



Translocation of 2,4-dichlorophenoxyacetic acid in three Canada thistle (*Cirsium arvense* [L.] Scop.) ecotypes
by Jae Ho Kim

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:

Tracer studies employing C14-labeled 2,4-dichlorophenoxyacetic acid (2,4-D-l-C14) were conducted on three Canada thistle (*Cirsium arvense* (L.) Scop.) ecotypes under three different environmentally controlled conditions and in the field. Ecotype response to 2,4-D sprays was also studied.

Data from radioautographic evidence indicated that three different relative humidity levels (low, intermediate and high) did, not significantly affect translocation of 2,4-D-C14. Differences in translocation of C14 between F1 and G1 ecotypes were significant at the one percent level of probability under all controlled growing conditions. In the field, a significant difference was, indicated between treatment dates, but not between ecotypes.

No significant difference in response to 2,4-D sprays was found among the three ecotypes. There was a significant interaction between ecotypes and growing conditions, however.

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This thesis is dedicated to my grandmother.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	v
LIST OF APPENDIX TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
INTRODUCTION	1
LITERATURE REVIEW	2
MATERIALS AND METHODS	13
RESULTS AND DISCUSSIONS	17
SUMMARY	32
APPENDIX	33
LITERATURE CITED	41

LIST OF TABLES

Number		Page
I	A visual evaluation of radioautographs from the entire plant of three Canada thistle ecotypes treated with 2,4-D-C ¹⁴	18
II	A visual evaluation of radioautographs from the entire plant of three Canada thistle ecotypes treated with 2,4-D-C ¹⁴	19
III	A visual evaluation of radioautographs from three Canada thistle ecotypes treated with 2,4-D-C ¹⁴ at three different dates in the field	23
IV	Response of three Canada thistle ecotypes to 2,4-D when growing under three different conditions.	30

LIST OF APPENDIX TABLES

Number		Page
I	Analysis of variance for translocation of 2,4-D-C ¹⁴ in three Canada thistle ecotypes including top, stem, and roots.	34
II	Analysis of variance for translocation of 2,4-D-C ¹⁴ in three Canada thistle ecotypes including top, stem, and roots.	35
III	Analysis of variance for translocation of 2,4-D-C ¹⁴ in field grown three Canada thistle ecotypes.	36
IV	Analysis of variance for response of three Canada thistle ecotypes to 2,4-D.	37
V	A visual evaluation of radioautographs from the entire plant of three Canada thistle ecotypes including top, stem, and roots.	38
VI	A visual evaluation of radioautographs from the entire plant of three Canada thistle ecotypes including top, stem, and roots.	39
VII	Dry weight of three Canada thistle ecotypes growing under three different conditions and treated with 2,4-D. .	40

LIST OF FIGURES

Number		Page
1	A visual evaluation of radioautographs from the entire plant of three Canada thistle ecotypes treated with 2,4-D.	20
2	Radioautographs of three Canada thistle ecotypes treated with 2,4-D-C ¹⁴ under three different conditions (from bottom to top, FI, YM, and G1 ecotypes; from left to right, greenhouse, chamber 1 and chamber 2)	21
3	Radioautographs of FI ecotype treated with 2,4-D-C ¹⁴ at three different dates in the field (from left to right, 5/24, 6/18, 7/1 treatment)	24
4	Radioautographs of YM ecotype treated with 2,4-D-C ¹⁴ at three different dates in the field (from left to right, 5/24, 6/18, and 7/1 treatment)	25
5	Radioautographs of G1 ecotype treated with 2,4-D-C ¹⁴ at three different dates in the field (from left to right, 5/24, 6/18, and 7/1 treatment)	26
6	Response of three Canada thistle ecotypes to 2,4-D when growing under three different conditions	31

ABSTRACT

Tracer studies employing C^{14} -labeled 2,4-dichlorophenoxyacetic acid (2,4-D- C^{14}) were conducted on three Canada thistle (Cirsium arvense (L.) Scop.) ecotypes under three different environmentally controlled conditions and in the field. Ecotype response to 2,4-D sprays was also studied. Data from radioautographic evidence indicated that three different relative humidity levels (low, intermediate and high) did not significantly affect translocation of 2,4-D- C^{14} . Differences in translocation of C^{14} between FI and GI ecotypes were significant at the one percent level of probability under all controlled growing conditions. In the field, a significant difference was indicated between treatment dates, but not between ecotypes.

No significant difference in response to 2,4-D sprays was found among the three ecotypes. There was a significant interaction between ecotypes and growing conditions, however.

INTRODUCTION

The development and use of 2,4-dichlorophenoxyacetic acid, as a selective herbicide, has proved to be of tremendous benefit to the modern farmer and rancher. It is particularly effective and most consistent in the control of certain annual broad-leaved weeds in small grains such as wheat and barley.

Although often used in the control of broad-leaved perennial weeds, results are sometimes inconsistent. Studies to determine the reason for these inconsistencies have led to the conclusion that inheritance, environment, edaphic factors and growth characteristics are all involved in causing differences of response (6,30,40).

The ultimate action of phenoxy type herbicides that must be moved (translocated) from the point of application on the foliage to the site of action in the growing points (4,6,7,30,38) is dependent on the quantity of herbicide reaching the sites where lethal action takes place.

This study involved the effect of humidity on penetration and translocation of 2,4-D in three Canada thistle (Cirsium arvense (L.)) ecotypes. It was one phase of an overall regional project involved with the relationship of temperature to the action of herbicides on Canada thistle as influenced by external and internal factors.

Canada thistle was the chosen plant in this study because of its widespread distribution and because a selected population was available for study.

Ecotypes of Canada thistle that had been tested and classified as resistant, intermediate and susceptible in response to 2,4-D were used in this study (16).

LITERATURE REVIEW

Translocation of Herbicides and Radioactive Tracers

The effectiveness of a systemic herbicide is dependent on its ability to reach and adversely effect the growth processes. The first obstacle to this occurrence is penetration into the epidermal cells. This obstacle directly influences the amount of herbicide available for translocation; however, few have actually studied translocation separate from penetration.

Much evidence (2,3,4,10,13,30) indicates that hormone-like herbicides applied to the foliage are moved from the mesophyll into the phloem in much the same way as assimilates. In the phloem, they move with the stream of food materials from the leaves to regions where the foods are being utilized.

Perkias, Nelson and Gorham (26) using autoradiography to study distribution of C^{14} -labeled photosynthate and C^{14} -labeled sucrose, glucose, and fructose in soybean plants found that the C^{14} of photosynthate was localized in the phloem; that of sucrose, introduced through the cut petiole, was concentrated in the xylem and the adjacent pith, and the C^{14} of fructose and glucose was localized mainly in the xylem. The investigators concluded that all translocation need not take place in the phloem but that some may take place in the pith and xylem.

Aronoff (1) used $C^{14}O_2$ applied to a limited area of soybean leaves to study translocation of the labeled sugar. He found that under normal conditions, movement of labeled sugar was greatest to the growing regions of the plant.

It is generally agreed that movement of the 2,4-D from the foliage is closely associated with translocation of food materials in the phloem (4,5, 6,7,20,25,30).

Crafts (4,6,7) concluded that 2,4-D was diffusing into the leaves entering the symplast, migrating to the phloem, and moving along in the assimilate stream with food materials. Many investigators (3,4,8,9,10) concluded that synthesis and export of food are the motivating forces for the export of labeled tracers from leaves. They also concluded that there is considerable variation among individual plants and individual experiments with respect to the extent of distribution of foliar-applied tracers into stem and roots.

The Rate of Movement of 2,4-D

Crafts (4,5) reported three years' work with C^{14} -labeled 2,4-D on bean, cotton, cucumber, and wild morning glory. The studies indicated that 2,4-D was absorbed and translocated into the bean roots in about three hours; movement into terminal buds required six hours.

Crafts (5) studying distance of movement of 2,4-D in treated leaves found that after four hours, movement was 11 inches; after 27 hours, 30 inches; 48 hours, 39 inches; in 8 days, to the full depth of excavation, that is, 6 feet.

Slife, et al. (37) reported that distribution of foliar-applied C^{14} -labeled 2,4-D in cucumber plants was achieved in 24 hours and changed little by the end of 4 or 8 days. Metabolic studies indicated that about 75% of the absorbed 2,4-D was converted into two major metabolites within 24 hours.

Effect of Time or Stage of Growth on Translocation

The age of the plants or physiological condition seems to influence translocation. For example, C^{14} -labeled 2,4-D was absorbed and moved more rapidly in young bean plants than in old ones (9).

Numerous investigators (4,5,6,7,20,23) found that no movement of 2,4-D took place from very young leaves that were still importing foods from more mature leaves.

Crafts (4) also reported in his studies using bean and cucumber plants that stage of growth was important on translocation of 2,4-D in plants. His studies showed that at the bud stage, transport was predominately downward; at flowering and fruiting it was into the flowers and fruits; in the ripe seed stage it was again predominately downward.

Linscott and McCarty (21) applying C¹⁴-labeled 2,4-D to iron weed (Vernonia baldwini Nutt.) at various stages of growth in the field and greenhouse found that during phases of rapid plant development C¹⁴ tended to concentrate in new leaves, axillary buds, petioles, and stems; but as plants approached full vegetative development, greater quantities were detected in rhizomes and roots and less in stems and leaves.

Effect of Nutrition of Plants on Translocation:

Mitchell and Brown (23) reported that the stimulus resulting from treatment with 2,4-D was not readily translocated from leaves of bean plants whose sugar contents were relatively low.

Rohrbough and Rice (31) using C¹⁴-labeled 2,4-D in studies on phosphorus nutrition of tomato plants concluded that 2,4-D was not readily translocated through tomato plants that were seriously deficient in phosphorus.

Rice and Rohrbough (28) found that if potassium-deficient plants were supplied with nutrients before or at the same time that 2,4-D was applied, greater stem curvature, greater growth inhibition, and greater movement of the labeled tracers occurred than occurred if the potassium was applied 24 hours later. They, therefore, concluded that translocation of 2,4-D is

markedly affected by the level of potassium in tomato plants.

Effect of Temperature on Translocation

Some of the effects of temperature on penetration and translocation are exerted through its effects on: physico-chemical processes--increased rate of diffusion, low viscosity, etc.; and physical factors--acceleration of photosynthesis, phloem translocation, protoplasmic streaming, and growth (8).

In general, optimum growth temperatures promoted penetration but a too high temperature together with low humidity was detrimental to absorption, due to increased evaporation of spray droplets (8).

Pallas (25) reported that when temperatures were increased from 20 to 30°C, absorption and translocation of 2,4-D acid and benzoic acid was increased.

As temperature was increased from 60 to 80°F there was a slight increase of uptake and translocation of foliar-applied atrazine in quackgrass (39).

When labeled simazine was fed to the roots of oat and cotton plants, absorption and translocation increased with increased temperature, relative humidity remaining constant. With the temperature constant, absorption and translocation increased with decreased relative humidity levels (36).

Prasad et al. (27) reported in their studies that greater uptake and translocation of foliar-applied dalapon occurred in beans at 43± 1°C than at 26± 1°C post treatment temperatures with constant relative humidity.

On the other hand, Morton (24) working with Mesquite seedlings found that no significant differences were found in the amount of 2,4,5-T absorbed and distributed at 70 and 80°F, but an increase occurred at 100°F after 72 hours.

Translocation was primarily basipetal from the point of application at 70°F, both acropetal and basipetal at 85°F, and a short distance acropetal at 100°F. He also showed that the quantities of 2,4,5-T translocated into untreated tissues at 100°F were less than at 70 and 85°F.

In many of these studies, penetration, absorption and translocation were not studied separately; therefore, the results reported for translocation may have been dominated by the penetration and/or absorption phases of the study.

Effect of Humidity on Translocation

The relative humidity of the environment seems to have an indirect effect on translocation in plants as indicated by the diverse findings of several investigators.

Recently Morton (24) studying the influence of humidity and temperature on foliar absorption, translocation, and metabolism, of 2,4,5-T by Mesquite seedlings reported that no increased absorption was found at the higher relative humidities. Although absorption was higher after 96 hours than after 24 hours, there was no change in the relative rates of absorption or translocation patterns at different humidity levels.

He also reported that there was better distribution of C¹⁴ in the Mesquite seedlings at humidity levels from 35 to 60% than at 75 to 100%.

Greater uptake and translocation of root applied atrazine was reported in quackgrass under low relative humidity than under higher relative humidity (39).

The above reports are in disagreement with Clor et al. (3) who used cotton and oats and Pallas (25) who used beans. Absorption and translocation of foliar applied radioactive 2,4-D and urea was greater under high

humidity (about 100%) than under normal greenhouse conditions.

Clor et al. (3) also reported that the radioactive compounds moved out of the treated organs and ascended acropetally throughout the shoot mainly in the xylem, more at high humidity than under low humidity levels.

Recently Prasad et al. (27) reported that greater amounts of 2,4-dichloropropionic acid (dalapon)- $2-C^{14}$ and C^{36} were absorbed and translocated at high (88 \pm 3%) than medium (60 \pm 5%) or low (28 \pm 3%) post treatment relative in barley, beans, etc. They reported also that high relative humidity favored stomatal opening, and that uptake of dalapon was greater through the abaxial than adaxial surface of leaf disks of zebrina coleus and nasturium under the same relative humidity.

Pallas (25) reported that the increased absorption and translocation at a higher humidity was correlated with the degree of stomatal opening.

Effect of Light on Penetration and Translocation

Currier and Dybing (8) suggested in their review of foliar penetration and translocation of herbicides that light might promote penetration directly by stimulating opening of stomata, indirectly by supporting photosynthesis and this makes more herbicide available for translocation.

Rice (29) reported in his studies using red kidney bean plants that there was no significant difference between the amount of the salt absorbed at 100 and 900 foot-candles of light when held at the same air temperature. The amount absorbed in the dark was significantly greater than the amount absorbed in the light.

Mitchell and Brown (23) found that 2,4-D was not translocated from leaves of young bean plants that had been depleted of carbohydrates by storage in the dark.

Recently Sargent and Blackman (33,34) working with phaseolus sp. found that varying the light level had little effect upon the amount of 2,4-D penetrating during light hours until the intensity exceeds 800 f.c. Above 1,000 f.c. there was a dramatic increase in penetration rate. They reported that under light conditions (800 f.c. - 1,200 f.c.), the amount of penetration of 2,4-D was markedly increased over dark conditions.

Effects of Surfactants on Translocation

Surfactants have not been shown to influence translocation of herbicides but much evidence (12,18,19,29) indicates that these compounds greatly increase the rate of penetration of herbicides and thus indirectly effect translocation.

Currier and Dybing (8) have suggested that the response to surfactants may be due to one or more of the following:

- (1) improving coverage of applied areas;
- (2) removing air films between spray and leaf surface;
- (3) inducing stomatal entry;
- (4) acting as co-solvents, etc.

Sargent and Blackman (33) studied foliar penetration using phaseolus sp. They reported that the addition of Tween-20 enhanced penetration into both leaf surfaces in beans; as the concentration was raised to about .05% the amount of 2,4-D entering correspondingly increased.

Differences in Translocation Among Plant Species

Leonard and Crafts (20) studying the uptake and distribution of C¹⁴-labeled 2,4-D in seven woody plants reported that in Coyote brush (Baccharis pilularis) 2,4-D-C¹⁴ was translocated slightly in February, intensively in April, less in May and June, and not at all in July. In February and March

its movement was downward from treated leaves, in April almost entirely upward. In Arroyo willow (Salix lasiolepis) little translocation of 2,4-D-C¹⁴ occurred before April 15; from late April until late summer, translocation or tracer was continuous. Translocation both upward and downward from treated leaves occurred from late April until September. Upward movement was predominant in April; in October all movement was downward.

In Manzanita (Arctostaphylos manzanita) 2,4-D-C¹⁴ absorbed and translocated, mostly upward in March, downward in May. In Toyon (Photinia arbutifolia) uptake was prominent from February through October; transport was primarily downward in February and March, upward from May through July, downward in Autumn.

They finally concluded that the pattern of translocation is different among different plant species, that different species require different treatments, and that a single application cannot be expected to control mixed brush population under certain conditions.

Gallup and Gustafson (13) studying translocation and absorption of 2,4-dichloro-5-iodophenoxyacetic acid containing radioactive iodine in oats, wheat, corn, beans, sunflower, dandelion, and plantain reported that the monocots absorb and translocate the indicators more slowly than dicots.

Fang and Butts (10) studying labeled 2,4-D in corn and wheat plants, also found absorption and translocation by these monocots slower than in beans, a dicot.

Variable Response of Plant Varieties to Herbicides

Hodgson (15,16,17) collected Canada thistle from 10 different locations in Idaho, Washington, Wyoming, and Montana. He described certain morphological differences exhibited by the 10 different ecotypes when grown in field

plots in Bozeman, Montana. He reported differences among the ecotypes in response to two herbicides.

Generally, the ecotypes were more susceptible to 2,4-D at the bud than at the bloom stage. G1 ecotype was designed as resistant compared to FI which was intermediate and YM which was susceptible to 2,4-D.

The response of these plants when grown in the greenhouse in California (11) was as follows: YM was most susceptible to 2,4-D, amitrol and picloram, whereas FI and G1 were most resistant to these three herbicides.

G1 was most resistant to dicamba, whereas FI and YM were intermediate and susceptible respectively.

Yellow foxtail plants have been (32) found to vary widely in growth habit, morphological characteristics and dalapon susceptibility. Santelmann and Meade (32) concluded that these variations might be at least a partial cause for failures in the use of herbicides for foxtail control.

Hamilton and Tucker (14) studied the response of selected and random plantings of Johnson grass to dalapon. They reported that strains of Johnson grass differed in their response to dalapon.

Sexsmith (35) reported that strains of hoary cress, particularly within the lens-podded species (Cardaria chalepensis (L.) Hand. & Maz.), varied greatly in leaf size and shape, spreading ability, stand density, and reaction to treatment with several herbicides. Without competition, heart-podded hoary cress (C. draba (L.) Desv.) spread farther and produced a denser stand than either lens-podded or globe-podded cress (C. pubescens (K. A. Meyer) and in general, was more susceptible to 2,4-D and 2,3,5-TBA than lens-podded cress. He also showed that within 12 strains of lens-podded hoary cress no relationship was found between susceptibility to 2,4-D or

2,3,6-TBA and any other characteristic.

Whitworth (40) reported that marked difference in the response of field bindweed (Convolvulus arvensis L.) plants to 2,4-D were found among plants grown from seed obtained from 19 states in the U. S., and one province in Canada. He concluded that these differences were not correlated with the origin nor with plant vigor, but that inherent physiological differences seemed to be responsible.

Metabolism of 2,4-D

There is much evidence that the 2,4-D molecule is metabolized within plant tissues (9,10,20,24).

Luckwill and Lloyd-Jones (22) studying selectivity of 2,4-D in red and black currants and varieties of apples and strawberries found that differences in selectivity are strongly correlated with differences in the ability of the tissues to oxidize the carboxyl and methylene carbons from the side chain of this growth regulator. Red currants oxidized up to 50% of the carboxyl carbon, and 20% of the methylene carbon of 2,4-D supplied to the leaves for seven days through cut petioles.

The susceptible black currant oxidized only 2% of the 2,4-D under comparable conditions. They concluded that oxidation destruction of 2,4-D in certain tolerant plants may partially explain selectivity.

Shaw et al. (38) stated that metabolism of 2,4-D includes (a) minor alteration in structure of the parent molecule, (b) partial degradation with loss of the acetic acid side chain, and (c) conjugation of 2,4-D or its metabolites with plant substrates.

Slife et al. (37) studying metabolism with cucumber plants, indicated

that considerably more C^{14} -labeled 2,4-D than C^{14} -labeled 2,4,5-T was absorbed by this species, but 75% of the absorbed 2,4-D- C^{14} was converted into two major metabolites within 24 hours. Evolution of $C^{14}O_2$ was approximately ten times greater from C^{14} -labeled 2,4-D treated plants than from 2,4,5-T- C^{14} treated plants. They concluded that the greater phytotoxicity of 2,4,5-T on these species seems to relate to the inability of the plants to detoxify this compound as rapidly as 2,4-D.

Materials and Methods

A. Plants and Development

Translocation of C¹⁴ labeled 2,4-D was studied in three Canada thistle ecotypes that had previously been classified by Hodgson (16) in field studies as susceptible, intermediate and resistant to 2,4-D. The ecotypes were identified by the county from which they were collected as YM (Yellowstone, Montana), G1 (Gallatin, Montana) and FI (Fremont, Idaho). G1 was most resistant and YM was most susceptible to 2,4-D in his studies. FI gave an intermediate response to 2,4-D.

Two-inch root sections with two or three buds on each were planted at the center of 4-inch square pots containing a prepared greenhouse soil mixture of fine sandy loam soil and peatmoss. Several pots of each ecotype were planted and placed in a shallow metal pan 6 feet by 8 feet. They were sub-irrigated with water containing 5 percent of N. P. K.; 20-20-20.

New young plants emerged about 5 to 10 days later depending on the condition of buds in the roots. All but one shoot was removed so that only one plant developed for treatment. After the plants reached the 19 to 20 leaf stage, uniform plants of each ecotype were selected and three of each ecotype were placed in each of two growth chambers, three plants of each ecotype were left in the greenhouse; thus three sets of growing conditions were established.

B. Growth Conditions

The following growing conditions were maintained as closely as possible:

Growing Condition	Light Intensity Light for 16 hours	Relative Humidity	Temperature	
			Light	Dark
Growth Chamber 1 (low humidity)	about 2000 f.c.	15 \pm 5 %	74 \pm 3°F	72 \pm 3°F
Greenhouse (intermediate)	variable and unknown	35 \pm 5 %	75 \pm 6°F	68 \pm 4°F
Growth Chamber 2 (high humidity)	about 2000 f.c.	65 \pm 5 %	75 \pm 4°F	71 \pm 4°F

Low humidity levels were obtained with a Dryomatic Model HRD 100 dehumidifier controlled by a Portlow wet-dry bulb programmer. At these low humidities, one-fourth inch glass beads were used to cover the surface of the soil, thereby reducing evaporation and frequency of watering. High humidity levels were obtained with a Walton mist humidifier controlled by a hair humiditat.

Accurate humidity control was not obtained during the experimental period, however, different levels were obtained. Temperature control in the greenhouse was not accurate and considerable variation occurred. While the experiment was conducted during the winter months, natural light was supplemented with incandescent light to give 16 hours of daylight in the greenhouse.

C. Treatment and Assay Method

Two 4-dichlorophenoxyacetic acid-1-C¹⁴ formulated as an oil-soluble amine was used in this study. The specific radioactivity was 1mc/mM.

The selected plants of each ecotype, YM, FI and G1 were placed under each growth condition for 21 days prior to treatment. Each plant was treated with a 10 ul aliquot of C¹⁴-labeled material containing .125 uc radioactivity. The treatment solution was applied to the upper surface of the leaf and was held in place by a lanolin ring. The preliminary tests were applied to the eighth leaf from the top and were placed to cover the midrib near the center of the leaf. Later treatments were made to the sixteenth leaf from the bottom and were placed adjacent to the midrib. A series of plants to be sprayed with 2,4-D at a rate of two pounds per acre were started and conditioned exactly the same as those treated with 2,4-D-C¹⁴.

Plants treated with C¹⁴-labeled material were harvested 96 hours after treatment. The leaf area treated was cut out using a cork borer and the plants were then washed free of all soil and divided into three sections by cutting--the top section cut just below the node of the treated leaf, the center section cut at the ground level, and the root section. After harvesting, the entire plant was immediately positioned for drying, according to S. Yamaguchi's air open drying method (41).

After drying, they were positioned on 8 x 10 inch mounting paper and placed in contact with Kodak Royal Blue X-ray film in film holders. After the films were exposed in the film box for 28 days, they were developed with a Kodak developer and fixer.

The degree of translocation was visually evaluated according to the intensity of exposure of the film.

The plants sprayed with 2,4-D and check plants were harvested 21 days after treatment. The entire plants were washed and then dried in a forced air dryer which maintained 80°C for 48 hours, and then weighed.

The comparisons were made by expressing the treated plant weights as a percentage of the untreated ones.

The experiment was conducted as a randomized complete block design with six replications. The data were statistically analyzed by an analysis of variance method and Duncan's multiple range test was used to establish significant differences between means.

D. Treatment of 2,4-D-C¹⁴ on Canada Thistle Ecotypes Growing under Field Conditions

This study was carried out on field plantings using uniform, healthy and well established plants of each ecotype of Canada thistle. The treatment was applied to the eighth leaf from the top of the plant using the method previously described. Plants were treated May 24, June 8, and July 1, 1966. The plants were approximately 2.5, 5.0 and 14.5 inches tall on the respective dates.

The plants were harvested by digging to a depth of about one foot and then washed, dried and mounted as already described.

RESULTS AND DISCUSSION

A. Translocation of 2,4-D-C¹⁴ in Three Canada Thistle Ecotypes

Preliminary tests were carried out in which 2,4-D-C¹⁴ was applied to plants growing under partly controlled conditions. Generally the eighth leaf from the top was treated. Results were variable and inconsistent; however, they indicated that significantly more 2,4-D-C¹⁴ moved throughout the FI ecotype than YM ecotype (Table I). Growing conditions effected translocation more than ecotype did. There was more 2,4-D-C¹⁴ absorbed and translocated in plants grown in the greenhouse than in those grown in either of the growth chambers (Table I). This was likely due to somewhat more vigorous plants because those grown in the growth chambers developed a slight chlorosis on the lower leaves.

In the major experiment although there was no difference in translocation of 2,4-D-C¹⁴ between different humidity levels within each ecotype, there was a highly significant difference in translocation of 2,4-D-C¹⁴ between ecotypes of Canada thistle (Table II, Fig. 1).

Ecotype FI was judged to have translocated more 2,4-D-C¹⁴ than G1 in all locations but no significant difference of translocation of 2,4-D-C¹⁴ was found between YM and G1 ecotypes except at the high humidity level (Table II, Figures 1 and 2).

Since G1 ecotype indicated the least translocation of 2,4-D-C¹⁴ this supports Hodgson's study (19) that G1 ecotype was most resistant to 2,4-D compared to FI and YM ecotypes under field conditions. On the other hand, since more translocation of 2,4-D-C¹⁴ was found in FI ecotype than in YM or G1 ecotype, this study contradicted Hodgson's results where FI ecotype

TABLE I. A visual evaluation* of radioautographs from the entire plant of three Canada thistle ecotypes treated with 2,4-D-C¹⁴.

Ecotype	Location			Average
	Greenhouse (unknown % R.H.)	Chamber 2 (35±5% R.H.)	Chamber 1 (65±5% R.H.)	
FI	15.4	9.2	7.5	10.70 a
G1	12.4	8.0	5.9	8.65 ab
YM	7.8	6.3	4.6	6.23 b
Average	11.86 a	7.83 b	6.00 b	25.89

^a Means followed by the same letter are not significantly different at 5% level. Duncan's range over the maximum number of means = 3.86.

* Visual rating of 0-10 was used with 0 representing no image and each plant assessed in 3 areas with the 3 ratings totaled.

(This experiment was conducted as a preliminary test, and under same light intensity that we carried out a final experiment).

TABLE II. A visual evaluation* of radioautographs from the entire plant of three Canada thistle ecotypes treated with 2,4-D-C¹⁴.

Ecotype	Location			Average
	Chamber 1 (15+5% R.H.)	Greenhouse (35+5% R.H.)	Chamber 2 (65+5% R.H.)	
FI	9.86 a	10.25 a	10.43 a	10.18 a
YM	7.21 b	7.96 b	8.90 a	8.02 b
G1	6.16 b	6.90 b	7.05 b	6.70 b
Average	7.74	8.36	8.79	

Same letters--Not significantly different at 5% level for comparing columns.
Underscores --Not significantly different at 5% level for comparing rows.

*--Visual rating of 0-10 was used with 0 representing no image and each plant assessed in 3 areas with the 3 ratings totaled.

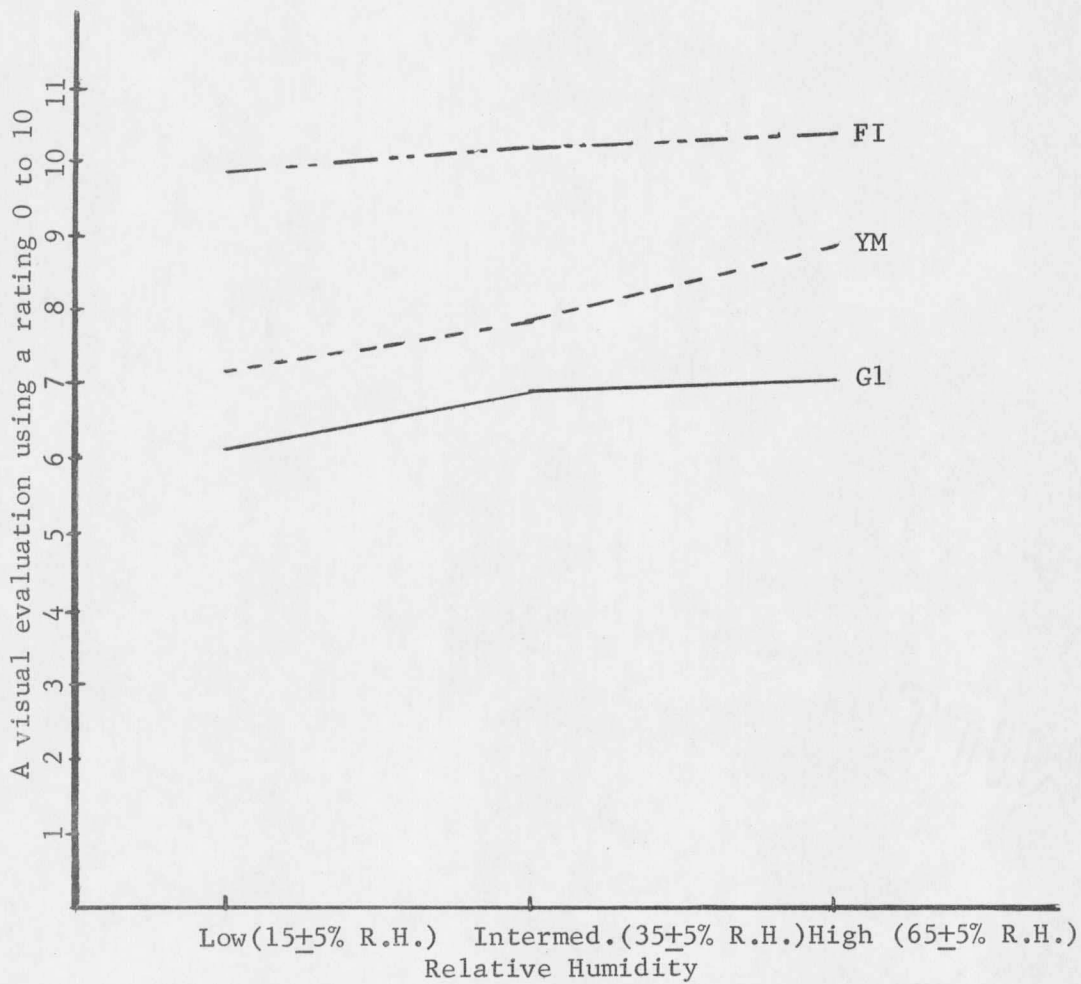


Fig.1. A visual evaluation of radioautographs from all parts of three Canada thistle ecotypes treated with 2,4-D-C¹⁴ under three different conditions.

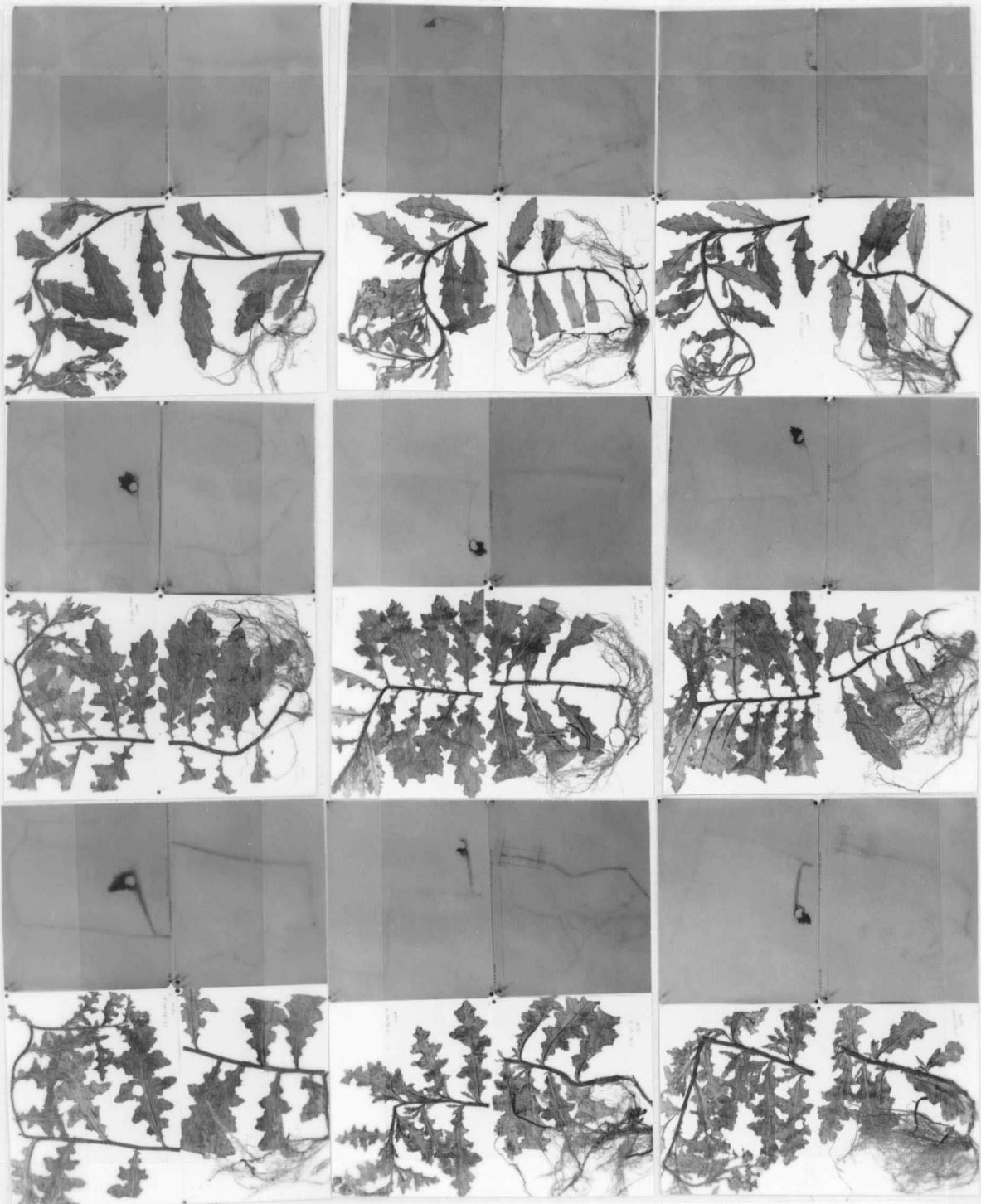


Fig. 2.. Radioautographs of three Canada thistle ecotypes treated with 2,4-D-C¹⁴ under three different conditions (from bottom to top, FI, YM, and G1 ecotypes; from left to right, greenhouse, chamber 1 and chamber 2).

was classified as intermediate in response to 2,4-D and YM ecotype susceptible. Less translocation of C^{14} may indicate less translocation of 2,4-D materials although some plants species metabolize 2,4-D to non-phytotoxic materials (24, 39, 40).

B. Translocation of 2,4-D- C^{14} in Three Canada Thistle Ecotypes in the Field

Although no significant difference among ecotypes occurred on a given date there was a highly significant difference in translocation of 2,4-D- C^{14} among treatment dates (Table III, Figures 3, 4, and 5).

More translocation of 2,4-D- C^{14} occurred from the June 18 treatment than from either of the other dates in all three ecotypes (Table III, Figures 3, 4, and 5). This result supports findings of other investigators (4, 5, 6, 7, 9, 20, 23) that stage of growth is very important in controlling certain weed species. FI ecotype indicated a tend to greater translocation of 2,4-D- C^{14} , even though the difference was not found to be significant (Table III).

Excellent radioautographs were obtained in this study through all treatment dates (Figures 3, 4, and 5). It was disappointing that none of the radioautographs made from plants growing under controlled environmental conditions were as good as those made from field grown plants; however, it might illustrate that excellent penetration and translocation of 2,4-D- C^{14} is possible in all ecotypes under ~~gcertaining~~ conditions.

TABLE III. Visual evaluation* of radioautographs from three Canada thistle ecotypes treated with 2,4-D-C¹⁴ at three dates in the field.

Ecotype	Treatment Time			Average
	5/24/66	6/18/66	7/1/66	
FI	18.0	26.8	16.5	20.43 a
YM	11.7	24.0	15.5	17.23 a
GI	10.5	26.0	13.1	16.53 a
Average	13.40 a	25.76 b	15.03 a	

Same letter--Not significantly different at 5% level.

^a Means followed by the same letter are not significantly different at 5% level. Duncan's range over the maximum number of means = 4.51.



Fig. 3. Radioautographs of FI ecotype treated with 2,4-D-C¹⁴ at three different dates in the field (from left to right, 5/24, 6/18, 7/1 treatment).



Fig. 4.. Radioautographs of YM ecotype treated with 2,4-D-C¹⁴ at three different dates in the field (from left to right, 5/24, 6/18, and 7/1 treatment).



Fig. 5. Radioautographs of G1 ecotype treated with 2,4-D-C¹⁴ at three different dates in the field (from left to right, 5/24, 6/18, and 7/1 treatment).

C. Effects of Humidity on Translocation of 2,4-D-C¹⁴ in Three Canada Thistle Ecotypes

The results indicate that the effects of different humidity levels on translocation of 2,4-D-C¹⁴ in three Canada thistle ecotypes were not significant (Table II). However, in Figure 1, a trend toward greater distribution of C¹⁴ in each ecotype under the highest humidity level was indicated.

Investigators (3, 24, 25, 27, 39) have reported that humidity effected translocation of C¹⁴ radioactive materials in other species. In our studies, no greater distribution of C¹⁴ was found under high humidity levels (65±5%) than medium (35±5%) and low (15±5%) humidity conditions. It is possible that effects of humidity on translocation of C¹⁴ was masked by other environmental factors which we had not considered. Also, it may indicate that the high humidity levels we maintained in this study were not high enough for promoting translocation of 2,4-D-C¹⁴.

In our studies, injury to the leaves at the spot treated with 2,4-D-C¹⁴ was found during the experimental period. While no exact measurements were made, it is believed that the injury was greatest on plants of YM ecotype. Injury was quite inconsistent throughout the experimental period. More injury was found under low humidity conditions than greenhouse and high humidity conditions. Injury was also observed on field grown plants, but it was much less extensive.

The injury occurred from one to three days following the application of 2,4-D-C¹⁴. The injury usually was confined to the lanolin ring, but in some areas extended beyond the ring towards the tip of the leaf.

It is possible that amounts of 2,4-D containing C¹⁴ radioactivity might be injurious to leaves. Still, we cannot explain why these injuries were not consistent throughout the experiment.

D. Response to 2,4-D in Three Canada Thistle Ecotypes

In preliminary tests, when the three ecotypes of Canada thistle were sprayed with 2,4-D at a rate of two pounds per acre, top growth of YM ecotype was most easily killed. Regrowth occurred first from this ecotype, but it cannot be assumed that root kills will occur because top growth is most susceptible.

A comparison of the three ecotypes under three different growing conditions revealed no significant difference in response to 2,4-D due to ecotypes, and a slight difference caused by growing conditions. A significant interaction between ecotypes and locations occurred in this study (Table IV, Fig. 6). G1 ecotype was most susceptible to 2,4-D under greenhouse conditions, but was resistant to 2,4-D under low and high humidity conditions in the growth chambers. Difference in light quality and greater temperature variability might account for these results.

The FI ecotype had a higher percent of the untreated plants than either of the other ecotypes, although the difference was not statistically significant as shown in Table IV. This suggests that FI ecotype should be most tolerant to 2,4-D. While such a conclusion partly substantiates Hodgson's (classifying FI ecotype as intermediate in response) results (15, 17) it is not in agreement with the greater translocation of C¹⁴ that occurred in the FI ecotype.

We can conclude from the data presented in Table IV that the three ecotypes of Canada thistle were more susceptible to 2,4-D under greenhouse conditions than under the other two conditions described (Table IV, Fig. 6).

We can assume that physio-chemical reactions to plants to fixed environmental conditions may be quite different from their response to natural environmental conditions. We realize that it is difficult to apply the results of studies which are conducted under artificially controlled conditions to natural environmental conditions.

The results of these studies indicate that certain humidity levels might influence the response of ecotypes of Canada thistle to 2,4-D and that G1 ecotype might be more sensitive than ecotypes FI and YM under certain humidity levels, but the data is not conclusive.

TABLE IV. Response* of three Canada thistle ecotypes of 2,4-D when growing under different conditions.

Ecotype	Location			Average
	Chamber 1 (15+5% R.H.)	Greenhouse (35+5% R.H.)	Chamber 2 (65+5% R.H.)	
FI	62.69 a	51.36 a	58.06 a	57.37 a
YM	58.21 a	45.16 a	54.09 a	52.48 a
GI	61.05 a	37.75 a	66.77 a	55.19 a
Average	60.65	44.75	59.64	

Same letters--Not significantly different at 5% level for comparing columns.
Underscores --Not significantly different at 5% level for comparing rows.
* Means of yield as percentage of untreated Canada thistle plants. . Duncan's multiple range test used for ecotypes within a location and locations within an ecotype.

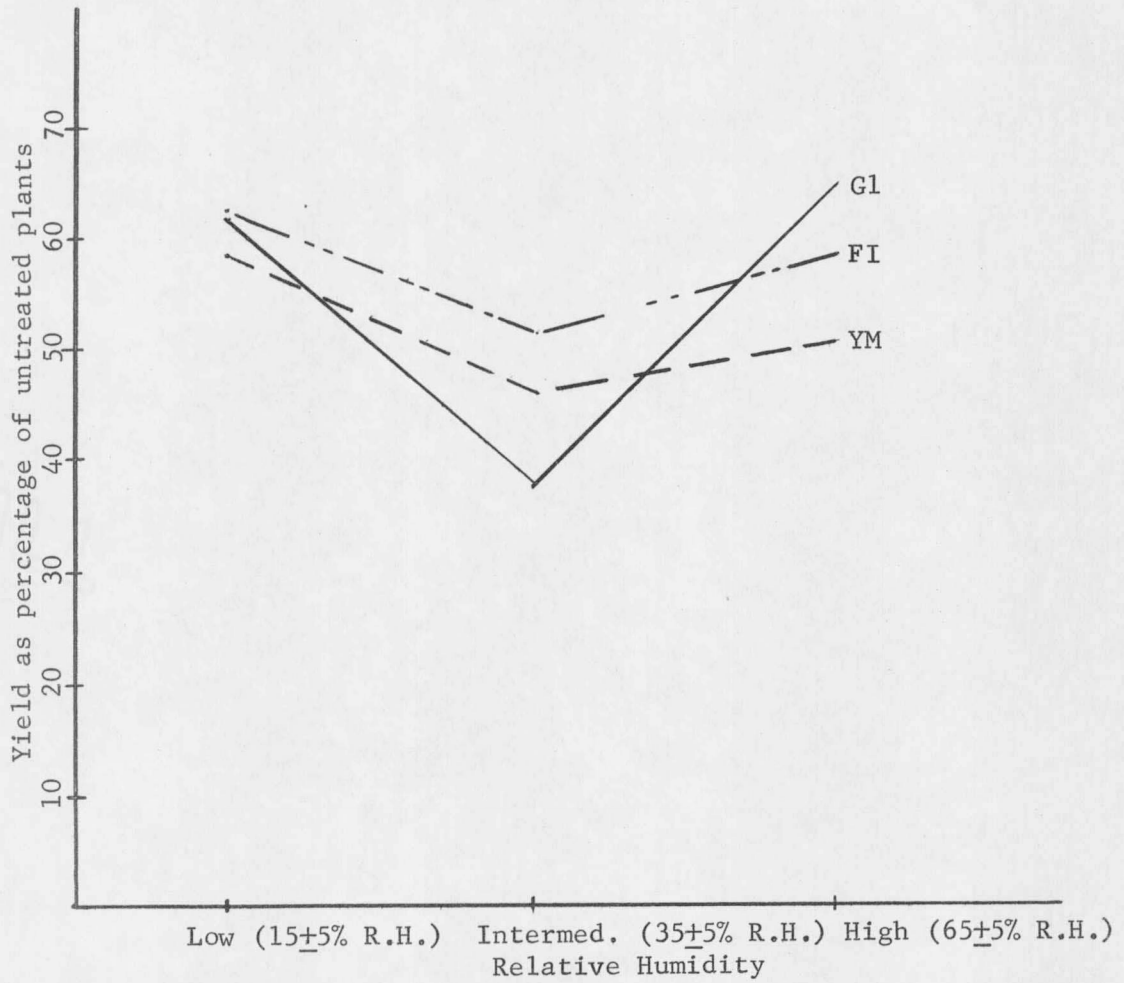


Fig.6. Response of three Canada thistle ecotypes to 2,4-D when growing under three different conditions.

SUMMARY

1. Canada thistle ecotypes previously classified as resistant, intermediate and susceptible to 2,4-D (FI, YM and G1) were used in this study.

2. They were propagated from rhizome sections about 2 inches long, grown in the greenhouse and then uniform plants selected which were subjected to three different growing conditions.

3. After a 21 day period of adaptation to their growing conditions the plants were treated with either 2,4-D-C¹⁴ or a two-pound per acre rate of 2,4-D amine. After 96 hours plants were prepared and radioautographs were made from the C¹⁴ treated plants.

4. Various humidity levels did not affect translocation of 2,4-D-C¹⁴ in each ecotype, but more translocation occurred in the FI ecotype than YM or G1 ecotype under greenhouse and low humidity conditions. Significantly more translocations of 2,4-D-C¹⁴ was found in FI ecotype than in G1 ecotype at all three growing conditions.

5. More translocation of 2,4-D-C¹⁴ occurred in plants treated June 12 than either younger or older plants under field conditions.

6. No significant difference in response to 2,4-D sprays was found among the three ecotypes. However, difference caused by growing conditions and a significant interaction between ecotypes and growing conditions occurred.

7. G1 ecotype was more susceptible to 2,4-D under greenhouse conditions than under low and high humidity conditions in growth chambers.

8. Generally all three ecotypes of Canada thistle were more susceptible to 2,4-D under greenhouse conditions than two other conditions.

APPENDIX

APPENDIX TABLE I. Analysis of variance for translocation* of 2,4-D-C¹⁴ in three Canada thistle ecotypes including top, stem and roots.

Source of Variance	Degree of Freedom	Sum of Square	Mean of Square	F.
Replication	4	210.10	52.70	5.685
Location	2	266.68	133.34	14.384 **
Ecotypes	2	149.43	74.71	5.933 **
Location x Ecotypes	4	39.49	9.87	1.060 NS
Error	32	317.69	9.27	
Total	45	984.10		

NS - Not significantly different at 5% level.

** - Significantly different at 1% level.

* - Data collected by a visual evaluation of radioautographs from three Canada thistle ecotypes treated with 2,4-D-C¹⁴ from preliminary test.

APPENDIX TABLE II. Analysis of variance for translocation* of 2,4-D in three Canada thistle ecotypes including top, stem, and roots.

Source of Variance	Degree of Freedom	Sum of Square	Mean of Square	F.
Replication	5	15.06	3.01	1.64 NS
Location	2	9.86	4.93	2.33 NS
Ecotypes within locations	6	113.29	18.88	8.99 **
Greenhouse	2	35.15	17.58	8.79 **
Growth Chamber 1	2	34.15	17.58	8.21 **
Growth Chamber 2	2	43.63	21.82	10.39 **
Error	40	84.34	2.10	
Total	53	222.55		

NS - Not significantly different at 5% level.

** - Significantly different at 1% level.

* - Data collected by a visual evaluation of radioautographs from three Canada thistle ecotypes treated with 2,4-D-C¹⁴.

APPENDIX TABLE III. Analysis of variance for translocation* of 2,4-D-C¹⁴ in field grown three Canada thistle ecotypes.

Source of Variance	Degree of Freedom	Sum of Square	Mean of Square	F.
Time (Rep.)	2	270.80	165.40	43.072 **
Ecotype	2	25.94	12.97	3.377 NS
Error	4	15.36	3.84	
Total	8	312.10		

NS - Not significantly different at 5% level.

** - Significantly different at 1% level.

* - Data collected by a visual evaluation of radioautographs from three Canada thistle ecotypes treated with 2,4-D in the field.

APPENDIX TABLE IV. Analysis of variance for response* of three Canada thistle ecotypes to 2,4-D.

Source of Variance	Degree of Freedom	Sum of Square	Mean of Square	F.
Replication	5	7853.08	1570.61	
Location	2	2846.58	1423.29	34.854 **
Ecotypes	2	211.16	105.58	2.585 NS
Location x Ecotypes	4	912.89	228.22	5.588 **
Error	40	1633.40	40.835	
Total	53	13457.11		

NS - Not significantly different at 5% level.

** - Significantly different at 1% level.

* - Data collected by yield as % of untreated Canada thistle plants.

APPENDIX TABLE V. A visual evaluation* of radioautographs from the entire plant of three Canada thistle ecotypes including top, stem, and roots.

Ecotypes	Locations	Replication						Average
		1	2	3	4	5	6	
FI	Greenhouse	20.0	16.0	17.0	5.0	19.0		15.40
	Chamber 2	11.0	6.5	15.0	3.5	10.0		9.20
	Chamber 1	10.2	6.0	11.0	2.5	8.0		7.50
YM	Greenhouse	12.0	7.0	14.0	14.0	15.0		12.40
	Chamber 2	7.0	11.0	11.0	5.0	6.0		8.00
	Chamber 1	4.5	6.0	12.0	2.0	5.0		5.90
G1	Greenhouse	13.0	10.0	4.0	6.0	6.0		7.80
	Chamber 2	10.0	4.5	9.0	3.5	4.0		6.30
	Chamber 1	9.0	3.0	4.5	4.0	2.0		4.65

Greenhouse--Unknown

Chamber 2 -- 35-50% Relative humidity.

Chamber 1 -- 60-75% Relative humidity.

* Data collected from preliminary test, using a rating of 0 to 10 (with 0 representing no image) on each of three plants parts.

APPENDIX TABLE VI. A visual evaluation* of radioautographs from the entire plant of three Canada thistle ecotypes including top, stem, and roots.

Ecotypes	Location	Replication						Average
		1	2	3	4	5	6	
FI	High	15.0	7.0	9.0	11.0	10.7	9.9	10.43
	Intermed.	9.0	12.0	9.5	12.5	9.3	9.2	10.25
	Low	10.0	9.5	8.0	11.0	11.1	9.6	9.86
YM	High	10.0	8.5	8.5	8.5	9.3	8.6	8.90
	Intermed.	7.0	10.5	6.5	8.0	7.1	8.7	7.96
	Low	7.0	7.0	7.5	7.5	6.9	7.4	7.21
G1	High	7.0	6.5	8.5	6.0	7.0	7.3	7.05
	Intermed.	6.0	6.0	4.7	7.0	9.8	7.4	6.90
	Low	7.0	6.0	3.2	8.0	6.2	6.6	6.16

High -- Chamber 2 (65+5% R.H.)

Intermed. -- Greenhouse (35+5% R.H.)

Low -- Chamber 1 (15+5% R.H.)

* A visual evaluation using a rating of 0 to 10 (with 0 representing no image) on each of three plants parts.

APPENDIX TABLE VII. Dry weight* of three Canada thistle ecotypes growing under three different conditions and treated with 2,4-D.

Ecotypes	% R.H.	Treatment	Replication						Av.
			1	2	3	4	5	6	
FI	High	Treated	3.80	11.63	10.52	15.07	12.90	9.90	58.06
		Untreated	11.87	15.18	20.36	18.67	21.00	20.20	
		Percent.**	32.01	73.61	51.66	80.71	61.40	49.00	
	Inter.	Treated	3.76	3.36	5.95	8.12	7.80	8.60	
		Untreated	9.13	11.56	9.38	11.85	16.00	15.00	
		Percent.	41.18	29.06	63.43	68.52	48.70	57.30	
	Low	Treated	4.22	8.26	6.86	7.62	9.80	9.20	
		Untreated	9.43	10.65	11.23	16.67	12.40	13.50	
		Percent.	44.75	77.55	61.08	45.71	79.00	68.10	
YM	High	Treated	4.10	5.16	7.56	11.47	12.30	12.50	54.09
		Untreated	10.51	11.96	14.09	16.37	22.50	19.50	
		Percent.	39.01	43.14	53.65	70.06	54.60	64.10	
	Inter.	Treated	2.71	5.09	4.81	8.07	6.90	8.70	
		Untreated	9.44	11.26	10.65	13.61	17.40	16.40	
		Percent.	28.70	45.20	45.16	59.29	39.65	53.00	
	Low	Treated	3.98	6.97	4.85	8.40	12.70	8.20	
		Untreated	10.17	11.26	8.41	11.87	14.80	10.10	
		Percent.	39.13	61.90	57.66	53.91	85.50	50.90	
GI	High	Treated	4.80	7.56	7.58	8.97	10.10	12.20	66.77
		Untreated	9.53	12.49	12.98	13.02	14.00	14.30	
		Percent.	50.36	65.21	58.80	88.89	72.10	85.30	
	Inter.	Treated	2.59	3.07	2.76	4.57	5.60	2.60	
		Untreated	7.11	8.44	8.96	10.77	10.00	10.60	
		Percent.	36.42	36.37	30.38	42.43	56.00	24.50	
	Low	Treated	3.22	5.97	6.26	7.69	7.40	7.10	
		Untreated	8.31	13.21	9.77	8.17	13.20	11.20	
		Percent.	38.74	45.19	68.96	94.12	56.00	63.30	

High -- Chamber 2 (65+5% R.H.)

Inter. -- Greenhouse (35+5% R.H.)

Low -- Chamber 1 (15+5% R.H.)

* --Dry weight in grams of entire plant.

** --Percentage of untreated.

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