



Differences in lipids on Canada thistle leaves as influenced by ecotype and site
by Jesse Moroni Hodgson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Crop and Soil Science

Montana State University

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Abstract:

Canada thistles (*Cirsium arvense* (L.) Scop.) have responded quite erratically to control treatments with 2,4-D and other herbicides.

The variability in amount of lipid present on leaves was investigated as a possible factor in causing differential response to 2,4-D. Plants representing ecotypes from various locations in Idaho, Montana, Washington and Wyoming that had been brought to Bozeman for earlier morphological and herbicidal studies were asexually propagated for this study. Field plots were established at three different geographic sites and lipids extracted from leaf samples of the actively growing leaves.

Significant differences in extractable lipids due to site, eco-type and date of sampling were found. Thistles from the site with the greatest wind movement and greatest evaporation produced the most lipid independent of temperature. The relative response of ecotypes was similar at the different locations. Lipid extracts ranged from 25.87 $\mu\text{g}/\text{cm}^2$ to 20.18 $\mu\text{g}/\text{cm}^2$ for the various ecotypes.

Three Canada thistle ecotypes that had the most lipid on their leaves were most resistant to 2,4-D spray treatments, while four ecotypes having the least lipid on their leaves were the most susceptible to 2,4-D. Two other ecotypes were inconsistent in the comparison.

The evidence indicates that the varied deposition of lipid on the leaves of Canada thistle is a factor affecting differential response of Canada thistle plants to 2,4-D spray treatments.

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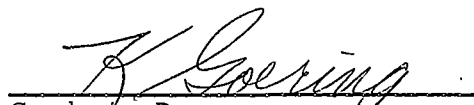
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TABLE OF CONTENTS

	<u>Page</u>
VITA	ii
ACKNOWLEDGMENT	iii
TABLE OF CONTENTS.	iv
LIST OF TABLES	v
LIST OF FIGURES.	vi
ABSTRACT	vii
INTRODUCTION	1
MATERIALS AND METHODS.	9
Culture and Treatment of Canada Thistle Ecotypes.	9
Sites Where Lipid Samples Were Produced	13
Lipid Extraction From Canada Thistle Leaves	14
Specific Leaf Weight Determination.	14
EXPERIMENTAL RESULTS AND DISCUSSION.	16
Response of Canada Thistle Ecotypes to Herbicides	16
Lipid Yield for Ecotypes, Sites, Dates.	20
Lipid Yield and 2,4-D Response.	24
Specific Leaf Weight.	27
SUMMARY.	32
LITERATURE CITED	35
APPENDIX	40

LIST OF TABLES

	<u>Page</u>
Table I. Site characteristics where the Canada thistle plants were grown	13
Table II. Response of Canada thistle ecotypes to three herbicide treatments, Bozeman, Montana.	17
Table III. Survival of Canada thistle from three chemical treatments for 4 years.	19
Table IV. The effect of ecotype and site on lipids on leaves of Canada thistle.	21
Table V. The effect of date of sampling on lipids on leaves of Canada thistle	23
Table VI. Comparison of leaf surface lipid and response of Canada thistle to 2,4-D foliage treatments.	25
Table VII. Heat accumulation at three sites where Canada thistle plants were grown and lipid yields.	28
Table VIII. The effect of ecotype and site on specific leaf weight of Canada thistle.	29
Table IX. Specific leaf weight of Canada thistle leaves as influenced by ecotype, harvest date, and site	31

APPENDIX TABLES

Table X. Sample yields of lipid from Canada thistle leaves from three sites in 1969.	41
Table XI. The effect of date of sampling on the yield of leaf lipids of Canada thistle	43
Table XII. Specific leaf weight of Canada thistle leaves as influenced by ecotype and site.	45
Table XIII. Specific leaf weights of Canada thistle leaves as effected by ecotype, date, and site	47

LIST OF FIGURES

	<u>Page</u>
Figure 1. Typical leaves of Canada thistle ecotypes	10

ABSTRACT

Canada thistles (Cirsium arvense (L.) Scop.) have responded quite erratically to control treatments with 2,4-D and other herbicides. The variability in amount of lipid present on leaves was investigated as a possible factor in causing differential response to 2,4-D. Plants representing ecotypes from various locations in Idaho, Montana, Washington and Wyoming that had been brought to Bozeman for earlier morphological and herbicidal studies were asexually propagated for this study. Field plots were established at three different geographic sites and lipids extracted from leaf samples of the actively growing leaves.

Significant differences in extractable lipids due to site, ecotype and date of sampling were found. Thistles from the site with the greatest wind movement and greatest evaporation produced the most lipid independent of temperature. The relative response of ecotypes was similar at the different locations. Lipid extracts ranged from 25.87 $\mu\text{g}/\text{cm}^2$ to 20.18 $\mu\text{g}/\text{cm}^2$ for the various ecotypes.

Three Canada thistle ecotypes that had the most lipid on their leaves were most resistant to 2,4-D spray treatments, while four ecotypes having the least lipid on their leaves were the most susceptible to 2,4-D. Two other ecotypes were inconsistent in the comparison.

The evidence indicates that the varied deposition of lipid on the leaves of Canada thistle is a factor affecting differential response of Canada thistle plants to 2,4-D spray treatments.

INTRODUCTION

Canada thistle (Cirsium arvense (L.) Scop.) is one of the most important weeds of the world (9) ^{1/}. It has a broad base of plant variability which enables it to adapt to a wide range of habitats (20). The development of control methods for Canada thistle began in America before 1796 (39). Stevens stated "Knowledge has been abundantly furnished in reference to them, their natural history, their injury to crops and the means of their destruction. There has not been, however, any systematic or general attempt to eradicate or even limit them; and it is to be feared that they will never be limited much less eradicated. All that can be done is to show that the thistle can be extirpated and this will secure good farmers from the pest, and enable the negligent ones to destroy them whenever and wherever it may be necessary. But, this will leave a host of shiftless ones whose inaction will permanently secure the continued existence of this formidable agricultural enemy."

Mr. Stevens may have understood farmers but probably did not fully appreciate the "thistle's" ability to persist and spread even in spite of the efforts made to control them. Canada thistles have spread widely since Mr. Stevens made the above prediction. Because of their vigor and prevalence control measures have been extensively sought for over 170 years in the United States. Control practices that have been developed and recommended include cultivation practices, cropping pro-

^{1/} Italic numbers in parentheses refer to "Literature Cited", p.

grams, and chemical treatments (19).

Chemical treatments to control Canada thistle involving modern growth regulator chemicals have been erratic and sometimes ineffective (42, 19). This report presents results of studies of the variable response of Canada thistle to the herbicides, 2,4-Dichlorophenoxyacetic acid (2,4-D), and 3-amino-1,2,4-triazole (amitrole) and to cultivation treatments. The variation in amount of leaf surface lipids as influenced by sites and ecotypes and the relationship of amount of lipid to plant response to 2,4-D treatment at one site is also reported.

LITERATURE REVIEW

Canada Thistle, History and Distribution

Cirsium arvense is indigenous to Europe, western Asia, and Northern Africa (9) and is known by a variety of common names throughout the world. The most often used are Canada thistle, creeping thistle, California thistle, field thistle, and cursed thistle (3, 8, 31). Canada thistle may have become the popular name in the United States because of the tradition that Frenchmen introduced it into Canada for feeding swine (9) and that from there it spread to the United States. No evidence to support this tradition was found.

According to Hansen (15), Canada thistle was first introduced to North America as an impurity in crop seeds in two Canadian provinces, Quebec and Ontario. It was likely introduced at various times and places with agricultural seeds brought by early colonizers since Dewey (9) reported it to have been spread throughout the greater part of Europe by the middle of the 18th century. He also reported it to be found in western Asia, northern India, Australia, and New Zealand. He cites reports of 1900 that showed it to be in all states of the United States bordering on, or north of, the 37th parallel.

The rapid spread of Canada thistle has continued since that time. Man has been a very active agent in its dispersal by planting seeds of contaminated forage and small grains (16). By 1952, it was reported to infest more acreage than any other noxious weed in a four state area of Montana, Idaho, Oregon, and Washington (11). Blankenship (5),

in 1901, reported it to be rare in Montana, with only five small patches. Heikes in 1956 reported 625,000 acres infested in Montana (18). By 1901, most of the 31 states that had reported infestations of Canada thistle had declared it to be a noxious weed (5). This did not deter its advance throughout the area of its adaptation as indicated by the increasing acreages. In 1957, it was listed as a noxious weed in the seed laws of all states of the United States except Alaska, Arkansas, Hawaii and New Mexico (43).

Nature of the Problem

Canada thistle propagates vegetatively by roots and is very aggressive in any field because of this characteristic. However, the plant is also a threat to other areas because of its seed production, which is its major means of spreading from farm to farm and from farms and roadsides to ranges and watershed areas (3, 15, 14).

Canada thistle causes serious yield losses in spring-sown small grains, peas, row crops, and pastures and is obnoxious in many other situations. It is well adapted to growing with spring grains and grows with this crop perhaps more often than with any other. The difference in adaptation of Canada thistle to spring wheat and alfalfa hay cropping programs was clearly shown in two experiments reported in 1958 (19) and 1959 (25). The percentage of Canada thistles increased progressively in continuous spring wheat cropping, but decreased in alfalfa mowed for hay. Other investigators have reported

alfalfa, perennial forage grasses and winter wheat to be the most effective competitive crops in controlling Canada thistle (41,48).

A vigorous stand of Canada thistle has the ability to withstand repeated cultivation or destruction of the shoots above the ground for periods up to 18 months (36). The recommended starvation practice by cultivation involves cultivation (cutting off of all shoots) to a depth of three to four inches every 21 days during one and one-half growing seasons (36, 19, 21).

The growth regulator type herbicide 2,4-D appeared in 1944 and was immediately tested to control Canada thistle. Researchers generally classed Canada thistle as intermediate in response to 2,4-D. It required repeated treatments for effective control (29). Bakke (2) reported 70 percent control in one year with a treatment of one to three pounds per acre applied at the early bud stage and a second treatment to control regrowth in September.

In an experiment conducted by the writer at two different locations in 1953 marked differences in response occurred (21). Canada thistle plants at the Newdale, Idaho site were much more resistant to 2,4-D than at the Bozeman, Montana site. The varied response apparently resulted from differences in the environments of the two sites and/or genetic differences of the thistle plants. The studies of Clausen, Keck, and Hiesey (7) indicated that wide variation in several species were controlled by the environment as well as heredity. They

stated that each species is composed of an assemblage of regional races that are heritably distinct ecotypes. An ecotype being a biotype arising as a result of its genotypical response to a particular habitat. The Canada thistle species is widely distributed in diverse habitats which has resulted in development of many ecotypes with different adaptations (20). Diverse morphological variations are also present. One particular group of ecotypes collected from various locations in the Western United States manifested variations in morphological characteristics as well as variable locally adaptive characteristics such as shoot emergence in the spring, shoot growth, time of flowering (20).

Leaf Surfaces and Response to Herbicides

Herbicides of the translocated type must penetrate the leaf epidermal cells and move in vascular tissue of the plant in order to kill the meristematic region of the roots (6). There are many physiological processes in plants involved in the toxicity and effectiveness of herbicides. This review will be limited to some of the aspects of absorption of foliar applied herbicides.

The plant cuticle presents a distinct barrier to absorption of foliar applied herbicides (27, 44). It is a complex continuous covering (not cellular) of fine films inserted between cutin lamellas (14). There are usually some pectic and cellulose substances near the cell surface. Stomata have been proposed as portals of entry of

herbicides through the cuticle (13, 14, 44). However, in a study of the variation in number and area of stomata of different Canada thistle ecotypes by Moore and Hodgson (26) a relationship of stomata numbers to response to 2,4-D was not found although significant differences in epidermal cell stomata ratios were found.

The structure and composition of the cuticle of a number of species have been investigated (27, 34, 35, 32). These studies revealed that the detailed structure such as thickness, amounts of surface and embedded waxes and amounts of pectic substances of the cuticle varied considerably from one species to another. Differences in susceptibility of weed species to 2,4-D (30) and amitrole (17) was attributed to differential cuticle structure and thickness. The permeability of the intact cuticle depends on such factors as cuticle thickness, the nature of the wax deposits, hydration of the cutin and the presence of ectodesmata (33). The amount of wax in the cuticle may be the major factor inhibiting penetration of water and other compounds (35, 37).

Environmental factors are often reported to affect cuticle formation but experiments showing such effects are few. Stevens (40) reported 20 percent variation in cuticle thickness in several varieties of cranberries in different seasons. From extensive studies of effect of age of leaf on cuticle Skoss (37) reported that deposition of cuticle on tobacco leaves was continuous until the leaf reached

morphological maturity and then ceased. Hull (23) found total wax content of leaflets of mesquite increased with increasing temperature. Cuticle weights from English ivy leaves grown in full sunlight were consistently greater than cuticle weights of leaves grown in the shade (37). The most cuticle was produced at a median temperature. At high temperature the greatest percentage of the cuticle was wax. Plants undergoing water stress produced cuticles containing a greater proportion of waxes and a greater cuticle deposit than did plants with more favorable moisture conditions. High relative humidity moisture on the leaf surface, and low moisture tension in the plant, favor more rapid foliar uptake of herbicides (28, 46).

Skoss (37) made separations of cuticle material into cutin and waxes. The waxes were that component of ground cuticles extracted in 95 percent ethanol. He reported cutin percentage varied more within than among different species studied.

As indicated above, environmental conditions under which cuticle development occurred and age of leaf resulted in cuticle variations within species as well as between species. The possibility that varied response of Canada thistle to 2,4-D might be influenced by cuticular variations among ecotypes is clearly implied from the above citations. The objective of this study was to determine the differences of amount of lipid on the surfaces of leaves of some ecotypes of Canada thistle and to study the amount of lipid as it might be related to response to 2,4-D.

MATERIALS AND METHODS

Horizontal root sections of Canada thistle ecotypes were selected from different locations in Montana, Idaho, Washington and Wyoming. Morphological and phenological variations among these ecotypes were studied earlier (20). Variations in leaf characteristics were quite extreme as may be observed in Figure 1. The ecotypes were designated according to locations from which they were obtained as follows: G1, G2, G3, and G4, from Gallatin County, Montana; AI from Ada County, Idaho; FI from Fremont County, Idaho; YM from Yellowstone County, Montana; FM from Fergus County, Montana; LW from Laramie, Wyoming and PW from Prosser, Washington. Type specimen of each of the ecotypes were placed in the Montana State University Herbarium.

One-foot root sections of each ecotype were planted at the center of a one square rod plot. There were four plots of each ecotype in random arrangement in each of four blocks. The plots were established on a Bozeman silt loam soil. This is a highly productive soil that was fallowed for three months before planting and was free of other Canada thistle plants. The plots were irrigated immediately and as needed thereafter to provide good growing conditions. Sodium chlorate was applied in a two-foot band around all square rod plots; however, occasional encroachment occurred. Many of the ecotypes had spread throughout the plots 12 months after planting.

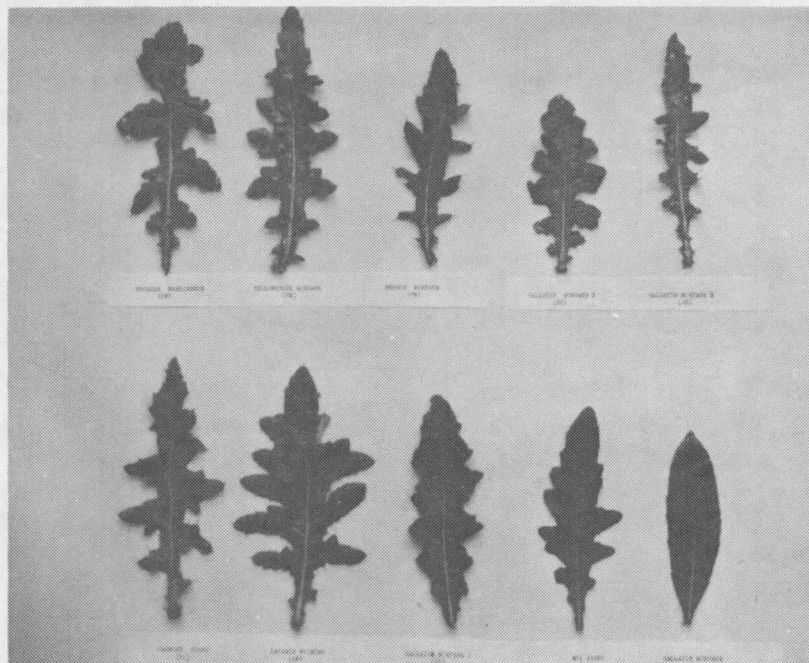


Fig. 1 Typical leaves of 10 Canada thistle ecotypes. Left to right: Top row - PW, YM, FM, G2, G3. Bottom row - FI, LW, G1, AI, G4.

Vigorous heavy stands were present on all plots two and one-half years after planting and the following herbicide treatments were applied on the four replicates of each ecotype: (a) 1.5 lb/A of 2,4-D, as the propylene glycol butyl ether ester at the bud-stage (June 15); (b) 1.5 lb/A of 2,4-D at the early bloom stage (July 10); and (c) 4 lb/A of 3-amino-1,2,4-triazole (amitrole) at the early bloom stage (July 10). The 2,4-D applications were repeated at the same stage of growth in each of the next 3 years with an additional treatment in September in the second and third years. Amitrole was applied once each year for four years also. There was also an untreated series of four replicates of each ecotype.

The population of Canada thistle was sampled each year by counting the shoots present on an area three by six feet at the center of each plot. The number of Canada thistles present in June prior to the first treatment were compared to later stands, to determine the effect of treatments on the various ecotypes. The data were analyzed by analysis of variance and means were compared by using Duncan's(10) multiple range test.

The study of the amount of lipids on Canada thistle leaves and the influence of ecotype and site on amount of lipid produced involved the same ecotypes, except G3. Three sites were selected for growing the plants and included the Bozeman and Huntley Agricultural Experiment Stations and a leased site at Newdale, Idaho. These three sites

were chosen to represent different aspects of climate that might be expected to cause variations in lipid deposition on the leaves. In an earlier experiment at Newdale and Bozeman results indicated that Canada thistles at Newdale were more resistant to 2,4-D than at Bozeman (21). The Newdale site characteristically has lower humidity, greater wind travel and greater evaporation than Bozeman. Both ecotypes (G1 and FI) from the above experiment (21) were included in these studies. The Huntley site represented a characteristically higher temperature site with intermediate humidity and evaporation. More complete descriptions of the sites are given in Table I.

Root sections of the Canada thistle ecotypes were planted at the three sites in 1965. The plantings included five replicates of nine ecotypes in a randomized block at each location. Leaf stomata and other growth characteristics of Canada thistle plants were studied in 1967 and 1968. In 1969, leaf samples were obtained from all replications at all locations for extraction of surface lipids. The samples were obtained at approximately the first bloom stage of the plants at each location. Four of the ecotypes were sampled a second time about four weeks after the first sampling.

Each leaf sample was composed of leaves from seven thistle shoots chosen at random from a single plot. The shoots were wrapped in damp towels, placed in ice chests and moved to the laboratory for further processing. Four sound leaves were selected from each shoot in the

Table I. Site characteristics where the Canada thistle plants were grown.

Site Characteristics		Location of Sites ^{a/}		
		Newdale, Idaho	Huntley	Bozeman
Location	Latitude	43° 53' N	45° 55' N	45° 40' N
	Longitude	111° 45' W	108° 15' W	111° 03' W
Elevation	Feet	4890	2889	4860
Soils PH		7.34	7.44	6.44
	Sand %	33	33	31
	Silt %	55	28	43
	Clay %	12	39	26
	OM	2.5	2.0	3.4
Frost free period	Days	100	127	108
Evaporation (May, June, July)	inches	24.9	22.4	20.3
Wind travel (May, June, July)	miles	8049	4920	6479

^{a/} Weather Stations: 5 miles from plots at Newdale
.5 mile from plots at Huntley
.7 mile from plots at Bozeman

area of the 15th to 22nd node above the crown for a total leaf sample from each plot of 28 leaves. A sub-sample of four leaves was used for specific leaf weight determinations (SLW) while the other 24 leaves were used for lipid extraction. The 24 leaves for lipid extraction were immediately fastened to metal clips by their petioles and suspended from a rocker shaft into quart jars filled with a 75 percent hexane and 25 percent ether solvent that had been distilled just prior to use. Anhydrous diethyl ether was used. Ten leaf samples or 1 complete replication of samples were extracted simultaneously. The action of the rocker kept the leaves moving in the solvent for 30 minutes. The extracted leaves were then removed intact and dried at 60° C for 48 hours and weighed.

Five, one square centimeter sections were punched from each of the four leaves to determine SLW as described by Barnes, et al. (4). The leaf sections were dried and weighed to determine the leaf weight per square centimeter for each plot which was the average of the 20 sections. The total weight of the leaves from which the lipid was extracted was then divided by the SLW of that sample to determine total leaf area extracted.

The lipid extracts were filtered through glass fiber filters and evaporated to near dryness on a vacuum flash evaporator. They were transferred to preweighed vials with chloroform and evaporated to dryness under a nitrogen sweep. The vials were then weighed, re-

evaporated and reweighed until a constant weight was obtained.

The net weights of lipid were then converted to lipid yield in $\mu\text{g}/\text{cm}^2$ of leaf for each sample. An analysis of variance was performed on the data and mean comparisons made by Duncan's multiple range test (10).

The extraction procedure did not assure that only leaf surface compounds were removed. However, most of the solvent samples remained completely colorless indicating little or no contamination with chlorophyll. It was assumed that any internal lipids that may have been extracted were inconsequential.

RESULTS AND DISCUSSION

The chemical response data was published in March, 1970 (22). It is necessary to briefly present it here in order to show the relationships of amount of lipid produced and the herbicide response among the ecotypes. The ecotypes as a group were much more susceptible to 2,4-D when applied at the bud stage than when applied at the bloom stage (Table II). The average control of all ecotypes was 22 percent greater when 2,4-D was applied at the bud stage. Two ecotypes, G2 and G4, showed a reverse trend with greater survival from bud stage treatments than from bloom stage treatments.

Amitrole at 4 lb/A was more effective than 2,4-D in limiting regrowth of these Canada thistle ecotypes except for AI. This ecotype was among the most resistant to all herbicide treatments and it was significantly more resistant to amitrole than any other ecotype.

The ecotypes were quite variable in their response to 2,4-D as influenced by date of treatment. Ecotype FM exhibited the most difference in response to 2,4-D at the two stages of growth ranging from 22 percent survival from bud stage treatments to 79 percent survival from bloom stage treatments. Growth data (20) showed it to be one of the latest to emerge in the spring, however, it attained bloom stage earlier than most other ecotypes. Thus the quantity and quality of lipids in the cuticle of FM could have varied between the two dates of spray application more than other ecotypes.

Table II. Response of Canada thistle ecotypes to three herbicide treatments, Bozeman, Montana.

Ecotype ^{a/}	Canada thistle survival from herbicide treatments ^{b/}				Ecotype Means
	Amitrole	2,4-D 1.5 lb/A		2,4-D	
	4 lb/A	Bud Stage	Bloom Stage	Average	
	Percent	Percent	Percent	Percent	Percent
AI	71 bc	60 b-e	75 b	67 a	69 o
G1	41 e-i	52 c-g	95 a	73 a	63 op
G4	33 g-m	75 b	64 b-d	70 a	57 p
G3	19 k-n	35 g-m	67 b-c	51 b	40 q
LW	20 k-n	39 e-k	58 b-f	49 b	39 q
FM	14 m-n	22 i-n	79 a-b	50 b	38 q
FI	11 n	36 g-l	66 b-c	51 b	38 q
G2	21 i-n	45 d-h	38 f-l	42 b	35 q
PW	16 l-n	30 h-n	53 c-g	42 b	33 q
YM	14 m-n	33 g-m	52 c-g	43 b	33 q
Means	26 x	43 v	65 z		45

^{a/} The ecotypes were designated by letters and numbers according to place of origin as follows: AI, Ada County, Idaho; G1, G2, G3, G4, Gallatin County, Montana; LW, Laramie, Wyo.; FM, Fergus County, Montana; FI, Fremont County, Idaho; PW, Prosser, Washington; YM, Yellowstone County, Montana.

^{b/} Data are averages of 4 reps. for 4 years for each ecotype and treatment. Numbers with no similar letters are significantly different as determined by Duncan's multiple range test at $P=0.05$.

Ecotypes G1 and G4 were consistently among the most resistant to herbicide treatments. They also had more shoots per square foot of soil area than others (20). This may indicate that their resistance was associated with their vigor of growth or their ability to initiate shoots. However, AI was also one of the most resistant to herbicides but was intermediate in number of shoots per square foot of area. Susceptibility or resistance of a particular ecotype to the herbicides may have been related to vigor of plants or adaptation to the environment in some ecotypes, but in others it seems to be related to genetic or physiological factors. Phenotypic variations were frequent among these Canada thistle ecotypes mostly because of different leaf forms or flower color (20). However, their response to herbicides did not appear to be related to any of these observed differences. Whitworth (47) found varied response of strains of field bindweed (Convolvulus arvensis L.) to 2,4-D and concluded that response was not related to vigor as determined by rate of growth.

Response of the ecotypes to herbicides in the treatment x year comparisons (Table III) indicates less survival of Canada thistle each succeeding year as 2,4-D treatments were continued.

There was a significant advantage to spraying 2,4-D at the bud stage of growth over spraying at the bloom stage for the first two years of the experiment. After the first two years, treatments at the bloom stage reduced stands as much or more than the bud stage treat-

Table III. Survival of Canada thistle from three chemical treatments for four years.

Treatment	Percent Canada ^a /thistle survival				Treatment Means
	1st yr.	2nd yr.	3rd yr.	4th yr.	
2,4-D, 1.5 lb/A Applied bloom stage	89 a	85 a	55 c	30 de	65 m
2,4-D, 1.5 lb/A Applied bud stage	72 b	59 c	31 de	9 g	43 n
Amitrole 4 lb/A Applied bud stage	29 de	41 d	21 ef	14 fg	26 o
Means for years	63 x	61 x	36 y	18 z	45

a/ Each datum is an average of 40 plots each year for each treatment. Figures with different letters differ significantly at the 0.05 level, according to Duncan's multiple range test; treatment x year comparisons may be made.

ments.

Amitrole at 4 lb/A resulted in 29 percent survival 11 months after the first treatment and controlled Canada thistle much better than 2,4-D at 1.5 lb/A. However, after 4 years of treatments at the bud stage, Canada thistle survival was about the same for amitrole or 2,4-D. When amitrole treatments were repeated, Canada thistle plants usually were still highly symptomatic of the previous treatment, and growth was retarded. This probably contributed to the limited effect of the follow-up treatments of amitrole.

Amount of Lipid on Canada Thistle Leaves

The amount of lipid extracted from Canada thistle leaves was significantly affected by site where plants were grown (Table IV). Lipid yield of Newdale plants was greater than that of Huntley or Bozeman plants and lipid yield of Huntley grown plants was greater than that of Bozeman plants. This might be expected according to the climatic effects of the three locations since evaporation and wind travel were greatest at Newdale (Table I). The data show a significant variation in amount of lipid on leaves among the nine ecotypes. The amount of lipid ranged from $29.77 \mu\text{g}/\text{cm}^2$ for G4 ecotype down to $21.44 \mu\text{g}/\text{cm}^2$ for FM with a continuous range between these two extremes. The data indicated a trend toward an ecotype x site interaction; however, it was not statistically significant at the .05 level of probability.

Table IV. The effect of ecotype and site on lipids on leaves of Canada thistle.

Ecotype ^{a/}	Yield of Lipid at 3 Sites ($\mu\text{g}/\text{cm}^2$)			Avg. ^{b/}
	Newdale	Huntley	Bozeman	
G4	31.50	32.02	25.75	29.77 a
A1	32.43	29.48	25.82	29.24 a
G1	32.25	28.92	24.55	28.57 a b
LW	29.06	28.06	25.17	27.43 a b c
F1	29.96	23.08	25.87	26.30 b c
PW	27.89	28.24	20.18	25.43 c d
G2	25.00	23.64	22.81	23.82 c d e
YM	23.97	22.08	22.92	22.99 d e
FM	<u>20.97</u>	<u>22.43</u>	<u>20.90</u>	21.44 e
Avg.	28.11 x	26.44 y	23.78 z	

^{a/} The ecotypes are designated by letters and numbers according to place of origin as follows: A1, Ada County, Idaho; G1, G2, G4, Gallatin County, Montana; LW, Laramie, Wyo.; FM Fergus County, Montana; F1, Fremont County, Idaho; PW, Prosser, Washington; YM, Yellowstone County, Montana.

^{b/} Means with similar letters are not different at $P = 0.05$

Amount of lipid on thistle leaves as influenced by two dates of sampling was quite variable among four of the ecotypes (Table V). At Newdale significantly less lipid was obtained on the second date. Leaves from Bozeman showed the same tendency while those at Huntley showed a tendency to increase in amount of lipid on the surface on the second date. It is not known why the date of sampling had such a variable influence on amount of lipid, since it is normally expected that older leaves exposed to more of the heat of the season would have a heavier cuticle (37). It is possible that cuticle at Newdale on the second date was composed of more materials that were insoluble in the hexane-ether solvents used or that wind abrasion may have caused some losses between dates of sampling. Also, there were more aphids at Newdale and although leaf samples were selected to avoid any effect of these insects they may have influenced the lipid yield. There was a significant interaction of date of harvest and ecotype. AI and GI both yielded more lipid on date one than on date two while YM yielded more lipid on date two than on date one. This implies that the production of lipids occurs earlier in some ecotypes than others and could lead to a definite differential response to herbicides applied at certain times. This effect probably carried over into the location x date of harvest x ecotype analysis resulting in significant differences in these responses.

Table V. The effect of date of sampling on lipids on leaves of Canada thistle.

Site and Date of Sampling ^{b/}	Lipid Yield ^{a/} of Ecotypes $\mu\text{g}/\text{cm}^2$				Average For:		Site
	A1	G1	G4	YM	Date 1	Date 2	
<u>Newdale</u>							
Date 1	32.4	32.3	31.5	24.0	30.0 a		
Date 2	<u>24.9</u>	<u>22.6</u>	<u>24.6</u>	<u>25.2</u>		24.3 bc	
Avg.	28.7	27.4	28.0	24.6			27.2 r
<u>Huntley</u>							
Date 1	29.5	28.9	32.0	22.1	28.1 a		
Date 2	<u>27.00</u>	<u>32.6</u>	<u>28.3</u>	<u>32.2</u>		30.0 a	
Avg.	28.2	30.8	30.2	23.1			29.1 q
<u>Bozeman</u>							
Date 1	25.8	24.6	25.8	23.9	24.8 bc		
Date 2	<u>22.6</u>	<u>25.1</u>	<u>20.7</u>	<u>19.4</u>		21.9 c	
Avg.	24.2	24.8	23.2	21.2			23.4 s

Average 27.1t 27.7t 27.1t 24.3u 27.6y 25.4z
^{a/} Means followed by similar letters are not significantly different at P = 0.05.

^{b/} Date 1 samples were taken at the first bloom stage at each location with first blooms occurring on these dates: Huntley, 6-23-69; Newdale, 7-1-69; Bozeman, 7-7-69. Date 2 samples were taken - Huntley, 7-29-69; Newdale, 7-31-69 and Bozeman, 8-5-69.

The results indicate that development of lipid on leaf surfaces of Canada thistle depends upon inheritance (ecotype) as well as upon the effects of environment as suggested by Skoss (37) for other plants. Increased wax deposits and cuticle thickness were reported to interfere with absorption of foliar applied herbicides by several authors (24, 34, 37).

The able assistance of Dr. L. L. Jackson and others of the Chemistry Department of Montana State University in the extraction of the lipids was very much appreciated. The wax samples have been given to Dr. Jackson for further studies of kinds and amounts of lipids present. Very preliminary results indicate at least 22 different compounds are involved. As his work is completed some of the variations found in the present data may be better understood.

Ecotype response to amitrole treatment showed no relationship to amount of lipid on the leaves. Smith, Bayer, and Foy (38) studied the appearance of metabolic products from amitrole in resistant and susceptible ecotypes from this study. They reported a more rapid breakdown of amitrole in the resistant ecotype. Inasmuch as the breakdown products were considered to be detoxification products, it was assumed that the rate of breakdown of amitrole by the two ecotypes explained their differential susceptibility to amitrole.

Canada thistle response to 2,4-D was apparently related to lipid produced for most of the ecotypes (Table VI). The correlation

Table VI. Comparison a/ of leaf surface lipid and response of Canada thistle to 2,4-D foliage treatments.

Ecotype	Lipid Yield <u>b/</u> ug/cm ²	2,4-D Survival <u>b/</u> %
Gl.	24.6 a	74 a
G4	25.8 a	70 a
AI	25.8 a	68 a
FI	25.9 a	51 b
FM	20.9 b	50 b
LW	25.2 a	49 b
YM	22.9 a b	43 b
G2	22.8 a b	42 b
PW	20.2 b	42 b

a/ Means bearing similar letters are not significantly different at $P = 0.05$.

b/ The correlation coefficient (r) of lipid yield vs. 2,4-D survival for all nine ecotypes = .610; r required at $P = 0.05 = .666$

The r value for seven ecotypes not including LW and FI = .801 which is sufficient at $P = 0.05$.

coefficient of 2,4-D response of ecotypes and leaf surface lipid was .610 while that required for significance at $P=0.05$ was .666. The data indicate that of seven of the nine ecotypes studied there was a positive correlation of the amount of lipid and response to 2,4-D treatment (Table VI). Ecotypes G1, G4, and AI produced highest yields of leaf lipids and greatest survival to 2,4-D treatment. Ecotypes YM, FM, PW, and G2 had lowest yield of leaf lipids and lowest survival from 2,4-D treatments. Ecotypes LW and FI did not follow the same pattern of lipid and 2,4-D response. Thus factors other than lipid on the leaves may be operating to cause a difference in plant response to the herbicides. Whitworth and Muzik (47) evaluated strains of field bindweed in response 2,4-D and selected a susceptible and resistant clone for detailed studies. They found pronounced morphological differences between the two clones but none of these differences were found to correlate with response to foliage application of 2,4-D. They reported the most pronounced differences between the susceptible and resistant clones occurred at the cellular level and they attributed the differential response of these two bindweed strains to undefined physiological processes.

Variation in lipid production at the different locations may have been caused by various factors of climate such as humidity, wind travel, temperature, light intensity or other factors (12). A comparison of degree days from April to leaf sampling dates shows that plants at the Newdale site had been subjected to lower temperature than at Huntley (Table VII). However lipid yield at Newdale was equal to that at Huntley and greater than that at Bozeman. On the other hand, wind travel and evaporation were greater at Newdale than at the other sites. It is possible that wind and evaporation stimulated plants at Newdale to produce additional lipid even though the temperature was lower at Newdale. No records of relative humidity were available for any of the sites. However, it was observed that relative humidity was lowest at Newdale, intermediate at Huntley, and highest at Bozeman for the season involved.

Specific Leaf Weight

Leaves of thistle plants grown at Bozeman had a significantly greater weight per unit area (SLW) than those from Huntley or Newdale (Table VIII). Also SLW of plants from Newdale and Huntley were about the same. The ecotypes varied less in SLW than in lipid yields. SLW varied from location to location, the over-all average being greater at Bozeman. The interaction of SLW and location was significant with some ecotypes showing a different order of SLW at different locations.

Table VII. Heat accumulation at three sites where Canada thistle plants were grown and lipid yield.

	Location of Sites		
	Newdale	Huntley	Bozeman
	Degree <u>a/</u>	days and harvest date	days and harvest date
Accumulated heat			
5-year average April 1 to July 1	987	1362	984
Apr. 1 to 1st sampling date	1153	1364	1144
	7- 1-69	6-23-69	7-7-69
Apr. 1 to 2nd sampling date	1914	2333	2050
	7-31-69	7-29-69	8-5-69
Lipid yield			
Lipid yield: 1st date	30.0 a	28.1 a	24.8 bc
(ug/cm ²)			
2nd date	24.3 b	30.0 a	21.9 c

a/ An arithmetic accumulation by days of degrees of mean temperature above a base of 40° F.

Table VIII. The effect of ecotype and site on specific leaf weight of Canada thistle.

Ecotype	Specific leaf weight ^{a/} mg/cm ²			Average
	Newdale	Huntley	Bozeman	
G2	6.84 a b	5.94 e i	6.79 a-c	6.53 m
LW	6.09 d-h	6.20 b-h	7.10 a	6.46 m
GI	6.41 b g	6.23 b-h	6.17 b-h	6.27 m
FI	6.27 a-e	4.75 l	6.64 a-d	5.99 n
G4	5.68 h-k	6.03 d-h	6.14 c-h	5.95 n
PW	5.92 e-i	5.17 j-l	6.31 b-h	5.88 n
AI	5.78 f-j	5.35 i-l	6.46 b-f	5.86 n
FM	5.75 g-j	5.11 k-l	6.32 b-h	5.73 n
YM	5.18 j-l	5.89 f-i	6.16 i-h	5.72 n

Avg.	6.02 x	5.68 y	6.47 z
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^{a/} Means bearing the same letter were was not significantly different at P = 0.05

SLW was generally highest on the second date of harvest (Table IX). This substantiates a point reported by Barnes (4) that the oldest active leaves have a higher SLW.

In some cases, the SLW and lipid yield appeared to be related but the correlation coefficient did not approach significance at $P = 0.05$.

Table IX. Specific leaf weight of Canada thistle leaves as influenced by ecotype, harvest date and site, 1969.

Site and Date of Sampling	Lipid yield ^a / of ecotypes mg/cm ²				Average		
	AI	G1	G4	YM	Date 1	Date 2	Site
Newdale							
Date 1	5.78	6.41	5.68	5.18	5.76		
Date 2	6.86	6.30	6.50	6.65		6.58	6.17a
Huntley							
Date 1	5.35	6.23	6.03	5.80	5.85		
Date 2	6.83	6.57	6.22	6.46		6.52	6.19a
Bozeman							
Date 1	6.45	6.17	6.14	6.16	6.23		
Date 2	6.83	6.96	6.45	6.74		6.74	6.49b
Average							
Average	6.35	6.44	6.17	6.17	5.95c	6.62d	

SUMMARY

Canada thistle is an aggressive perennial weed growing in the northern half of the United States and many other countries. It is well adapted to a wide variety of habitats in this area. It is a weed of croplands, roadsides, ditchbanks, rangelands, pastures and forests, and is spread and introduced into new areas by wind and by man when he plants contaminated forage and small-grain seeds.

Because of its aggressive nature and its wide distribution many kinds of control measures have been sought for over 170 years in the United States. Certain cultivation treatments have been developed by which infestations of Canada thistle in arable land can be controlled. Competitive cropping, particularly with alfalfa, has been shown to be effective in its control. Chemical sprays have also been extensively investigated for the control of Canada thistle. The growth regulator type herbicide, 2,4-D has been used widely for control of Canada thistle and results have been highly variable. The varied response between patches of Canada thistle to 2,4-D have been attributed to differences in environment and inheritance.

These studies were conducted to more closely define differences that might exist in the population of Canada thistle in response to herbicides and to study the influence of ecotype and site on the amount of lipid on the leaves of Canada thistle and its influence on response of certain ecotypes to 2,4-D.

Canada thistle plants were selected from 10 locations in Montana, Idaho, Washington and Wyoming and were asexually propagated in field plots at Bozeman, Montana. After a three year establishment period herbicide treatments of 2,4-D and amitrole were applied to the different plants. The ten ecotypes exhibited a wide range of response to these herbicides. The range of survival was from 67 to 42 percent after four years of repeated 2,4-D treatments and was 71 to 11 percent after four years of repeated amitrole treatments. Certain ecotypes exhibited more resistance to the herbicides than others through the experiment.

The same group of ecotypes in an earlier study had been found to vary significantly in their epidermal cell-stomata ratios. However, no relationship could be established between the epidermal cell-stomata ratios and response of the ecotypes to herbicides.

Lipid content of leaf cuticle is an important factor in the absorption of herbicides and differences in amount of lipid may be one of the reasons for differential response of plants to herbicide treatment. The amount of lipid present on leaves of Canada thistle ecotypes that had been included in the herbicide response studies was investigated. The amount of lipid on leaf surfaces of the Canada thistle ecotypes was found to vary significantly because of site where the thistles were grown, because of the ecotypes themselves, and because of date of sampling.

The thistles produced the most lipid at the site having the greatest wind travel and greatest evaporation and lowest humidity. Specific leaf weight of Canada thistle plants also varied with site and ecotype but was not related to lipid content.

The amount of leaf surface lipid of seven of the nine ecotypes was correlated positively with survival of 2,4-D treatments. Among the seven ecotypes those with the most lipid exhibited the greatest survival to the 2,4-D treatment. Two ecotypes with high lipid on the leaves were among the lowest group in survival to 2,4-D treatments.

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A P P E N D I X

Table X. Sample yields of lipid from Canada thistle leaves from three sites in 1969.

Site & Replication	Lipid in $\mu\text{g}/\text{sq cm}$ for each ecotype									
	G1	G4	AI	YM	G2	FI	FM	LW	PW	Total
Bozeman										
1	23.41	25.20	22.86	17.30	22.09	24.98	21.40	23.72	18.60	199.56
2	23.03	27.91	24.72	24.49	25.79	23.14	26.00	27.19*	22.91*	225.18
3	24.43	25.35	28.60	22.29	21.36	30.04	19.02	22.15	21.25	215.39
4	24.50	25.44	22.51	26.64	22.74	23.48	15.10	27.91	16.41	204.73
5	27.37	24.87	30.41	23.86	22.09	27.70	22.10	24.90	21.73	225.02
Total	122.74	128.77	129.10	114.58	114.07	129.34	104.52	125.87	100.89	1069.88
Average	24.55	25.75	25.83	22.92	22.81	25.87	20.90	25.17	20.18	23.78
Huntley										
1	24.67	30.76	33.91	27.08	28.68	30.35	24.68	30.45	31.97	262.55
2	35.14	34.74	30.18	19.24	24.47	24.53	25.25	36.27	42.69	272.51
3	36.94	31.62	31.69	22.65	20.36	23.69	18.43	24.25	19.56	229.19
4	24.67	30.07	26.04	22.05	23.54	18.68	21.64	25.01	18.29	209.99
5	23.18	32.92	25.56	19.40	21.13	18.16	22.14	24.33	28.68	215.50
Total	144.60	160.11	147.38	110.42	118.18	115.41	112.14	140.31	141.19	1189.74
Average	28.92	32.02	28.48	22.08	23.64	23.08	22.43	28.06	28.24	26.44
Newdale										
1	34.26	28.60	36.79	21.20	35.12	30.63	24.30	26.04	30.29	267.23
2	40.97	38.06	31.49	37.50	27.86	38.67	17.47	37.05	29.53	298.60
3	25.61	30.13	31.21	19.48	25.60	29.75	24.93	30.44	29.81	246.96
4	25.42	37.15	25.12	17.68	14.89	24.73	17.15	27.90	30.05	220.09
5	34.98	23.54	37.52	23.97*	21.51	26.01	21.02	23.83	19.75	232.13
Total	161.24	157.48	162.13	119.83	124.98	149.79	104.87	145.26	139.43	1265.01
Average	32.25	31.50	32.43	23.97	25.00	29.96	20.97	29.05	27.89	28.11
Grand Total	428.58	446.36	438.61	344.83	357.23	394.54	321.53	411.44	381.51	3524.63
Ecotype Mean	28.57	29.77	29.24	22.99	23.82	26.30	21.44	27.43	25.43	26.11

*Calculated value for missing plot.

Table X. Sample yields of lipid from Canada thistle leaves from three sites in 1969 (cont'd).

Analysis of Variance

Source of Variance	Degree of Freedom	Sum of Square	Mean Square	F
Total	134	4295.14		
Replications within sites	12	1268.40	105.70	
Sites	2	430.42	215.21	16.92**
Ecotypes	8	1023.91	127.99	10.01**
Ecotype x site	16	345.28	21.58	1.69
Error	96	1227.13	12.78	

** Significant F test at $P = 0.01$

Table XI. The effect of date of sampling on the yield of leaf lipids of Canada thistle plants.

Site & Replication	Micrograms of lipid from leaves of four ecotypes on 2 dates									
	Date 1					Date 2				
	G1	G4	AI	YM	Total	G1	G4	AI	YM	Total
Bozeman										
1	23.41	25.20	22.86	17.30	88.77	24.88	20.53	19.06	20.99	85.46
2	23.03	27.91	24.72	24.49	100.15	21.79	22.87	21.83	18.17	84.66
3	24.43	25.35	28.60	22.29	100.67	29.07	17.51	26.39	18.98	91.95
4	24.50	25.44	22.51	26.64	99.09	20.87	22.25	24.71	20.46	88.29
5	27.37	24.87	30.41	23.86	106.51	28.72	20.27	21.18	18.28	88.45
Total	122.84	128.77	129.10	114.58	495.19	125.33	103.43	113.17	96.88	438.81
Average	24.55	25.75	25.82	22.92	24.76*	25.07	20.67	22.63	19.38	21.94
Huntley										
1	24.67	30.76	33.91	27.08	116.42	37.92	24.99	23.82	30.83	117.56
2	35.14	34.74	30.18	19.24	119.30	23.48	26.72	20.13	27.43	97.76
3	36.94	31.62	31.69	22.65	122.90	29.88	20.51	24.12	33.51	108.02
4	24.67	30.07	26.04	22.05	102.83	38.11	33.81	36.39	34.82	143.13
5	23.18	32.92	25.56	19.40	101.06	33.60	35.38	30.48	34.33	133.79
Total	144.60	160.11	147.38	110.42	562.51	162.99	141.41	134.94	160.92	600.26
Average	28.92	32.02	29.48	22.08	28.13	32.60	28.28	26.99	32.18	30.01
Newdale										
1	34.26	28.60	36.79	21.20	120.85	23.75	19.56	28.82	27.65	99.78
2	40.97	38.06	31.49	37.50	148.02	20.80	27.53	22.30	25.12	95.75
3	25.61	30.13	31.21	19.48	106.43	19.76	20.82	23.70	21.67	85.95
4	25.42	37.15	25.12	17.68	105.37	23.91	23.76	27.69	27.49	102.85
5	34.98	23.54	37.52	23.97*	120.01	24.77	31.17	22.18	24.01	102.13
Total	161.24	157.48	162.13	119.83	600.68	112.99	122.84	124.69	125.94	486.46
Average	32.25	31.50	32.43	23.97	30.03	22.60	24.57	24.94	25.19	
Grand Total	428.58	446.36	438.61	344.83	1658.38	401.31	367.68	372.80	383.74	3183.91
Average	28.57	29.77	29.24	22.99		26.75	24.51	24.85	25.58	

*Calculated value for missing plot.

Table XI. The effect of date of sampling on the yield of leaf lipids of Canada thistle plants (cont'd).

Analysis of Variance

Source of Variance	Degree of Freedom	Sum of Square	Mean Square	F
Total	119	3769.87		
Replications within sites	12	945.61		
Sites	2	679.23	339.62	31.50**
Ecotypes	3	208.61	69.54	6.45**
Dates	1	147.07	147.07	13.64**
Ecotype x site	6	51.96	8.66	.80
Ecotype x dates	3	278.90	92.97	8.62**
Dates x sites	2	294.17	147.09	13.64**
Ecotype x date x sites	6	237.44	39.57	3.67**
Error	86	926.88	10.78	

** Significant F test at $P = 0.01$

Table XIII. Specific leaf weight of Canada thistle leaves as influenced by ecotype and site.

Site & Replication	Specific leaf weight for each ecotype mg/cm ²									Total
	G1	G2	G4	LW	PW	YM	AI	FM	FI	
Bozeman										
1	6.45	6.73	5.87	6.66	5.59	5.08	5.19	5.90	6.70	54.17
2	5.46	6.85	5.71	6.93	5.91	5.52	6.26	6.06	5.78	54.48
3	6.36	6.55	6.76	7.01	7.42	6.35	7.95	7.02	7.72	63.14
4	6.22	6.50	6.31	7.94	6.15	6.83	6.84	6.43	6.54	59.76
5	6.35	7.34	6.06	6.94	6.48	7.04	6.49	6.21	6.47	59.38
Total	30.84	33.97	30.71	35.48	31.55	30.82	32.70	31.62	33.21	290.93
Average	6.17	6.79	6.14	7.10	6.31	6.16	6.45	6.32	6.64	
Huntley										
1	5.83	6.00	5.72	6.25	5.50	5.77	5.49	4.52	4.95	50.03
2	7.10	5.76	6.43	6.79	6.22	4.80	5.31	5.65	4.69	52.75
3	6.78	5.86	5.87	5.29	5.75	7.26	5.42	4.81	4.62	51.66
4	5.22	6.15	6.10	6.48	5.37	5.50	5.16	5.47	4.73	50.18
5	6.24*	5.94	6.03	6.20	5.41	5.73	5.35	5.11	4.75	50.76
Total	31.17	29.71	30.15	31.01	28.25	29.06	26.73	25.56	23.74	255.38
Average	6.23	5.94	6.03	6.20	5.17	5.89	5.35	5.11	4.75	
Newdale										
1	6.35	6.30	5.72	4.96	5.78	5.60	5.37	5.64	6.30	52.02
2	6.53	6.38	5.60	5.33	6.12	5.32	5.86	5.59	6.71	53.44
3	5.72	6.97	5.68	7.29	5.42	4.59	5.99	6.12	7.21	54.99
4	6.26	7.74	6.24	6.69	5.84	5.20	5.14	5.89	6.64	55.64
5	7.19	6.82	5.15	6.20	6.46	5.18*	6.52	5.49	5.97	54.98
Total	32.05	34.21	28.39	30.47	29.62	25.89	28.88	28.73	32.83	271.07
Average	6.41	6.84	5.68	6.09	5.92	5.18	5.78	5.75	6.57	
Grand Total	94.06	97.89	89.25	96.96	89.42	85.77	88.34	85.91	89.78	817.38
Average	6.27	6.53	5.95	6.46	5.80	5.72	5.86	5.73	5.99	6.03

*Calculated value for nursery plot.

Table XII. Specific leaf weight of Canada thistle leaves as influenced by ecotype and site,
(cont'd).

Analysis of Variance

Source of Variance	Degree of Freedom	Sum of Square	Mean Square	Cal F
Total	134	71.87		
Replications within sites	12	21.56	1.797	15.23
Ecotypes	8	14.30	1.788	15.15**
Sites	2	11.01	5.505	46.65**
Site x Ecotype	16	20.17	.8543	7.239**
Error	96	4.83	.11802	

** Significant F test at P = 0.01

Table XIII. Specific leaf weights of Canada thistle leaves as effected by ecotype, date of harvest, and site, 1969.

Site and Replication	Specific leaf weight of four ecotypes on two dates mg/cm ²									
	Date 1					Date 2				
	G1	G4	AI	VM	Total	G1	G4	AI	YM	Total
Huntley										
1	5.83	5.72	5.49	5.77	22.81	6.24	6.56	6.55	7.50	26.85
2	7.10	6.43	5.31	4.72	23.56	6.69	5.90	6.31	5.41	24.31
3	6.78	5.87	5.42	7.26	25.33	6.49	5.93	6.03	6.27	24.72
4	5.22	6.10	5.16	5.50	21.98	6.79	5.60	7.89	7.23	27.51
5	6.23*	6.03*	5.35*	5.73	23.34	6.66	7.11	7.39	5.91	27.07
Total	31.16	30.15	26.73	28.98	117.02	32.87	31.10	34.17	32.32	130.46
Average	6.23	6.03	5.35	5.80		6.57	6.22	6.83	6.46	
Newdale										
1	6.35	5.72	5.37	5.60	23.04	6.00	6.52	6.81	7.62	26.95
2	6.53	5.60	5.86	5.32	23.31	5.98	7.57	6.92	6.36	26.83
3	5.72	5.68	5.99	4.59	21.98	6.66	5.61	6.44	6.36	25.07
4	6.26	6.24	5.14	5.20	22.84	6.62	5.49	7.21	7.47	26.79
5	7.19	5.15	6.52	5.18*	24.04	6.24	7.31	6.92	5.46	25.93
Total	32.05	28.39	28.88	25.89	115.21	31.50	32.50	34.30	33.27	131.57
Average	6.41	5.68	5.78	5.18		6.30	6.50	6.86	6.65	
Bozeman										
1	6.45	5.87	5.19	5.08	22.59	6.15	6.35	6.09	7.27	25.86
2	5.46	5.71	6.35	5.52	23.04	7.16	5.95	6.82	6.51	26.44
3	6.36	6.76	7.95	6.35	27.42	7.62	7.26	8.48	7.16	30.52
4	6.22	6.31	6.34	6.83	25.70	6.67	6.12	6.31	6.58	25.68
5	6.35	6.06	6.49	7.04	25.94	7.22	6.56	6.43	6.18	26.39
Total	30.84	30.71	32.32	30.82	124.69	34.82	32.24	34.13	33.70	134.89
Average	6.17	6.14	6.46	6.16		6.96	6.45	6.83	6.74	
Grand Total	94.05	89.25	87.92	85.69	356.92	99.19	95.84	102.60	99.29	396.92

*Calculated value for nursery plot.

Table XIII. Specific leaf weights of Canada thistle leaves as effected by ecotype date of harvest and site, 1969, (cont'd).

Analysis of Variance

Source of Variance	Degree of Freedom	Sum of Square	Mean Square	Cal F
Total	119	64.39		
Replications within sites	12	11.75	.979	3.48
Sites	2	2.59	1.30	4.63*
Ecotype	3	1.69	.563	2.00 NS
Harvest date	1	13.34	13.34	47.47**
Ecotype x date	3	2.32	.773	2.75*
Ecotype x site	6	.91	.152	.54 NS
Ecotype x site x date	6	7.63	1.272	4.53**
Error	86	24.16	.281	

** Significant F test at $P=0.01$

* Significant F test at $P=0.05$

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