0.0	0.0	6416 c	6034 cd
Cyanazine	2.2	6.7	7.3	4.8	3.9	0.3	0.0	5601 b	5099 b
Cyanazine	3.4	9.0	8.7	7.5	7.8	0.5	0.0	6642 c	6272 de
Cyanazine + oil	2.2+9.4L	9.6	9.3	8.4	8.1	1.9	0.6	5613 b	5226 bc
Cyanazine + oil conc.	2.2+2.3L	10.0	8.9	8.6	7.4	4.0	0.5	5958 b	6366 de
Cyanazine + surfactant	2.2+1.2L	7.0	8.5	4.9	7.1	0.5	0.3	5758 b	5444 bc
Cyanazine + vegetable oil	2.2+2.3L	--	8.8	--	7.7	--	0.8	--	6272 de
Cyanazine + alachlor + oil	1.1+2.2+9.4L	8.9	9.6	6.4	9.1	1.3	0.7	5833 b	6818 e
Atrazine + simazine + oil	1.1+2.2+9.4L	7.6	8.0	6.8	7.3	1.6	0.6	5676 b	5996 cd

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]0 = no control or injury; 10 = complete kill.

severe corn injury than did the surfactant or vegetable oil. Although crop injury was observed initially, final grain yields were not significantly reduced with any adjuvant-herbicide combination. Cyanazine plus alachlor plus oil provided good control with the exception of the infestation late in 1972. Atrazine plus simazine plus oil more effectively controlled fall panicum in this experiment than in the stage of growth study; however, more corn injury also was observed.

The adjuvant treatments were also examined in the greenhouse on corn with 4 leaves and fall panicum plants with 4 to 6 leaves. Application of 2.2 kg/ha of cyanazine caused injury (Table 7). Less corn injury resulted from the cyanazine and the cyanazine plus vegetable oil treatment compared to cyanazine plus other adjuvants; however, erratic control of the fall panicum occurred. Crop oil, oil concentrate, and surfactant appeared to enhance corn injury and grass control. In the greenhouse, addition of the surfactant resulted in fall panicum death in contrast to similar treatments in the field.

These data indicate that the use of a phytobland additive increased both fall panicum control and corn injury. The degree of selectivity did not appear to be broadened. Although corn treated with cyanazine plus oil or alachlor plus oil usually resulted in yields comparable to the hand-weeded controls, the use of cyanazine alone appears consistently less injurious. Cyanazine at 3.4 kg/ha applied

Table 7. Influence of adjuvants on cyanazine action on the dry weight of greenhouse-grown corn with 4 leaves and fall panicum with 4 to 6 leaves.

Cyanazine (kg/ha)	Adjuvant	Rate (L/ha)	Dry weight[a] Corn (mg/3 plants)	Dry weight[a] Fall panicum (mg/cup)
0.0	None	--	301 a	470 a
2.2	None	--	248 bc	197 b
2.2	Oil	9.4	226 c	103 b
2.2	Oil conc.	2.3	217 c	118 b
2.2	Surfactant	1.2	221 c	0 c
2.2	Veg. oil	2.3	263 b	174 b

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

to the early stages of plant growth (corn with less than 5 leaves and fall panicum with less than 4 leaves) eliminated competing fall panicum and provided sufficient soil residual for long season control and maximum yields. By the late treatment, the fall panicum had tillered to form a dense foliage and resulted in unacceptable weed control. In addition, increased corn injury suggests late season treatments should not be used.

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CHAPTER 3

UPTAKE, MOVEMENT, AND METABOLISM OF CYANAZINE IN FALL PANICUM, GREEN FOXTAIL, AND CORN

Abstract

Greenhouse studies indicated that root absorption after postemergence applications of cyanazine (2-[[4-chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile) enhanced phytotoxicity to fall panicum (*Panicum dichotomiflorum* Michx.), green foxtail (*Setaria viridis* L.), and corn (*Zea mays* L.). Less ^{14}C-cyanazine was taken up by the foliage of corn than by the weed species. A lower concentration of parent cyanazine in corn leaves was also evident. The addition of a phytobland oil to the treatment solution resulted in increased foliar cyanazine absorption 1 and 5 days following treatment. Although rapid metabolism occurred in corn roots, the large amounts of cyanazine absorbed via the root system resulted in internal concentrations of parent cyanazine similar to that observed in the weed species. Cyanazine translocation was mainly acropetal from the point of application. The basis of selectivity is not solely based on the differential foliar uptake of cyanazine, but also on the proportion taken up by the foliage and roots. Under conditions favoring uptake by roots, the margin of selectivity may be reduced.

Introduction

Until recently, selective postemergence herbicides have been ineffective on fall panicum and green foxtail (8, 11, 13, 14). Although green foxtail is less tolerant to triazine herbicides than fall panicum, the threat of cornfields invaded with another weedy grass should be avoided. Cyanazine offers a possible control measure for these weedy grasses (8).

The influence of the microclimatic conditions on the foliar uptake of atrazine is well documented. Investigators have shown an increase in atrazine or cyanazine activity due to high relative humidity, increased temperature, and the presence of additives (5, 10, 12, 15, 16). Other researchers have suggested the importance of soil interception of atrazine resulting in root uptake to obtain optimum control of annual grasses (7, 8, 9, 10, 11, 12, 15, 16). In a field trial in 1972 on a sandy loam soil, significant reductions in corn growth were observed following cyanazine plus oil treatments.[1] Rain amounting to 7.8 cm fell for 11 of the 15 days following treatment. Conditions favorable for greater root absorption by green foxtail and corn resulted in additional phytotoxicity.

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[1]Kern, A. D., W. F. Meggitt, and R. C. Bond. 1972. Green foxtail control in Northern Michigan. N. Cent. Weed Contr. Conf. Res. Rep. 29:144.

The objectivies of this study were to determine the contribution of foliar and root absorption to cyanazine toxicity and to determine the role that translocation and metabolism play in selectivity.

Materials and Methods

Fall panicum and green foxtail seeds, collected in Michigan, were planted in 473-ml cups and allowed to grow 6 to 7 cm in height. 'Michigan 396' corn seed was planted in 946-ml cups and allowed to attain the 4-leaf stage. The grasses and corn were grown in the greenhouse and thinned to 15 plants and 3 plants per cup, respectively.

Treatments designed to cover only the foliage were accomplished by placing a 0.8 to 1.0-cm layer of vermiculite on the soil to intercept the herbicide spray. After the treated foliage had dried, the vermiculite was removed. In the foliage plus root treatments the spray was allowed to contact soil and foliage. Treatments were made with 235 L/ha at 2.1 kg/cm^2 pressure and a phytobland oil[2] at 4% (v/v) or 9.4 L/ha was added. The treatments were assigned in a completely randomized design. The plants were harvested 9 days following treatment and fresh weights were measured. Data reported are the means of two experiments with four replications each.

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[2]Sun 11E is a phytobland oil produced by the Sun Oil Company, Philadelphia, Pennsylvania.

Foliar application of ^{14}C-cyanazine. To obtain optimum cuticular wax deposition, fall panicum, green foxtail, and corn were grown outdoors during the spring and summer months. Plants of fall panicum and green foxtail at the 4 to 5-leaf stage and corn in the 4-leaf stage were brought to the laboratory and selected for uniformity for the ^{14}C-cyanazine absorption and translocation study. A 5-µl drop containing 0.1 µCi of ^{14}C-cyanazine was placed inside a lanolin enclosure on the second leaf of corn. Similar droplets were applied between two lanolin bars placed perpendicular to the length of the grass leaves. The ring-labeled ^{14}C-cyanazine had a specific activity of 3.86 µCi/nM and was 97% pure.[3] Following treatment, the plants were moved to a growth chamber with a 16-hr day and a light intensity of 19 klux. Day temperature was maintained at 25 C and night temperature at 22 C. The plants were supplied with a modified Hoagland's no. 1 solution (6) and randomized daily. The treatments were replicated three times and the experiments were repeated.

After 1 and 5 days, the treated plants were sectioned into three parts. The treated leaf acropetal to the point of application was removed and immediately placed into a freezer at -5 C. Similarly, the rest of the aerial

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[3] ^{14}C-labeled cyanazine was supplied by Shell Development Company, Modesto, California.

plant portion basipetal to the spot and the root portion were separated and frozen. The plant portions were homogenated in a Sorvall Omni-Mixer for 2 min in 10 ml of 80% methanol, and the homogenate was filtered through glass wool as suggested by Beynon et al. (2, 3, 4). Following reextraction the residue was collected for dry weight and methanol-insoluble residue analysis by the Schoniger combustion method (17). The volume of the methanol-soluble filtrate was reduced in vacuo and 10 ml of water added. The aqueous fraction was partitioned with chloroform. The volumes of the fractions were reduced in vacuo and 500 µl of cold methanol added. Aliquots of 50 µl were assayed by liquid scintillation spectrometry. Another 200 µl were spotted onto 250 nm thick (20 by 20 cm) silica gel F-254 (Brinkmann Instruments) thin layer chromatography (TLC) plates. The aqueous fractions were developed in ethyl acetate:H_2O:formic acid (70:4:4, v/v/v) and then radioautographed. The lipophylic fractions were developed in acetone:chloroform (35:65, v/v). The radioactive spots on the TLC plate were removed and radioassayed by liquid scintillation spectrometry. The scintillation solution consisted of 0.1 g of dimethyl POPOP [1,4-bis 2-(4-methyl-5-phenyloxazolyl)-benzene], 5.0 g of PPO (2,5-diphenyloxazole), 50.1 g of naphthalene, 380 ml of toluene, 380 ml of 1,4-dioxane, and 240 ml of absolute ethanol.

Root uptake of ^{14}C-cyanazine. The plant species were germinated in the greenhouse in a sand medium and transferred

to a Hoagland's no. 1 solution containing 5 μCi/L of ^{14}C-cyanazine (cyanazine concentration was 3.5 x 10^{-7} m). Based on previous observations of root weights, volumes of solution were adjusted to 10 mls for the weed plants and 200 ml for corn. The plants were allowed to remain in the ^{14}C-labeled solution for 3 days. The plants were then removed, and the roots were washed in three consecutive water baths. The harvested material was frozen as previously described prior to analysis. At harvest two plants from all previous treatments were quickly frozen with dry ice, freeze-dried, and radioautographed.

Results and Discussion

In greenhouse studies, green foxtail was more sensitive to cyanazine than was fall panicum. Significant reductions in fresh weight of the three species indicate the importance of root uptake on cyanazine phytoxicity (Table 1). Although the addition of a phytobland oil masked the significance of root uptake in fall panicum, a marked response was shown at the 2.2 and 3.4 kg/ha rate. Increased corn injury due to soil interception was observed with cyanazine at 3.4 kg and cyanazine plus oil at 2.2 kg and 3.4 kg + 9.4 L/ha (Figure 1).

Foliar application of ^{14}C-cyanazine. Fall panicum absorbed 3 to 5 times as much ^{14}C-cyanazine from foliar application than did green foxtail. Corn leaves took up only about 35 and 20% as much labeled herbicide at 1 and 5 days,

Table 1. Fresh weight of plants 9 to 11 days following foliar and foliar + root treatments of cyanazine to corn with 3 leaves and 6 to 7.5-cm fall panicum and green foxtail.[a]

Cyanazine (kg/ha)	Oil (L/ha)	Fresh weight					
		Corn		Fall panicum		Green foxtail	
		Foliar	Foliar + root	Foliar	Foliar + root	Foliar	Foliar + root
		(gm/3 plants)		(gm/cup)		(gm/cup)	
0.0	0.0	19.8 a	19.4 a	5.8 a	6.1 a	6.7 a	7.0 a
1.1	0.0	17.1 ab	16.3 bc	4.0 a	3.4 a	3.6 b	0.6 cde
2.2	0.0	17.5 ab	15.4 bcd	3.9 a	1.2 bc	1.2 cd	0.2 de
3.4	0.0	17.6 ab	14.2 de	2.0 b	0.5 c	--	--
1.1	9.4	11.7 ef	10.9 ef	1.4 bc	0.1 c	1.5 c	0.1 de
2.2	9.4	13.4 e	9.3 fg	1.2 bc	0.4 c	1.3 c	0.0 e
3.4	9.4	11.6 ef	8.3 g	--	--	--	--
	Mean[b]	15.5	13.4	3.0	2.0	2.9	1.6

[a]Means within species with similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]F value for method of application is significant at 5% level.


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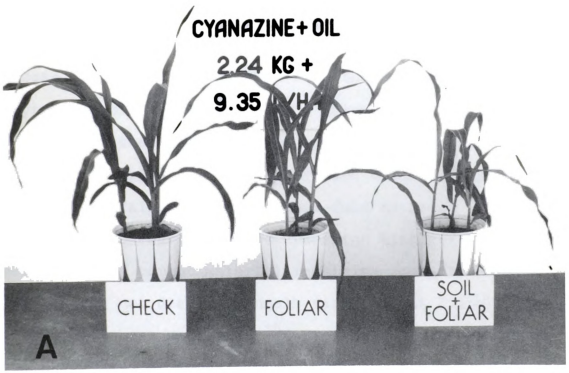
Figure 1. Comparison of cyanazine phytotoxicity to corn and fall panicum 9 to 10 days following foliar treatment. The plants on the left of each plate are the controls. The center plants received only foliage treatments. The soil and foliage of the cup on the right were both allowed to intercept the herbicide spray. (A) Corn treated with 2.2 kg plus 9.4 L/ha of cyanazine plus oil. (B) Fall panicum treated with 3.4 kg/ha of cyanazine.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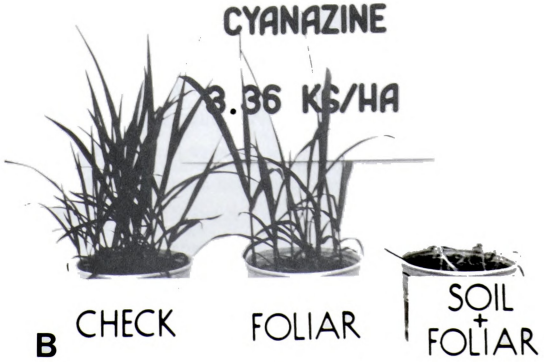
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respectively, as green foxtail and about 5% as much as fall panicum (Table 2). However, after 5 days a significant increase in absorption resulted from the addition of the phytobland oil. This may be the reason for foliar injury observed in the field following postemergence applications of cyanazine plus oil. Oil increased uptake of cyanazine two-fold in fall panicum and four-fold in green foxtail.

Following foliar ^{14}C-cyanazine applications, the percentage of parent material remaining among the three species was not distinguishably different at 1 and 5 days (Table 3). Less unaltered ^{14}C-cyanazine was recovered at the 5-day harvest in all three species. The nature of the metabolism appears to be different among the species. Corn harvested 5 days following treatment had more distinguishable metabolites than did fall panicum or green foxtail. The origin in the separation system included some breakdown products; however, they were not further separated. Fall panicum contained a metabolite in the aqueous fraction which moved only slightly from the origin and could not be separated from it. Greater absorption and less metabolism accounted for the high concentration of parent cyanazine in fall panicum both 1 and 5 days following foliar treatment (Figure 2). Less absorption and a lower concentration of unaltered cyanazine in corn appeared to be the basis for selectivity to foliar treatments.

Root uptake of ^{14}C-cyanazine. Corn absorbed more ^{14}C-cyanazine than fall panicum and green foxtail when

Table 2. ^{14}C-cyanazine absorption by shoots of the seedlings of three species 1 and 5 days after foliar treatment.[a]

Species	Treatment Duration (days)		^{14}C-uptake Without oil (dpm/mg)	^{14}C-uptake With oil (dpm/mg)
Fall panicum	1		13,525 bcd	24,463 a
	5		14,588 bc	35,979 a
		Mean[b]	14,056	30,174
Green foxtail	1		2,311 e	9,794 bcd
	5		4,725 cd	17,484 b
		Mean	3,518	13,639
Corn	1		827 e	730 e
	5		805 e	3,865 cd
		Mean	816	2,298

[a]Means followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

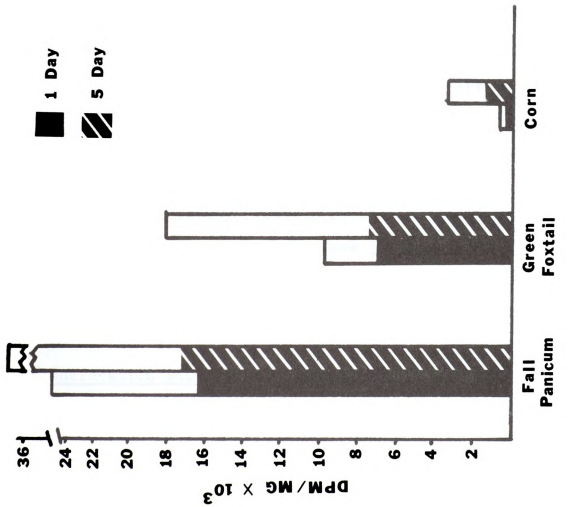
[b]F value for oil vs. no oil is significant at the 5% level.

Table 3. Metabolism from foliar ^{14}C-cyanazine application to three species 1 and 5 days after treatment.[a]

Species	Percentage of total ^{14}C recovered			
	Parent (%)	Origin (%)	Metabolites (%)	Methanol-insol. (%)
1 day				
Fall panicum	66.6 a	22.9 ab	9.2 cd	1.4 ab
Green foxtail	64.9 a	31.5 a	2.4 de	0.0 b
Corn	73.2 a	15.9 bc	9.9 cd	0.7 ab
5 day				
Fall panicum	47.9 ab	38.0 a	13.5 bc	0.5 ab
Green foxtail	40.1 b	37.3 a	22.6 b	0.0 b
Corn	40.8 b	25.4 a	30.5 a	3.3 a

[a]Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Figure 2. Absorption of ^{14}C-cyanazine and concentration of ^{14}C in three plant species 1 and 5 days following foliar treatment. The height of the total bar represents the total accumulation and the darkened portion within the bar indicates the amount of unaltered parent cyanazine.


1 Day
5 Day
DPM/MG X 10³
36
24
22
20
18
16
14
12
10
8
6
4
2
Fall Panicum
Green Foxtail
Corn

transferred to a nutrient solution containing the labeled cyanazine (Table 4). Fall panicum had the highest percentage of parent cyanazine in the shoots and corn the lowest (Table 5). Although large amounts of radioactivity were found in corn roots compared to other species (Table 4), much more was metabolized (Table 5). Green foxtail roots contained large amounts of unaltered cyanazine. This could explain the greater sensitivity of green foxtail than fall panicum to cyanazine. Fall panicum roots altered cyanazine to products other than parent cyanazine and contained a large amount of methanol-insoluble residue. Although a large amount of cyanazine was metabolized, a loss in the margin of selectivity resulted from root treatments to the plants in the 4-leaf stage (Figure 3). It should be noted that corn plants took up 40 times more nutrient solution than fall panicum or green foxtail plants. As a result, more herbicide uptake and translocation would likely occur.

Translocation studies. Cyanazine was rapidly translocated in fall panicum, green foxtail, and corn following foliar applications (Figures 4 and 5). When a phytobland oil was added, greater cyanazine movement was observed (Figure 4). In all plants, ^{14}C moved acropetally in the treated leaf with minimal basipetal movement. Root uptake studies indicate uniform translocation acropetally from roots to areas of highest transpiration. The translocation pattern of cyanazine is consistent with other s-triazine herbicides.

Table 4. Distribution of activity in seedlings of three species 3 days after supplying the roots with ^{14}C-cyanazine.[a]

Species	^{14}C distribution	
	Shoot (dpm/mg)	Root (dpm/mg)
Fall panicum	331 a	387 ab
Green foxtail	560 ab	500 ab
Corn	900 b	2020 c

[a]Means followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 5. Metabolism from root application of ^{14}C-cyanazine to three species 3 days after treatment.[a]

Species	Percentage of total ^{14}C recovered			
	Parent (%)	Origin (%)	Metabolites (%)	Methanol-insol. (%)
Shoot				
Fall panicum	73.8 a	19.8 bc	0.0 cd	6.4 cd
Green foxtail	61.6 ab	18.5 bc	2.5 c	17.2 bc
Corn	53.8 b	7.1 d	21.6 b	17.4 bc
Root				
Fall panicum	28.6 c	33.7 a	0.0 cd	37.6 a
Green foxtail	57.2 ab	21.0 ab	0.0 cd	21.8 ab
Corn	9.2 d	21.6 ab	58.4 a	10.8 c

[a]Means within columns followed by similar letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Figure 3. Uptake and the concentration of cyanazine in the shoot and root portions of corn, fall panicum and green foxtail seedlings which were grown in nutrient solution containing ^{14}C-cyanazine.

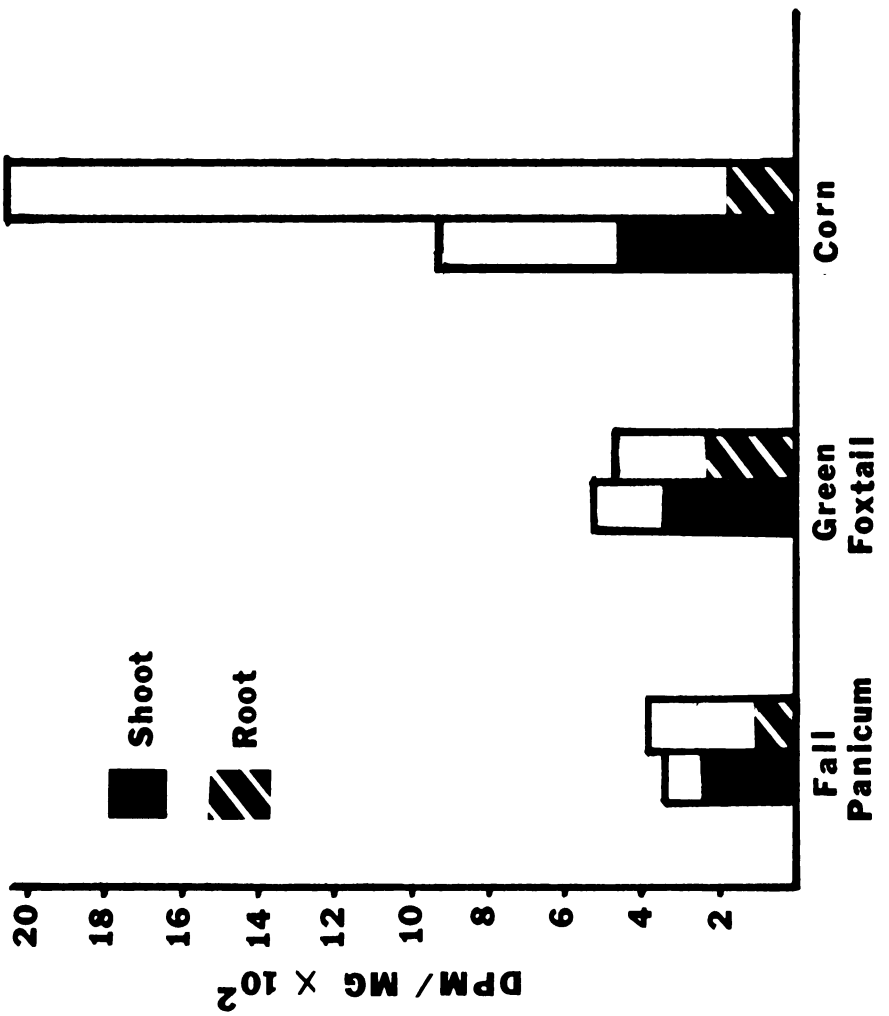
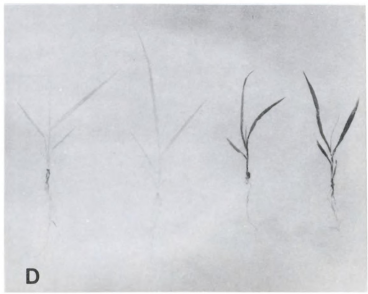

DPM/ MG X 10^2
20
18
16
14
12
10
8
6
4
2
Shoot
Root
Fall Panicum
Green Foxtail
Corn

Figure 4. Translocation of ^{14}C-cyanazine in fall panicum and green foxtail. The treated plants (A) and corresponding radioautograph (B) show fall panicum and green foxtail 5 days following a foliar treatment with cyanazine. Each set of grass species have the cyanazine treatment (left) compared to cyanazine plus oil (right). The treated plants (C) and corresponding radioautograph (D) show fall panicum and green foxtail 3 days following placement in a nutrient solution containing ^{14}C-cyanazine.

A

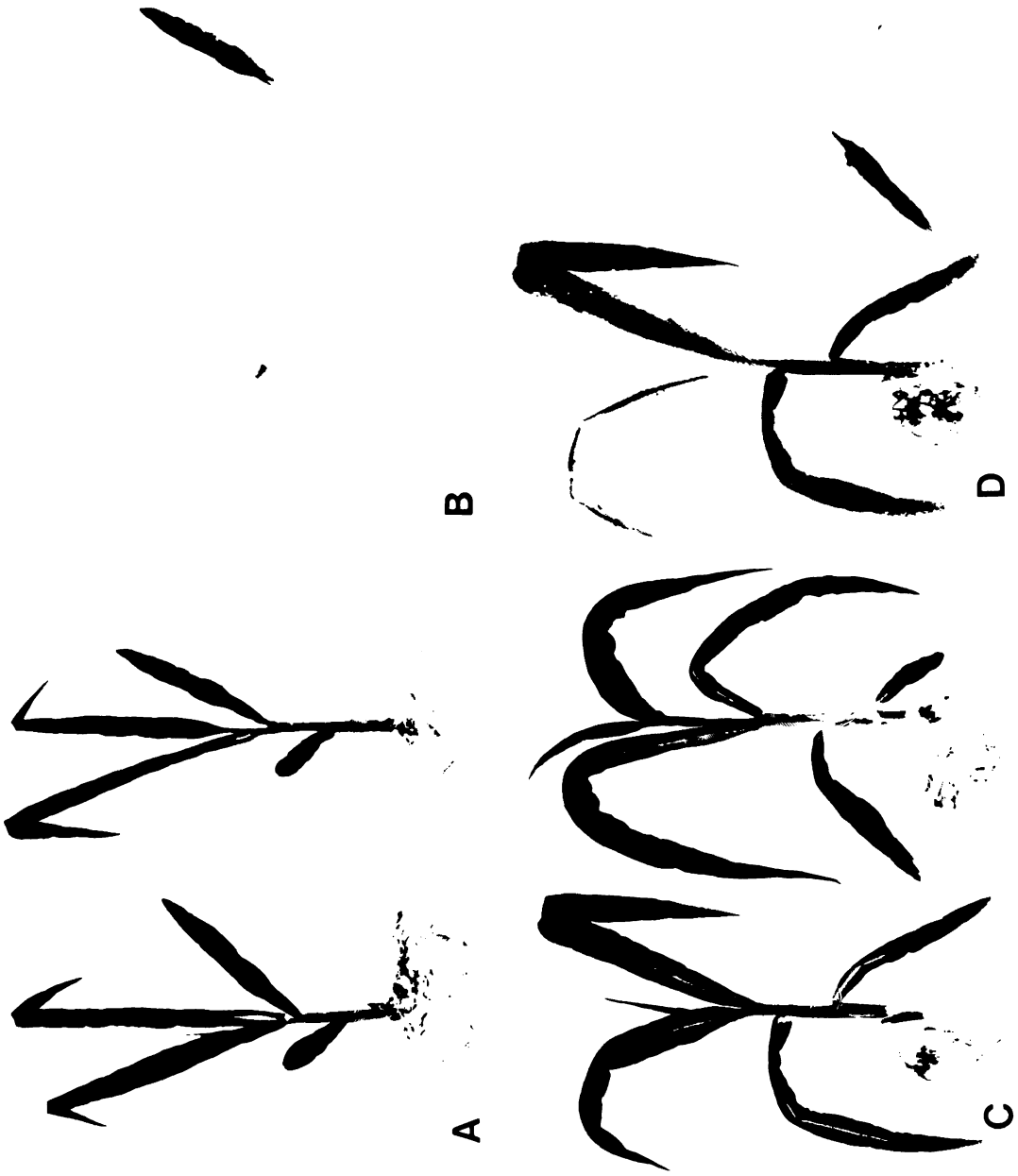
B

C



D

Figure 5. Translocation of ^{14}C-cyanazine in corn. The treated plants (A) and corresponding radioautograph (B) show corn treated for a 5-day duration with cyanazine (left) compared to cyanazine plus oil (right). The treated plants (C) and corresponding radioautograph (D) show acropetal movement 5 days after supplying the roots with ^{14}C-cyanazine (left) and leaf with ^{14}C-cyanazine plus oil (right).


A
B
C
D

Phytotoxicity following postemergence application of cyanazine was the result of both foliar and root absorption. In relation to the weed species, corn absorbed less ^{14}C material from foliar applications and more from root treatment. Slower inactivation of cyanazine by fall panicum and green foxtail appears to play a role in selectivity following root treatments. However, selectivity is not solely based on foliar uptake, but also on the proportion taken up by foliage and roots. Under conditions favorable for root uptake the margin of selectivity may be reduced.

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CHAPTER 4

CYANAZINE METABOLISM IN CORN, FALL PANICUM, AND GREEN FOXTAIL

Abstract

The metabolism of cyanazine (2-[[4-chloro-6-(ethylamino)-*s*-triazin-2-yl]amino]-2-methylpropionitrile by corn (*Zea mays* L.), fall panicum (*Panicum dichotomiflorum* Michx.), and green foxtail (*Setaria viridis* L.) was compared to determine the contribution of metabolism to selectivity. Cyanazine metabolism by plants with 4 leaves was examined following foliar or root treatments with ^{14}C-cyanazine. Parent material was separated from metabolites by thin-layer chromatography.

Five days following foliar application, 32.5 and 41.1% of a water-soluble metabolite were found in fall panicum and green foxtail, respectively. In corn two metabolites with 19.1 and 25.7% of ^{14}C activity were found in the water-soluble fraction. Only 12.0 and 6.2% of the same breakdown products were found in corn after a single day of treatment. Small quantities of other metabolites were also found. Metabolism of root-applied cyanazine appears to differ from foliar treatments in the weedy grasses as more parent cyanazine was recovered. Green foxtail had

equal amounts (61.1 and 57.2%) of unaltered cyanazine present in both shoot and root portions. The roots of corn and fall panicum contained 78 and 60% less cyanazine, respectively, than did the shoots. Rapid metabolism of cyanazine by corn roots provided evidence for an active detoxification mechanism.

Introduction

Fall panicum can metabolize atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], a widely used corn herbicide, to peptide conjugates (16, 17). Green foxtail also has a greater tolerance to atrazine than other species in the same genus (11, 16). Similar studies indicate that the two species could not significantly detoxify simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine] or cyanazine[1] (11, 16). In addition to dealkylation, the primary pathways responsible for the resistance of corn to atrazine are glutathione conjugation in shoots and hydrolytic reactions in the roots due to benzoxazinone (3, 12, 13, 16, 17). It has been suggested that dealkylation to less toxic metabolites are more evident in plants that are susceptible to triazine herbicides (14).

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[1]Thompson, R. P. and F. W. Slife. 1973. Uptake and degradation of ^{14}C-Bladex and ^{14}C-atrazine by four crop and two weed species. Weed Sci. Soc. Abstr. No. 142.

Corn grown in cyanazine treated soil for 32 and 70 days contained chlorotriazines and hydroxytriazines including the dealkylated derivatives (3, 5). Cyanazine applied to corn sap was hydrolyzed resulting in loss of the nitrile group but showed no evidence of glutathione or cysteine conjugation. The researchers concluded that 2-hydroxy-4-(1-carboxy-1-methylethylamino)-6-ethyl-amino-1,3,5-triazine was a direct breakdown product from cyanazine during the incubation. They also suggested that the extracted amide-cyanazine from corn plants was a result of uptake of that compound from the soil. Similar products were found in wheat (Triticum aestivum L.) and potatoes (Solanum tuberosum L.) as in corn. However, wheat leaves contained significant amounts of the chloro acids which otherwise only occurred in the roots of corn (5).

The purpose of this study was to determine the nature of differences in cyanazine metabolism between corn and the susceptible fall panicum and green foxtail. The significance of root and foliar degradation pathways compared within each species were also of interest.

Materials and Methods

Fall panicum, green foxtail, and corn seeds were germinated in the greenhouse, after which the seedlings were grown outside. Fall panicum and green foxtail plants with 4 to 5 leaves and corn with 4 leaves were selected and brought to the laboratory for ^{14}C-cyanazine treatment. A

5-µl drop containing 0.1 µCi of ^{14}C-cyanazine was placed inside a lanolin enclosure on the second leaf of corn. Droplets were placed between two lanolin bars on the second leaf of the weed species. A 4% phytobland oil[2]-water solution was used as the carrier. The uniformly ring-labeled ^{14}C-cyanazine had a specific activity of 3.86 µCi/nM and was 97% pure.[3] Following treatment, plants were moved to a growth chamber with a 16-hr day at 25 C and a light intensity of 19 klux. Night temperatures were 22 C. The plants were supplied with a modified Hoagland's no. 1 solution (8) and randomized daily.

After 1 and 5 days, the treated plants were sectioned into three parts. The treated leaf acropetal to the point of application was removed and immediately placed into a freezer at -5 C (1). Similarly, the rest of the aerial plant portion basipetal to the spot and the root portion were separated and frozen. The plant portions were homogenated in a Sorvall Omni-Mixer for 2 min in 10 ml of 80% methanol, and the homogenate filtered through glass wool as suggested by Beynon et al. (3, 4, 5). The volume of the methanol-soluble filtrate was reduced in vacuo and

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[2]Sun 11E phytobland oil is produced by Sun Oil Company, Philadelphia, Pennsylvania.

[3]^{14}C-labeled cyanazine was supplied by Shell Development Company, Modesto, California.

10 ml of water added. The aqueous fraction was partitioned with chloroform. The volumes of the fractions were reduced *in vacuo* and 500 µl of cold methanol added. Aliquots of 200 µl were spotted onto 250 nm thick (20 by 20 cm) silica gel F-254 (Brinkmann Instruments) thin-layer chromatography (TLC) plates. The aqueous fractions were developed in ethyl acetate:H_2O:formic acid (70:4:4, v/v/v) and then radioautographed. The lipophylic fractions were developed in acetone:chloroform (35:65, v/v). The radioactive spots on the TLC plate were removed and radioassayed by liquid scintillation spectrometry. The scintillation solution used consisted of 0.1 g of dimethyl POPOP [1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene], 5.0 g of PPO (2,5-diphenyloxazole), 50.1 g of naphthalene, 380 ml of toluene, 380 ml of 1,4-dioxane, and 240 ml of absolute ethanol. The activity in each spot was calculated as the percent of total activity from the TLC plate. Percentage data were transformed to arc sin for statistical analysis. Metabolites were identified by comparing Rf's to published Rf's in cyanazine degradation schemes (1, 2, 3, 4, 5, 6, 7, 9).

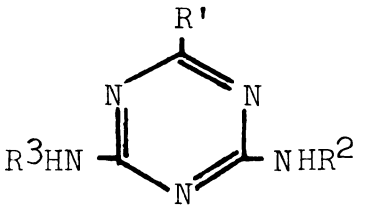
The plants for the root uptake studies were germinated in the greenhouse in a sand medium and transferred to a Hoagland's no. 1 solution containing 5 µCi/L of ^{14}C-cyanazine (cyanazine concentration was 3.5×10^{-7} M). Based on previous observations of root weights, volumes of solution were adjusted to 10 ml for the weed plants and 200 ml for corn. The plants were allowed to remain in the

^{14}C-labeled solution for 3 days. The plants were then removed and the roots were washed in three consecutive water baths. The harvested material was frozen prior to analysis. Data reported are the means for two experiments with three replications.

Results and Discussion

The distribution of metabolites following development in thin-layer chromatographic (TLC) systems is shown in Table 1. A number of distinguishable metabolites (A to O) were compared to cyanazine breakdown products as identified by Beynon *et al.* (3, 4, 5). Five compounds in trace or small amounts (B, D, E, F, N) were located by the TLC system, but could not be compared to other published Rf values (7, 9). Thin-layer chromatographs of the water-soluble fractions indicated that a difference in the nature of metabolism of foliarly applied cyanazine existed between corn and the weed species 1 and 5 days after treatment (Table 2). Following foliar treatments, corn appeared to metabolize cyanazine to near equal ratios of the stable products A and C. In contrast, fall panicum and green foxtail metabolism resulted in a large percentage of A. The percent of stable product increased over time in all species. Similar percentages of parent cyanazine were found in the three species 1 and 5 days after foliar treatment (Table 3). The large percentage of J in green foxtail indicated a dealkylated product. The Rf values did not reveal the

Table 1. Thin-layer chromatography and structures of ^{14}C-cyanazine and metabolites.



Compound reference	Group structures[a]			Rf	
	R^1	R^2	R^3	Methanol-soluble System I[b]	Chloroform-soluble System II[c]
Cyanazine	Cl	$C(CH_3)_2CN$	C_2H_5	.98	.80
A	OH	$C(CH_3)_2COOH$	H	.00	.00
B				.04	--
C	OH	$C(CH_3)_2COOH$	C_2H_5	.08	--
D				.10	--
E				.13	--
F				.18	--
G	OH	H	C_2H_5	.27	--
H	OH	H	H	.36	--
I	Cl Cl	$C(CH_3)_2COOH$ $C(CH_3)_2CONH_2$	C_2H_5 H	.83	--
J	Cl	$C(CH_3)_2CN$	H	.90	.65
K	Cl	$C(CH_3)_2CONH_2$	C_2H_5	.93	.32
L	OH	$C(CH_3)_2CN$	C_2H_5	--	.05
M	Cl	$C(CH_3)_2CONH_2$	C_2H_5	--	.13
N				--	.18
O	Cl	H	C_2H_5	--	.55

[a]Structures taken from Beynon et al. (4).

[b]System I is ethyl acetate-H_2O-formic acid (70:4:4).

[c]System II is acetone-chloroform (35:65).

Table 2. Percentage of recovered water-soluble forms of ^{14}C-cyanazine 1 and 5 days after foliar treatment to three plant species.

		Percentage of total ^{14}C recovered from TLC plate					
		Corn		Fall panicum		Green foxtail	
Compound	Location (Rf)	1 day (%)	5 day (%)	1 day (%)	5 day (%)	1 day (%)	5 day (%)
A	.00	12.0	19.1	19.9	32.5	34.5	41.1
B	.04	6.6	4.7	2.2	5.5	2.3	3.4
C	.08	6.2	25.7	0.5	3.0	--	1.6
D	.10	--	--	0.6	1.7	1.1	--
E	.13	--	tr[a]	--	--	--	--
F	.18	--	--	1.5	--	--	0.6
G	.27	--	tr	1.2	tr	--	--
J	.90	--	--	--	0.9	--	--
K	.93	--	--	--	--	--	0.3
Cyanazine	.98	0.6	--	1.4	1.5	1.3	3.7

[a]tr = trace

Table 3. Percentage of recovered chloroform-soluble forms of ^{14}C-cyanazine 1 and 5 days after foliar treatment to three plant species.

		^{14}C Percentage of total recovered from TLC plate					
		Corn		Fall panicum		Green foxtail	
Compound	Location (Rf)	1 day (%)	5 day (%)	1 day (%)	5 day (%)	1 day (%)	5 day (%)
A	.00	0.6	7.4	1.0	4.9	1.8	1.1
L	.05	--	--	tr[a]	0.5	--	0.3
M	.13	--	--	tr	tr	--	0.4
N	.18	--	--	--	--	0.4	1.0
K	.32	--	--	0.9	0.7	1.4	--
O	.55	--	0.4	0.3	--	4.5	--
J	.65	--	--	--	--	--	12.9
Cyanazine	.98	73.2	40.8	62.5	45.2	55.7	36.4

[a]tr = trace

presence of J in fall panicum. A larger number of metabolites were found in the weed species than in corn. The susceptibility of the annual grasses to cyanazine may be due to this difference in metabolism to the stable products.

Supplying the roots with ^{14}C-cyanazine failed to produce significant recoverable amounts of the stable product A in the shoots of corn as compared to foliar ^{14}C-cyanazine application (Table 4). However, large amounts of A and C were found in the corn roots. This suggests a very active detoxification mechanism in the root system. In contrast, all recovered radioactivity in the water-soluble fraction of fall panicum and green foxtail was compound A. Small amounts of the dealkylated cyanazine J were found in the chloroform fraction in green foxtail (Table 5). The percentage of parent material in the shoot portion was comparable among the three species. However, the roots of corn and fall panicum contained less cyanazine than did the shoots. Green foxtail had large amounts of unaltered cyanazine present in both shoot and root portions. As previously reported this may be the reason for the increased sensitivity of green foxtail to cyanazine (10). The occurrence of the dealkylated hydroxy-acid in the weed species supports the report that dealkylation reactions are more evident in triazine-susceptible plants (14).

Although breakdown of cyanazine occurred over the time of 1 to 5 days, the data suggested that a difference in metabolism among the three species was not the sole

Table 4. Chromatography and percentage of total ^{14}C recovered in the water-soluble fraction 3 days after supplying the roots of three species with ^{14}C-cyanazine.

Compound	Location (Rf)	Percentage of total ^{14}C recovered from TLC plate					
		Corn		Fall panicum		Green foxtail	
		Shoot (%)	Root (%)	Shoot (%)	Root (%)	Shoot (%)	Root (%)
A	.00	7.1	17.6	--	30.4	14.5	12.3
B	.04	4.4	9.8	--	--	--	--
C	.08	19.8	39.4	--	--	--	--
E	.13	tr[a]	tr	tr	--	--	--
F	.18	tr	--	--	--	--	--
G	.27	tr	--	--	--	--	--
I	.83	--	0.6	--	--	--	--
J	.90	tr	--	--	--	--	--
K	.93	0.8	--	--	--	--	--
Cyanazine	.98	0.8	--	--	--	--	--

[a]tr = trace

Table 5. Chromatography and percentage of total ^{14}C recovered in the chloroform-soluble fraction 3 days after supplying the roots with ^{14}C-cyanazine.

Compound	Location (Rf)	Percentage of total ^{14}C recovered from TLC plate					
		Corn		Fall panicum		Green foxtail	
		Shoot (%)	Root (%)	Shoot (%)	Root (%)	Shoot (%)	Root (%)
A	.00	1.3	3.7	--	0.4	tr[a]	tr
M	.13	--	0.8	--	--	--	--
N	.18	--	0.9	--	--	--	--
O	.55	0.6	--	--	--	--	--
J	.65	0.1	--	--	--	1.6	7.0
Cyanazine	.98	53.7	9.7	73.6	28.6	61.6	57.2

[a]tr = trace

basis for selectivity from foliar treatments. However, rapid metabolism of cyanazine by roots of corn and fall panicum reveals an active detoxification mechanism in the roots of these species. Greater sensitivity of green foxtail to cyanazine may relate to its inability to alter the parent compound. It appeared that sufficient amounts of root-applied cyanazine reached the site of action in fall panicum to provide effective kill.

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CHAPTER 5

CORN TOLERANCE TO SOIL-APPLIED CYANAZINE

Abstract

Tolerance of greenhouse-grown corn (Zea mays L.) to cyanazine (2-[[4-chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile) and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] were compared when grown in Conover sandy loam soil. Reductions in dry weight were obtained following preemergence applications and postemergence applications of cyanazine allowed to contact the soil, under both low and high soil moisture conditions. Results of this study indicate that corn tolerance to cyanazine is largely influenced by root uptake. During periods of active plant growth, conditions favorable for rapid root uptake of cyanazine were responsible for the greatest corn injury.

Introduction

Postemergence applications of cyanazine have provided good control of annual grasses (2, 3). However, in some instances weed control has been variable and corn injury has occurred. Herbicide rate, stage of plant growth, soil interception of spray, rainfall, and adjuvants have

been suggested as factors in determining the degree of cyanazine injury to corn (1, 2, 3, 5).

Injury to corn seedlings treated with atrazine has been reported under cold, wet conditions (7). The researchers concluded that low temperatures decreased the rate of detoxification from foliarly-absorbed atrazine in the stressed plants. They suggested that reduced detoxification by the roots did not contribute to the death of the corn.

The interception of the postemergence spray by the soil and the subsequent herbicide uptake by plant roots may also play a role in determining plant responses to postemergence herbicide applications. Greater control of giant foxtail (Setaria faberii Herrm.) in the 4 to 5-leaf stage was achieved when atrazine was applied to wet soil followed by simulated rainfall than when rainfall did not occur (6).

Although root absorption of cyanazine was found to supplement foliar uptake resulting in corn phytotoxicity, the extent of direct root uptake was not measured (4). Previous studies with cyanazine suggested that rapid root uptake could result in an overloaded detoxification mechanism.[1]

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[1]Kern, A. D., W. F. Meggitt, and D. Penner. 1974. Absorption, translocation, and metabolism of foliar applied cyanazine in corn, fall panicum, and green foxtail. Weed Sci. Soc. Amer. Abstr. No. 194.

Occurrence of cyanazine injury may result if sufficient soil residual were available at a time of rapid uptake of water and the herbicide.

The objective of this investigation was to determine the role that root uptake of cyanazine plays in corn injury due to postemergence applications. The effects of application rate and soil moisture on tolerance to soil-applied cyanazine prior to corn emergence or when the corn had four leaves was determined.

Materials and Methods

'Michigan 396' corn seed was planted in 946-ml cups containing a Conover sandy loam soil with 2.4% organic matter and grown in the greenhouse. The corn was thinned to three plants per cup and grown at 23 to 27 C with supplemental lighting of 12.9 klux during a 16-hr day.

Preemergence herbicide applications were made immediately after planting. Postemergence herbicide treatments were made directly to the soil with a pipette when the corn had four leaves. Applications of 20 ml of the herbicide solution was made to dry soil. The mixture was applied slowly to insure against absorption by the base of the corn shoot.

The plants were supplied with a modified Hoagland's no. 1 solution every 3 days. Soil moisture levels were maintained under low and high regimes of 25 and 50 ml water per pot, respectively. If plants under the low

moisture conditions began to wilt, small amounts of water were added.

Shoot dry weight was recorded when the plants had 5 to 6 leaves. One preemergence study was harvested at the 3, 4, and 5-leaf stages for dry weight comparison over time. Thus, corn treated with preemergence applications of the herbicides were grown in treated soil for 16 to 25 days. The corn was harvested 10 days after the postemergence treatments. Data presented are the means of two experiments with three or four replications.

Results and Discussion

Both soil moisture level and herbicide rate had a pronounced effect on corn tolerance to preemergence applications of cyanazine (Table 1). Reduction in dry weight was greatest under high soil moisture conditions. Although cyanazine rates of 1.1 to 4.5 kg/ha reduced corn growth, applications of 5.6 and 6.7 kg/ha resulted in about a 50% loss in dry weight of 25-day-old corn grown under high soil moisture conditions. Similar reductions in dry weight from cyanazine were obtained under the low moisture regime. In comparison, excellent tolerance to atrazine was observed. Rapid conversion of absorbed atrazine to hydroxy-atrazine was probably responsible for good corn tolerance under these conditions. During preliminary experiments which gave similar results, reduced corn vigor was not visually observed until the plants reached the 3 to 4-leaf stage.

Table 1. Effect of preemergence applications of cyanazine and atrazine to corn grown in the greenhouse under two moisture regimes.[a]

Treatment rate (kg/ha)	Dry weight	
	Low moisture (g/3 plants)	High moisture (g/3 plants)
Cyanazine		
0.0	1.60 a	2.32 a
1.1	1.44 abc	1.92 bc
2.2	1.33 bcd	1.71 cd
3.4	1.27 cde	1.76 cd
4.5	1.12 de	1.55 d
5.6	1.11 e	1.27 e
6.7	1.13 de	1.08 e
Atrazine		
2.2	1.55 ab	2.09 ab
4.5	1.51 ab	1.94 bc
6.7	1.54 ab	2.28 a

[a]Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

The data in Table 2 indicates that corn harvested in the 5-leaf stage showed the greatest rate response to preemergence applications of cyanazine. This effect may be explained by the increased time interval for cyanazine uptake and the more rapid cyanazine uptake by the larger plants. Experimental conditions permitted cyanazine leaching into the root zone.

With the exception of the 4.5 kg/ha rate under low moisture, corn showed good tolerance to atrazine applied to the soil when the corn had four leaves (Table 3). However, rates of 2.2 and 4.5 kg/ha of cyanazine significantly reduced the growth of corn 10 days following soil application. Corn at this stage appeared unable to detoxify the large amounts of cyanazine absorbed from the soil rapidly enough to prevent injury. Similar responses have been observed in the field 10 to 20 days after cyanazine treatment.[2] However, the corn appeared to compensate for the reduction in growth as the growing season continued.

The coefficient of correlation and linear regression (Figure 1 and 2) indicated that the decrease in corn shoot dry weight as influenced by an increase in cyanazine was not as great under the low moisture regime as under

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[2]Kern, A. D., W. F. Meggitt, and R. C. Bond. 1972. Green foxtail control in Michigan. N. Cent. Weed Contr. Conf. Res. Rep. 29:144.

Table 2. Reduction in dry weight of corn harvested at three different stages of growth following preemergence treatments of cyanazine.[a]

Cyanazine (kg/ha)	Dry weight		
	3-leaf	4-leaf	5-leaf
	(% of control)		
0	100.0 a	100.0 a	100.0 a
2	100.0 a	93.5 ab	92.6 b
4	97.7	85.9 b	67.1 c
6	78.2 b	81.2 bc	60.4 c

[a]Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 3. Influence of atrazine and cyanazine on corn growth 10 days after soil treatment when the corn had four leaves.[a]

Treatment	Rate (kg/ha)	Dry weight	
		Low moisture (gm/3 plants)	High moisture (gm/3 plants)
Control	0.0	1.78 a	2.29 a
Atrazine	2.2	1.57 ab	2.13 ab
Atrazine	4.5	1.45 bc	1.98 ab
Cyanazine	2.2	1.33 cd	1.56 c
Cyanazine	4.5	1.18 d	1.30 cd

[a]Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

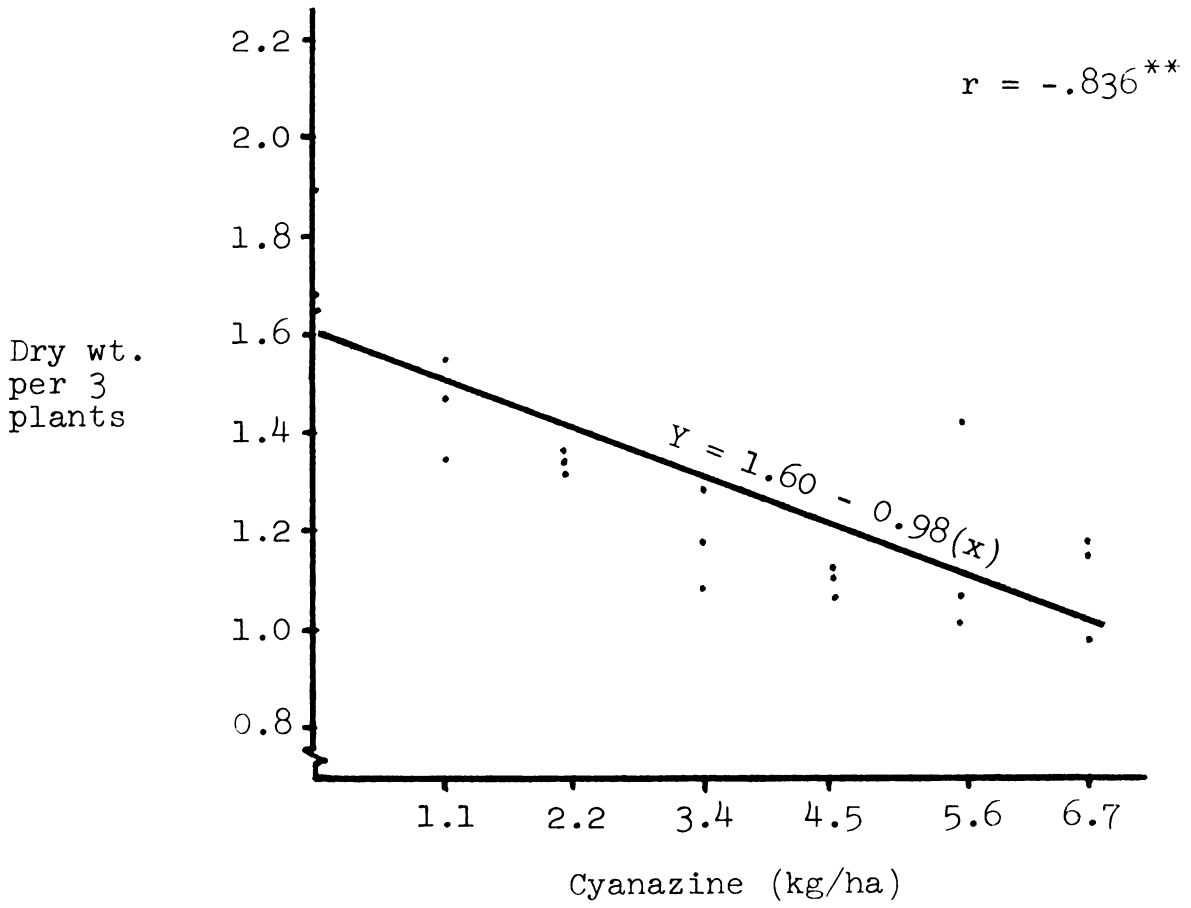


Figure 1. Relationship between the rate of cyanazine application and plant dry weight 10 days after treating corn with four leaves and maintained under low soil moisture conditions.

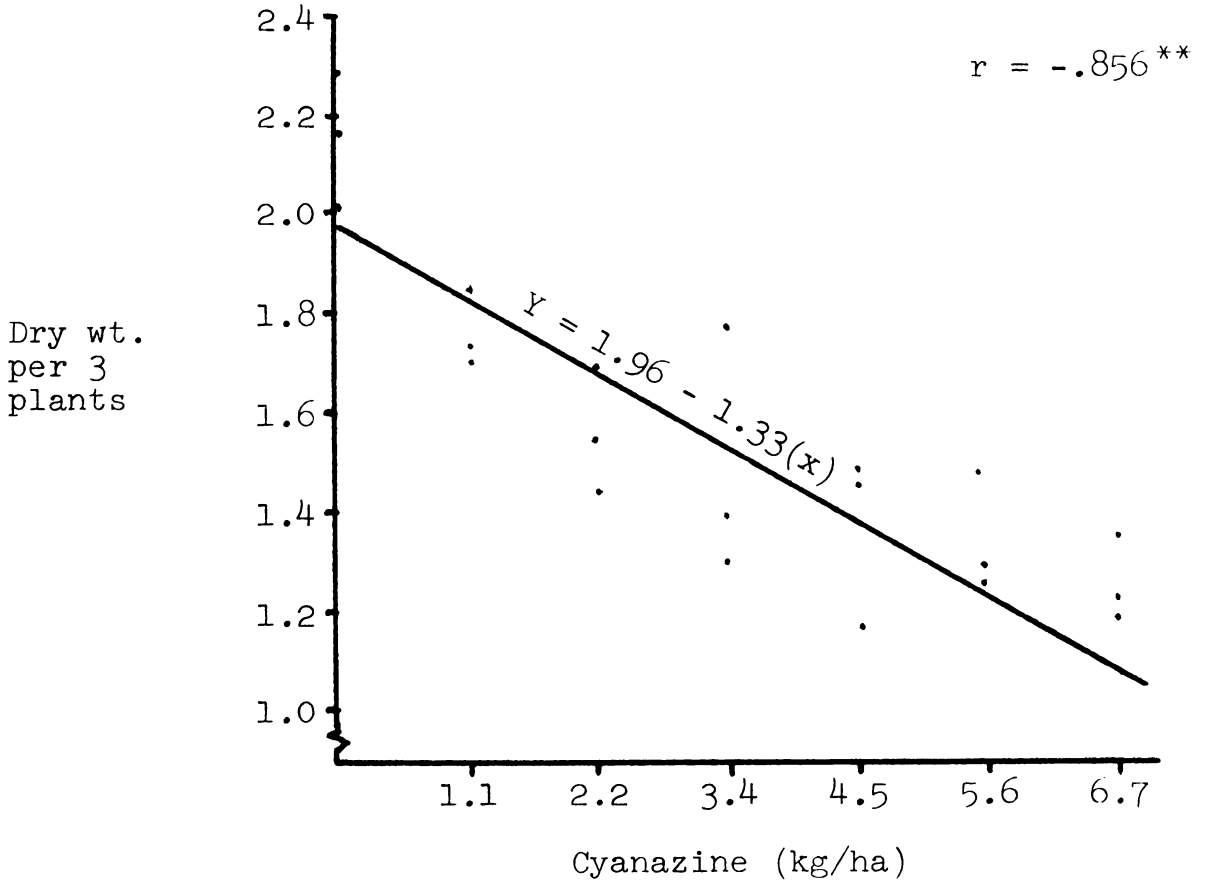
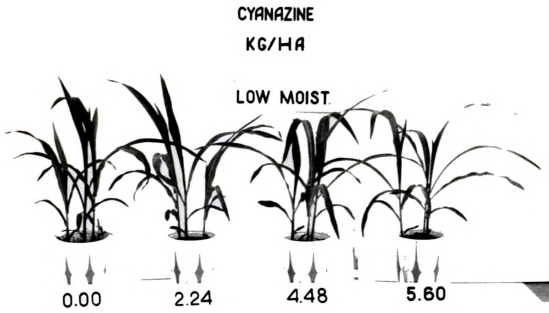


Figure 2. Relationship between the rate of cyanazine application and plant dry weight 10 days after treating corn with four leaves and maintained under high soil moisture conditions.

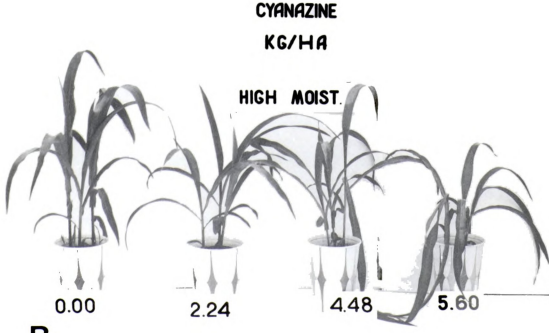
high moisture conditions. Repeated experiments gave similar results with significant values. Figure 3 shows that corn injury from cyanazine applied to the soil resulted in smaller and less turgid plants. Although color was not always an obvious injury indicator on marginally injured plants, corn treated with 5.6 and 6.7 kg/ha of cyanazine were distinguishably more chlorotic. The same plants grown under high moisture conditions became flaccid.

Results of this study indicate that corn tolerance to cyanazine is largely influenced by root uptake. Soil type, rainfall after treatment, and weed or crop situations favoring soil interception of the spray are contributors to cyanazine phytotoxicity to corn from postemergence treatments. The physiological stage of plant growth and other factors may be responsible for greater cyanazine uptake resulting in increased injury.

Figure 3. Response of corn to soil-applied postemergence treatments of cyanazine. The treated plants indicate responses 10 days after treatment to soils maintained under low (A) and high (B) moisture regimes.


CYANAZINE
KG/HA
LOW MOIST.
0.00
2.24
4.48
5.60

A


CYANAZINE
KG/HA
HIGH MOIST.
0.00
2.24
4.48
5.60

B

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CHAPTER 6

SUMMARY AND CONCLUSIONS

In the field, postemergence treatments of cyanazine at 3.4 kg/ha applied to corn with less than five leaves and fall panicum with less than four leaves eliminated competing fall panicum and provided sufficient soil residual for full-season control and maximum yields. Control was reduced significantly when treatments were applied at later stages of growth. In addition, greater corn injury resulted when cyanazine was applied to corn with six to seven leaves. Yield reductions as a result of late treatments were due to both crop injury and weed competition.

Greenhouse and field studies showed that phytobland oils increased both fall panicum and corn injury by cyanazine. These observations were confirmed with radiotracer studies. It appeared that selectivity from postemergence cyanazine treatment was partially due to the slow entry of the herbicide into the corn leaves. With oil large amounts of the herbicide were concentrated in the leaves of corn 5 days after foliar treatment. This may be the reason for foliar injury observed in the field following postemergence applications of cyanazine plus oil. Because of the narrowed

margin of selectivity and erratic results, the addition of adjuvants to cyanazine are not recommended.

Corn absorbed less cyanazine from foliar applications and more from root treatments when compared to the weed species. Absorbed cyanazine was rapidly translocated acropetally from point of application to areas of highest transpiration in both weeds and corn.

Slower inactivation of cyanazine by fall panicum and green foxtail plays a role in selectivity following root treatment. Both the shoots and roots of green foxtail contained larger amounts of unaltered cyanazine following foliar and root applications of the herbicide. Greater sensitivity of green foxtail than fall panicum to cyanazine is related to its inability to alter the parent compound.

Following foliar and root treatments of cyanazine, corn appeared to metabolize the herbicide to two stable hydroxy compounds. Greater amounts of the breakdown products were found 5 days after the foliar treatment than after one day of treatment in all three species. In contrast to corn, fewer metabolites were found in the weedy grasses. Sufficient amounts of root-applied cyanazine reached a sensitive site in fall panicum for effective kill.

Although treatments restricted to the foliage reduced fresh weight, complete fall panicum and green foxtail control was the result of both foliar and root absorption.

Similarly, greater corn injury was observed when soil interception of the herbicide was allowed. Therefore, corn tolerance is influenced by root uptake of the postemergence spray. Field conditions favoring cyanazine movement into the root zone of vigorously growing corn results in rapid uptake of the herbicide overloading the detoxification mechanism resulting in injury.

In conclusion, selectivity is not solely based on foliar uptake, but also on the proportion taken up by foliage and roots. Many factors influence the contribution of each pathway of uptake to selectivity. Under conditions favorable for root uptake, the margin of selectivity may be reduced. Crop population, weed density, soil type, and rainfall are factors contributing to the effectiveness of cyanazine for annual grass control and on phytotoxicity to the crop. These factors influence the amount of soil interception from postemergence application of cyanazine.

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APPENDICES

APPENDIX A

Modified Hoagland's No. 1 Solution

1.	1 M KH_2PO_4	2 ml/L
2.	1 M KNO_3	2 ml/L
3.	1 M $Ca(NO_3)_2 \cdot 4H_2O$	3 ml/L
4.	1 M $MgSO_4 \cdot 7H_2O$	2 ml/L
5.	1.5 g/L $MnCL_3 \cdot 4H_2O$	
	2.5 g/L H_3BO_4	
	0.1 g/L $ZnCl_2$	1 ml/L
	0.05 g/L $CuCl_2 \cdot 2H_2O$	
	0.05 g/L MoO_3	
6.	26.3 g/L Sequestrene^R	1 ml/L
	pH 6.5 to 6.8 with 1 M NaOH	

APPENDIX B

Table B. Effect of spray volume and simulated rainfall on the efficacy of cyanazine at 2.2 kg/ha with and without oil on 9 to 10-cm fall panicum.[a]

Treatment	Rate (kg/ha)	Fresh weight[b]		
		Application spray volume		
		234 L/ha (g/cup)	935 L/ha (g/cup)	935+L/ha[c] (g/cup)
Control	--	6.7 ab	7.0 a	6.7 ab
Cyanazine	2.2	5.4 bc	3.2 cde	3.8 cd
Cyanazine+oil	2.2+9.4 L/ha	3.6 cd	2.6 de	1.1 e

[a]Plants were blocked as to density.

[b]Means followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[c]Simulated rainfall equal to 2.5 cm was added immediately after treatment.

APPENDIX C

Table C. Influence of rainfall and relative humidity on the control of 10-cm fall panicum 25 days following treatment with cyanazine at 2.8 kg/ha with and without oil.

Condition	Visual control rating[a]	
	Cyanazine (%)	Cyanazine + oil (%)
Control	0.0	0.0
Treated control	4.8	6.0
Rain[b]	4.3	7.3
$(Rain)_2$	5.3	8.0
$(Rain)_2$-no soil contact[c]	3.0	8.0
High humidity[d]	2.3	6.5

[a]0 = no control; 10 = complete control or kill.

[b]Simulated rainfall equal to 2.5 cm was added immediately after foliage treatment had dried.

[c]Washing of herbicide was prevented by tilting cup to side.

[d]High relative humidity of 75 to 80 was maintained with a vaporizer. A relative humidity of 40 to 45 was maintained for the other treatments.

APPENDIX D

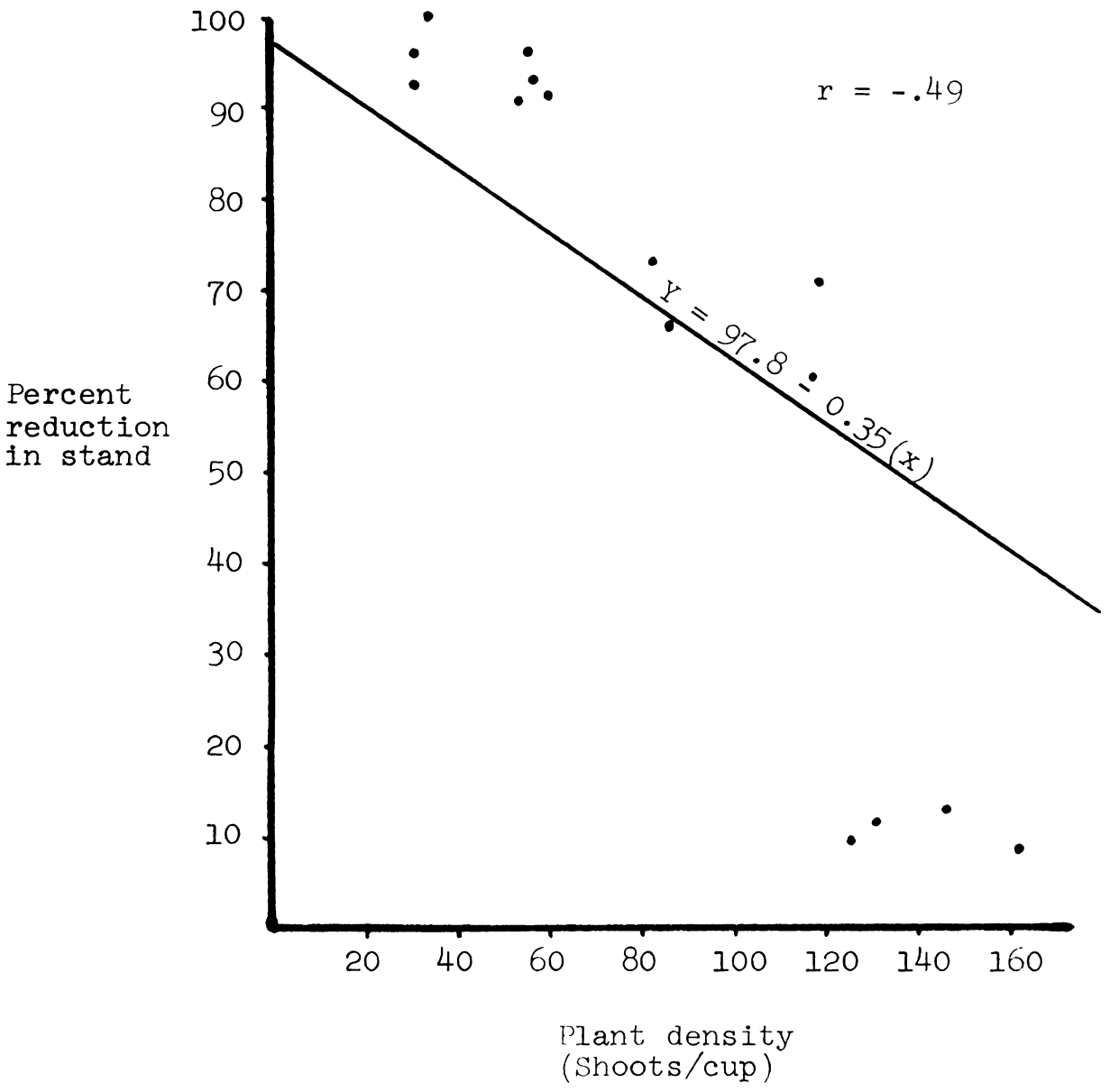

Figure 1. The relationship between plant density and the percent reduction in stand of fall panicum 14 days following cyanazine treatment of 3.4 kg/ha to 8-cm tall plants.