CARBOHYDRATE LEVELS IN LATERAL ROOTS OF *CIRSIUM ARVENSE* (L.) SCOP. FOLLOWING VARIOUS CULTURAL AND CHEMICAL TREATMENTS

by

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INTRODUCTION

After about twenty years of selective herbicide use in Western Canada, hard to kill perennial weeds such as Canada thistle (*Cirsium arvense* (L.) Scop.) have become an increasingly dominant part of the weed problem. Their resistance to common herbicides, their hardiness and competitive ability under adverse conditions, and their ability to regenerate from a perennial root system, make them a serious problem to the farmer.

The competitive ability and persistence of a perennial weed is largely due to its ability to draw on the reserve food materials and viable buds of its underground perennial roots to produce new leafy shoots. Even if the top growth of the weed is destroyed repeatedly the weed can still recover. Because of the concentration of food reserves in the roots of a perennial plant no control measure can be wholly successful unless it destroys the root system.

In theory there are three ways in which this destruction can be accomplished. First the root system could be mechanically destroyed by pulling or cultivating, thus exposing it to desiccation or actually removing it from the field. With most perennial weeds this would be impractical or impossible because of the depth of the roots, the large areas involved and the possibility of increasing the problem by spreading viable roots to a previously uninfested part of the field.

The second and most ideal method of destruction would be the application of a suitable herbicide to the plant or the soil which would so disturb the metabolism of the plant that death would occur. There is little doubt that many of the herbicides now available could cause this type of effect if they were translocated to all parts of the root system. However none of the presently available herbicides appear to be translocated in sufficient quantity to cause any serious damage to the roots. Consequently, in root systems which are often several feet in length, the herbicide destroys only a small fraction of the total root and the remaining healthy roots are able to sustain new leafy shoots.

The third and most successful method is the depletion of the food reserves of the root--a process commonly referred to as root starvation. Each time the plant produces new shoots it utilizes more of its organic reserves to obtain the energy necessary for this new growth. If, by repeated cultivation or other means, the supply of carbohydrates in the roots can be reduced below the amount necessary to sustain new leafy shoots until they reach the surface, then the plant will die.

In utilizing the principle of root starvation for perennial weed control it is important to have an adequate knowledge of the carbohydrate levels in the weed. Fluctuations in carbohydrate level are governed by such things as day length, moisture, nutrients, length of season and time of flowering and seed set. Thus, in order to make adequate control recommendations, it is important to know the seasonal trends of carbohydrates under local conditions as well as how these trends are altered by control measures.

The study described here was intended to establish the normal seasonal trends of carbohydrates in the lateral roots of Canada thistle under southern Manitoba conditions. Several treatments involving cropping, cultivation, herbicides, and combinations of these were studied to determine how they would affect the normal seasonal trend of carbohydrates in the roots of this weed species.

REVIEW OF LITERATURE

The resistance of Canada thistle (*Cirsium arvense* (L.) Scop.) to control measures has generally been attributed to its deep perennial root system. Aslander (3) recognized that, "eradication of perennial weeds must aim at the destruction of the perennial structures." Recognizing that herbicides were not translocated into the roots from sprayed foliage he concentrated on finding herbicides which would penetrate the soil to reach the perennial structures directly. The importance of destroying the perennial root system was also stressed by Hardy (15) and Pavlychenko (31).

To survive repeated cultivations a perennial plant must have enough organic food reserves in its storage organs to send a new leafy shoot to the surface. If these organic reserves reach low levels at certain seasons or at certain stages of development then these should be strategic times to start eradication procedures. With this principle in mind, the seasonal fluctuations in the organic reserves of various plants have been investigated by Arny (2) and others. A review of results obtained from investigation of other weedy plants is helpful in interpreting the carbohydrate fluctuations in Canada thistle.

Some authors have been unable to establish any relationship between stage of growth and carbohydrate levels. Linscott and McCarty (22) found that the carbohydrate content of roots of ironweed (Vernonia baldwinii) was not necessarily related to stages of growth. Their results indicated that root weight was a better indicator of plant vigor than root carbohydrate levels. They concluded that reductions in ironweed vigor as a result of annual mowing were caused by a reduction in the available energy material. However this was indicated by a decrease in total dry weight of the root system rather than a permanent decrease in carbohydrate percentage.

In other investigations, although definite patterns of carbohydrate fluctuations have been detected, the authors have not correlated them with a particular stage of growth. In studies of Johnson grass (Sorgum halepense (L.) Pers.) sucrose levels decreased steadily until early bloom stage and then increased sharply. In the same plants, however, total carbohydrate and glucose levels reached a maximum at the early bloom stage (26). Loomis (23) suggested early season control measures for dandelion after finding that carbohydrate levels in dandelion (Taraxacum officinale Weber) roots reached a low point two weeks after the start of vegetative growth in the spring. Total sugars and total starch were investigated in hoary cress (Cardaria draba var repens) by Barr (7). He found that sugars increased until July 6 and starch increased until August 1. Both fractions then decreased until mid September before increasing to their maximum levels on December 3.

Bindweed (Convolvulus arvensis (L.), a deep rooted perennial, has long been a serious weed in the United States. As a result, in 1935, the United States Department of Agriculture organized a series of uniform cooperative bindweed control experiments at five locations. Several of these projects included investigations of fluctuations in the percentage of storage carbohydrates, both seasonally and after various treatments (4, 5, 6, 10, 11, 12, 43). In these experiments, the total sugars plus starch-dextrin fraction was the most reliable indicator of food reserves (11). This fraction was found to have the same general trend at all five locations if variations in the start of spring growth were taken into account (6). In general the carbohydrate reserves tended to decrease from April 15 to May 1. They then increased to a maximum by July 1 after which they gradually declined. They found that the increase in carbohydrates from May 1 to July 7 could best be prevented by cultivation at twelve to fourteen day intervals (43).

Comparatively few attempts have been made to determine seasonal fluctuations in the organic reserves of Canada thistle. However, the results that have been obtained are quite consistent and indicate a strong relationship between low levels of carbohydrate and time of flowering.

The dry matter, ash, and carbohydrate reserves of Canada thistle were investigated by Rogers (37). He found that the low point in total carbohydrate percentage occurred during the latter part of June at the time of flowering.

Similar results were obtained by Welton et al. (45) who found low levels of carbohydrate to be correlated with the flowering stage of Canada thistle in Ohio. They also found that when the tops of the thistles were clipped the carbohydrates in the roots reached lower levels than in the unclipped plants. In the same plants the nitrogen level decreased until July 1, then began to increase.

In Minnesota, the total readily available carbohydrate fraction decreased rapidly from mid April until early May then continued to decline, but at a more moderate rate, until it reached its lowest level on July 13 when the plants were in the bloom stage (2). After flowering there was a rapid replenishment of the available carbohydrate until fall. As the temperature lowered in the fall there were marked decreases in the percentage of true starch and increases in total sugars. This effect was also noted by Barr (5) in his studies of bindweed root reserves. Arny (2) also detected a slight decline in organic nitrogen reserves which continued until mid August before the reserve nitrogen levels were replenished.

The introduction of hormone type herbicides (such as 2,4-D) in 1945 stimulated a great deal of research on control of susceptible weeds. Many studies were conducted investigating the effect of these chemicals on the carbohydrate levels of Canada thistle and other weed plants (13, 17, 18, 20, 21, 26, 27, 32, 35, 39). In general 2,4-D caused an increase in respiration immediately after application. This is reflected by a buildup of sugars at the expense of starches. In most cases the eventual result of an application of 2,4-D is the lowering of the reserve carbohydrate levels. It is uncertain whether or not this lowering of carbohydrate levels is an important factor in the death of a treated plant. Several workers have concluded that the lowered level of carbohydrate is a contributing factor, though not the prime factor, involved (18, 22, 35).

Canada thistle control measures involving cropping, cultivation or herbicides have been described by many workers (9, 17, 25, 34, 36). All of these measures depend on repeated destruction or suppression of the top growth in order to weaken underground structures. Whenever cultivation or herbicides are used to eradicate a perennial weed by depleting its root reserves, it is important to know how frequent treatments must be to obtain the maximum carbohydrate depletion. Frequent cultivation is not necessarily the most effective way to deplete carbohydrate reserves (12). Frazier (12) found that in field bindweed very little of the root reserves were expended by the plant in forming the rhizome as compared to the expenditure necessary to form the leafy shoot. This is in accordance with Timmons (42) who reported that twice as many cultivations at emergence were required to obtain the same control as cultivation twelve days after emergence. This emphasizes the importance of knowing the trends of organic reserves both seasonally and after cultivation or chemical treatments.

One of the difficulties in investigating the carbohydrates of plants is to find a suitable method for rapid and accurate determination of carbohydrate levels. Most methods of analysis involve two steps. These are the hydrolysis of the polysaccharides and disaccharides and the quantitative estimation of the resulting reducing sugars.

If enzyme hydrolysis is used, the enzyme treatment chosen will depend on the polysaccharide being analysed. A combination of saliva and acid hydrolysis was used by Frazier (11) in his study of bindweed roots. Since a primary storage form in ironweed is sucrose, an invertase hydrolysis was employed by Linscott (22). Both the invertase hydrolysis for sucrose and the saliva hydrolysis of starch are described by Loomis and Shull (24). A method for hydrolysis of the starch-dextrin-maltose fraction with takadiastase is described by Weinmann (44).

Acid hydrolysis is less selective in its action. The polysaccharide fraction hydrolyzed depends on the acid used, its strength, the temperature of the mixture and the length of time the acid is allowed to act on the plant material. Methods of acid hydrolysis using hydrochloric acid are outlined by Loomis and Shull (24). These procedures have been followed with modifications in the analysis of Canada thistle

(2) and bindweed roots (5). Klingman et al. (20) used sulphuric acid in their hydrolysis of the polysaccharide in wild garlic. Pirt and Whelan (33) found sulphuric acid to be less destructive to glucose than hydrochloric acid of the same normality. They found the optimum conditions for acid hydrolysis were 1.5N sulphuric acid at 100° C. for two hours.

After the polysaccharides have been hydrolyzed by either acid or enzyme the resulting reducing sugars are estimated. The most commonly used methods of reducing sugar estimation are metallic ion reduction procedures. These procedures estimate the reducing power of a solution by measuring the amount of cupric or ferric ions reduced. The Munsen-Walker (28), Somogyi (40, 41) and ceric sulphate (16) procedures are of this type. These procedures were used in studies on reserve carbohydrates by Frazier (11) McWhorter (26), Linscott (22) and Arny (2).

The use of dinitrosalicylic acid (DNS) was first described by Sumner (42) who used it to measure reducing sugars in diabetic urine. The DNS reagent combines with reducing sugars to form a colored reaction product. The optical density of the resulting solution can be related to the concentration of reducing sugars present. This technique has been further improved (29)(19)(8) and its reliability has been demonstrated.

METHODS AND MATERIALS

This study, conducted in 1964-65, was located on Red River clay soil near Rosser, Manitoba. The site chosen, approximately one half an acre in extent, was located in a farmer's field and was uniformly covered by an established infestation of Canada thistle. In 1963, the year prior to the start of this study, the field was sown to oats and sprayed with a low rate of 2,4-D. There were few weeds in the plot area other than a heavy stand of Canada thistle. At the beginning of the study in May 1964 the average density of the infestation was 28 Canada thistle shoots per square yard.

Ten 2-year treatments designated as A to J were begun in this infestation in 1964. These treatments were replicated four times in a randomized block design. Each plot was twelve feet wide and thirty feet long. Adjacent plots were separated by a two foot border strip and a four foot roadway separated adjacent replicates. All treatments received one cultivation with regular field equipment each spring before growth commenced.

The ten treatments are listed below by letter designation:

A. Weedy check both years.

(1) Canada thistle were allowed to grow undisturbed with no treatment except clipping of the flower heads to prevent dissemination of seed.

B. Summerfallow both years.

(1) The plots were rototilled each time the plants reached 2 - 3 inches in height. This involved six rotovations in 1964 and five in 1965.

C. Defoliation with paraquat both years.

(1) One pound per acre of paraquat (1,1-dimethyl-4,4-bipyridilium dichloride) was used to remove top growth each time the shoots reached 2 to 3 inches in height. Paraquat was applied the same number of times that rototilling was carried out in treatment B.

D. Summerfallow and MCPA in 1964 followed by oats and MCPA in 1965.

(1) The plots were rototilled as in treatment B on May 25, June 11, and July 9.

(2) The Canada thistle were then allowed to recover until a few shoots were budding before they were sprayed with MCPA amine (2-methyl-4-chloro phenoxyacetic acid) at 16 ounces per acre.

(3) Oats were seeded the second season and sprayed in the three leaf stage with MCPA amine at 16 ounces per acre.

E. Summerfallow and amitrole T in 1964 followed by oats and MCPA in 1965.

(1) The plots were rototilled on May 25, June 11, and July 9.

(2) The Canada thistle were allowed to recover until a few shoots were budding before spraying with amitrole T (3-amino, 1,2,4-triazole plus ammonium thiocyanate) at 4 pounds per acre.

(3) Oats were seeded in the second season and sprayed at the three leaf stage with MCPA amine at 16 ounces per acre.

F. Summerfallow and dicamba in 1964 followed by oats and dicamba in 1965.

(1) The plots were rototilled on May 25, June 11, and July 9.

(2) The Canada thistle were allowed to recover until a few shoots were budding before spraying with dicamba (2-methoxy-3,6 dichloro benzoic acid) at 4 pounds per acre.

(3) Oats were seeded in the second season and sprayed in the three leaf stage with dicamba at 4 ounces per acre.

G. Oats untreated both seasons.

H. Oats and MCPA both seasons.

(1) Oats were sown and sprayed at the three leaf stage with MCPA amine at 16 ounces per acre both seasons.

I. Oats and dicamba both seasons.

(1) Oats were sown both years and sprayed in the three leaf stage with dicamba at 4 pounds per acre in 1964 and with dicamba at 4 ounces per acre in 1965.

J. Oats and picloram both seasons.

(1) Oats were sown both years and sprayed in the three leaf stage with picloram (4-amino-3,5,6-trichloropicolinic acid) at 2 pounds per acre in 1964 and with picloram at 2 ounces per acre in 1965.

The oats, variety Russell, were sown on May 26, 1964 and May 20, 1965 using a pony press drill. No fertilizer was sown the first season but eighty pounds of 11-48-0 were sown the second season. This was in accordance with recommendations made by the Manitoba provincial soil testing laboratory from soil tests made the previous fall.

The oats were sprayed June 26, 1964 and June 21, 1965 when they were in the three leaf stage. The summerfallow treatments which involved spraying (D, E, F) were sprayed on August 23, 1964 and August 19, 1965. MCPA, dicamba, and picloram were applied in 5.5 gallons of total solution per acre at 40 pounds per square inch (p.s.i.) pressure. Amitrol T and paraquat were applied in 20 gallons of total solution per acre at 40 p.s.i. All herbicide rates are in pounds or ounces of active ingredient or acid equivalent per acre.

When the oats reached maturity, four random samples of one square yard each were harvested from each plot for yield determinations. In all cases the three outside rows were left unharvested to eliminate "border effects".

In 1965 the amount of thistle control was visually estimated using a 0-10 rating system (0 indicating no control and 10 indicating no visible thistle shoots). These ratings were used to assess the degree of control after two years.

Root samples for carbohydrate analysis were taken from the plots at three to four week intervals throughout both growing seasons. This involved nine sampling dates in 1964 and six sampling dates in 1965. Since only roots having visible top growth could be located and sampled, sampling dates tended to be spaced according to growing conditions. In some instances, especially in the second season, control measures had reduced the Canada thistle stand to the extent that root samples large enough for carbohydrate analysis could not be obtained from all treatments.

Only the surface foot of soil was sampled since it was found that nearly all the lateral storage roots were located between the five and ten inch depths. Sampling was done with a spade and a minimum of four holes were dug in each plot. The roots from the four or more holes were composited to form a single sample per plot. Only living lateral roots were sampled. Vertical stem sections, rootlets and dead and decaying roots were discarded. Each sample was put in a bottle of ice water to keep the roots cool and moist while they were being transferred from the field to the laboratory.

In the laboratory the root samples were washed free of soil under cold running water. The washed roots were cut into approximately one quarter inch lengths and dried for 16 hours at 70° C. in a vacuum oven. The dried roots were readied for analysis by grinding in a micro-wiley mill. Duplicate samples of 80 to 100 milligrams of this material, depending on the carbohydrate content, were used in the analysis for carbohydrates.

The analysis of the dried root material consisted of an acid hydrolysis of the polysaccharides followed by a colorimetric estimation of the resulting reducing sugars. The colorimetric reagent used was 3,5-dinitrosalicylic acid.

The root materials were hydrolyzed in two steps. An initial hydrolysis of thirty minutes at 100° C. in .02N sulphuric acid was used to remove free reducing sugars and easily hydrolyzable fructosans. This was necessary in order to minimize the destruction of this fraction by the more severe hydrolysis necessary to hydrolize dextrans and starch. The material from this first hydrolysis was filtered and the residue was rehydrolyzed for two hours at 100° C. in 1.5N sulphuric acid as described by Pirt and Whelan (33). The reducing sugars from each hydrolysis were estimated separately and added to obtain the total carbohydrate in each sample.

The use of 3,5-dinitrosalicylic acid (DNS) in the estimation of reducing sugars has been described by Sumner (42) and Noelting and Bernfeld (29) and was extensively tested and found reliable by Bendelow (8). The detailed method used in this study was a modification of that described by Bendelow (8). The hydrolysates from each of the hydrolyses described above were neutralized with 2.0N sodium hydroxide and made up to a total volume of 100 mls. A 2 ml. aliquot of this was added to 2 mls. of the DNS reagent in a test tube. The DNS-reducing sugar reaction product was developed by placing the tubes in a boiling water bath for exactly five minutes. The tubes were then quickly cooled in ice water and the 4 mls. of reaction product were diluted with 20 mls. of water. The optical density of the resulting dilution was read at 510 millimicrons against a reagent blank made by using 2 mls. of water in place of 2 mls. of sugar solution. The concentration of sugars represented by each optical density reading was determined by reference to a standard curve of known glucose concentrations. The total amount of reducing sugar in each sample was referred to as the carbohydrate content of the roots and was expressed as a percent of the dry weight of the roots.

RESULTS AND DISCUSSION

The estimates of root carbohydrate content expressed as a percentage of the dry weight of the roots are given for all treatments in Appendices 1 and 2. The infestation was assumed to be uniform at the start of the experiment and the first sample, taken on May 27, 1964 was a general composite sample and is used as the starting carbohydrate level for all treatments. For ease in discussing the carbohydrate trends, the ten treatments have been grouped under five headings on the basis of similarities between treatments.

(1) Seasonal trends of carbohydrate in the roots of untreated Canada thistle.

Treatment A was an untreated weedy check. These thistles were allowed to grow naturally and were not affected by any control measures or crop competition. Consequently carbohydrate fluctuations in their roots were considered to represent the normal seasonal fluctuations, affected only by climate and soil conditions and normal intraspecific competition.

The normal seasonal fluctuations were observed to follow a fairly specific pattern in both 1964 and 1965 (Fig. 1). In 1964 the carbohydrate levels declined rapidly from a spring high of 42.6 per cent on May 27 to a low of 30.9 per cent which occurred at the July 8 sampling date. Similarly, in 1965, the carbohydrate level declined from

39.7 per cent on May 14 to a low of 25.1 per cent on July 8. In both years the lowest levels of carbohydrate occurred at the July 8 sampling date. Field notes taken during both growing seasons show that most of the flower buds opened and the flowers were fertilized in the period from July 7 to July 16. Apparently the period from the start of growth in the spring until flowering constitutes a reproductive phase of growth during which the use of storage carbohydrate in cell differentiation and cell growth far exceeds its production by photosynthesis. This results in a net depletion of the carbohydrate in the roots.

After flowering with the completion of active growth and cell differentiation there was an almost immediate buildup of carbohydrates. In 1964 this buildup was slight from July 8 to July 29 but then continued at a much more rapid rate to a peak of 48 per cent on Oct. 1. In 1965 the buildup of carbohydrates was rapid from July 8 to the peak level of 39.6 per cent on Oct. 15. Since night temperatures as low as 20° F. became prevalent in the Sept. 15 to Oct. 15 period it seems evident that the late summer buildup of carbohydrate in treatment A was terminated by the prevention of photosynthesis resulting from frost damage to the shoots.

As a result of an exceptionally warm fall in 1964 sampling was continued until Nov. 12. After the late summer and fall accumulation of carbohydrate had been terminated by fall frosts a considerable decrease occurred. The carbohydrate level decreased from 48 per cent on Oct. 1 to 40 per cent by Nov. 12. This indicated a continued utilization of carbohydrate in the absence of replacement by photosynthesis. Late fall utilization of carbohydrates probably results from normal respiration and the production of buds and vegetative shoots. Canada thistle are known to produce underground buds and shoots up to three inches long in the fall (38). These remain dormant in the soil over winter and emerge the following spring. The generally lower carbohydrate levels in 1965 may be the result of larger than usual carbohydrate utilization during the exceptionally warm weather of the previous fall.

No actual late fall decrease in carbohydrates was observed in 1965. However, sampling was not continued as late in the fall of 1965 and this decrease may have gone undetected.

Results of this study indicate fairly predictable fluctuations in the seasonal levels of carbohydrate in the roots of untreated Canada thistles. These fluctuations are correlated with seasonal changes such as frost and with the growth stage of the plant. If differences in the dates of the start of spring growth and the dates of flowering caused by differences in climate are taken into account, the seasonal trends of carbohydrate reported in this study are similar to the trends found in studies by other workers. Arny (2), Rogers (37), and Welton et al. (45) each found that carbo-

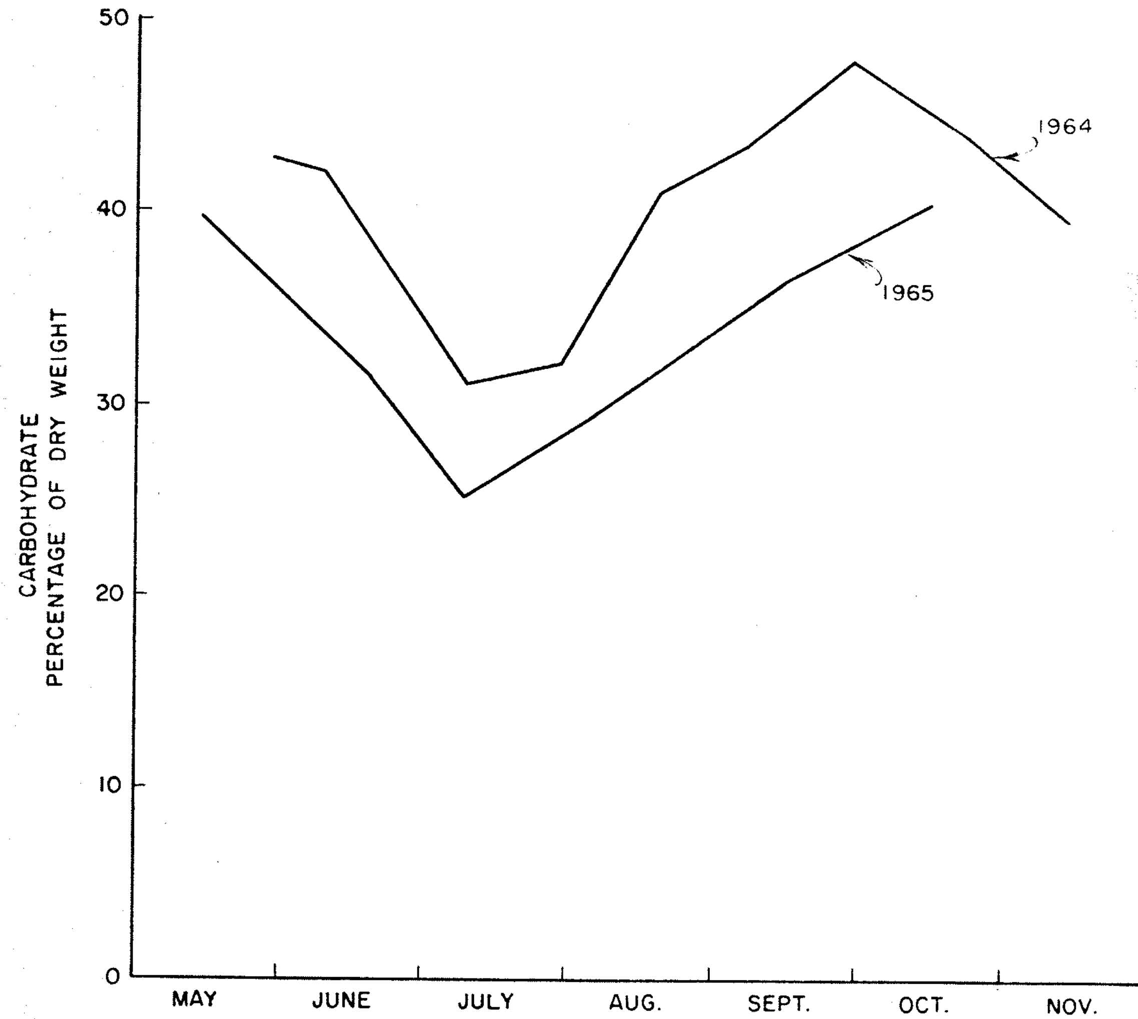

FIG. I

TREATMENT "A"

The seasonal trend of carbohydrate in untreated Canada thistle roots.

hydrate levels decreased until flowering time and then immediately increased. The date of flowering varied from June 1 to July 1 depending on the location of the study.

(2) The effect of summerfallow and defoliation treatments on the seasonal trend of carbohydrate in Canada thistle roots.

In a perennial weed storage carbohydrate is utilized in sending new shoots to the surface. If these new shoots are continually destroyed before they can replenish the carbohydrate reserve by photosynthesis then the carbohydrate level in the roots should decline progressively. Repeated shoot destruction was used in treatments B and C in an attempt to decrease carbohydrate content. In treatment B the above ground shoots were destroyed by repeated cultivation (each time they became 2 to 3 inches high) while in treatment C this destruction was accomplished by spraying with paraquat.

The effect of these treatments on the level of carbohydrate in 1964 is shown in Figures 2 and 3.

The effect of treatments B and C in 1964 was to cause increased utilization of carbohydrates in the early part of the season. This would be due to the fact that carbohydrate was constantly being utilized without replacement by photosynthesis. As a result the lowest carbohydrate level, which occurred on July 8, was 9 per cent lower than in the weedy check. In the cultivated plots (treatment B) this low level was maintained until Aug. 1 while in the paraquat plots (treatment C) it was maintained until Aug. 18. If photosynthesis was effectively prevented by these treatments no increase in carbohydrate would be expected. However, the late summer and fall buildup of carbohydrate observed in the weedy check was not prevented by either defoliation treatment. From a low point of 22.3 per cent the levels in both treatments had increased to 38 per cent by Sept. 8 and by Oct. 1 the level in treatment C was at almost the same level as the weedy checks.

Frazier (12) has shown that in bindweed more root reserves are utilized in forming the above ground shoot than in forming the rhizome and that as a result root reserves were depleted more rapidly if the shoots were allowed to grow for a time after emergence before they were cultivated. Consequently in the present study Canada thistle shoots were destroyed when they were 2 to 3 inches high. The intention was not only to prevent replenishment of reserves by photosynthesis but also to allow maximum depletion of reserves by allowing some shoot growth before cultivation. However, the large accumulation of carbohydrate in late summer and fall would seem to indicate that these small shoots were extremely efficient producers of carbohydrate. Results indicate that carbohydrate levels could have been decreased more rapidly by more frequent treatments at least after the July-August low period.

Carbohydrate declined prior to July 8 and increased after July 8 in spite of the fact that cultivation and paraquat treatments were made at approximately the same intervals and at the same stage of growth during both periods. This apparent difference in the efficiency of carbohydrate production may be explained in two ways.

In the latter part of the season, from July until October, the plants tend to form rosettes having larger more succulent leaves, rather than the upright shoot with smaller leaves which is typical of the early part of the season. These rosettes may be more efficient producers of carbohydrate and this could account for the late summer buildup.

The duration and intensity of sunlight will affect the photosynthetic activity of any plant. During July and August the amount of radiant energy received by the plants is much greater than during May and June and could account for substantial increases in photosynthesis. However, the fact that the carbohydrate level in these treatments and in the weedy check continues to increase throughout September and part of October after the intensity and duration of sunlight has begun to decline seems to indicate that sunlight is not a major controlling factor.

Although there was a large increase in carbohydrate percentage recorded in treatments B and C in the late summer of 1964 the degree of control obtained from these treatments was quite high. Field notes taken during the growing season indicated a substantial reduction in stand density. In the 1965 season thistle shoots were so few on both treatments that adequate samples could not be collected for carbohydrate analysis. Visual control ratings at the end of 2 years of 8.3 and 8.5 (10 indicates complete eradication) also indicate good control (Appendix 3). It would seem that in this case the trend of carbohydrate percentage was not a good indication of the vigor of the Canada thistle stand.

Linscott and McCarty (22) in a study of the effects of 2,4-D and mowing on ironweed concluded that reductions in ironweed vigor as a result of mowing were caused by a decrease in the dry weight of the root system rather than a permanent decrease in carbohydrate percentage. Similarly in the present study the fact that roots could not be collected from all plots indicates a decrease in the amount of roots present and the total dry weight of the roots may have been a better indicator of the vigor of the Canada thistle stand than was the carbohydrate percentage.

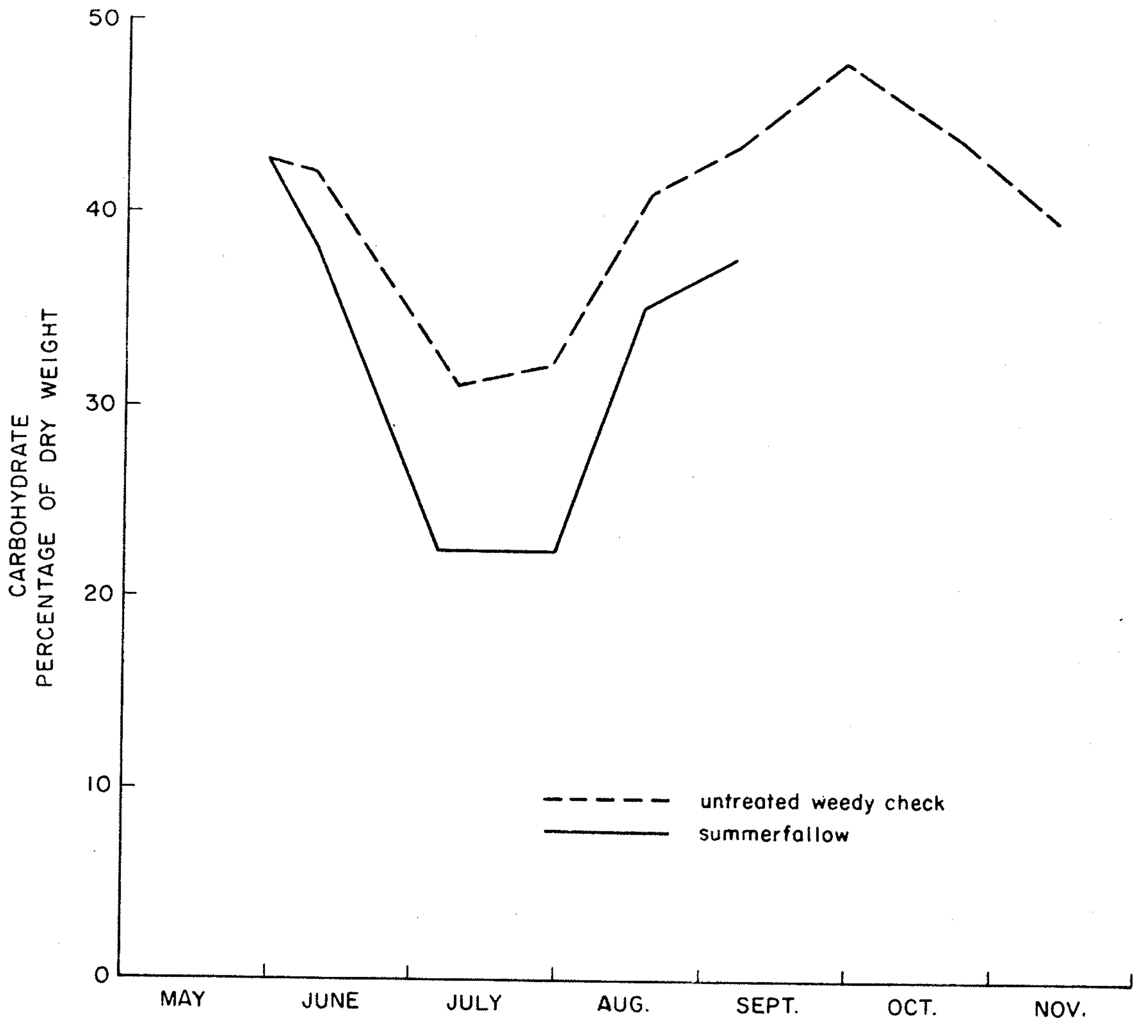

FIG. 2

TREATMENT "B"

The effect of regular cultivation on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

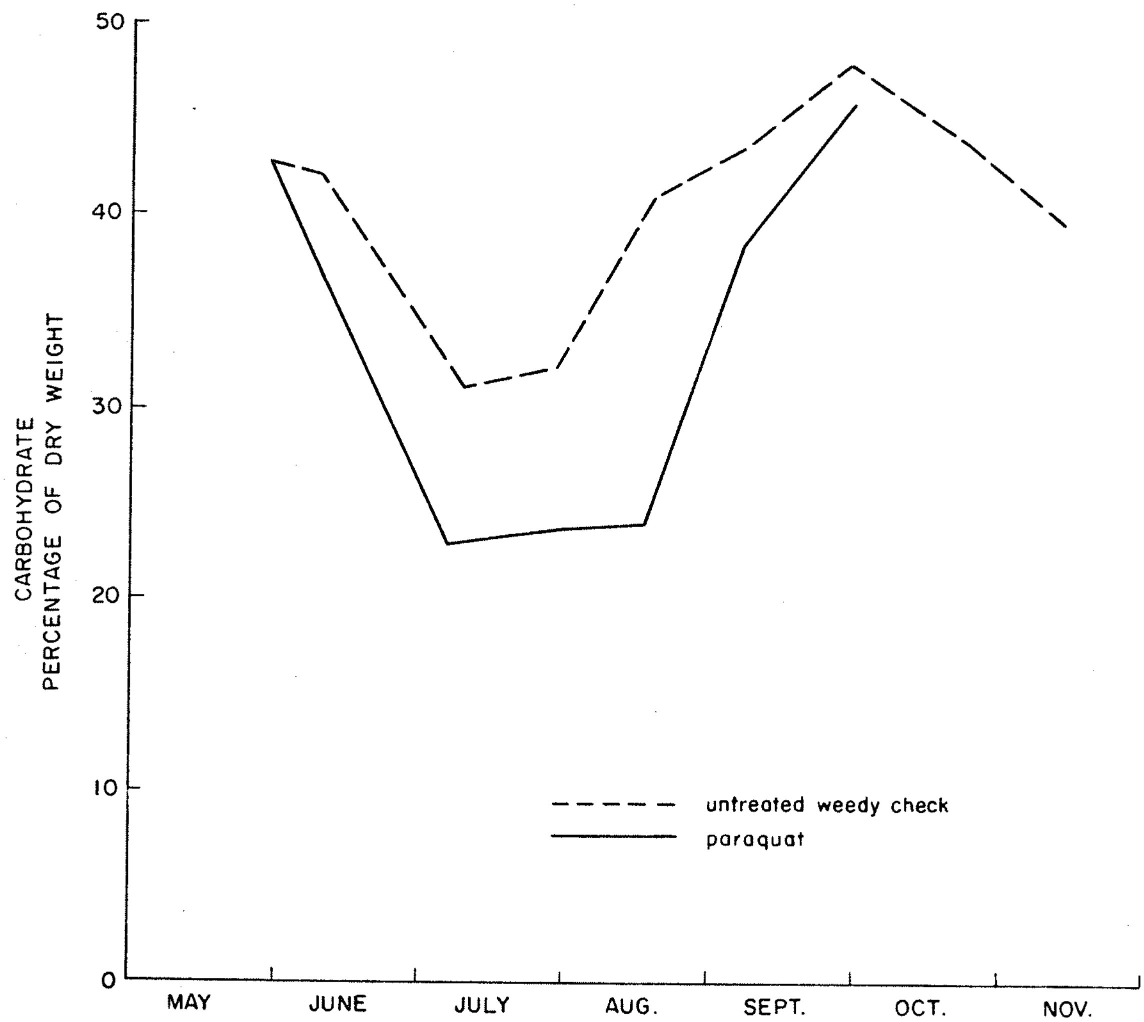

FIG. 3

TREATMENT "C"

The effect of regular defoliation with paraquat (1 pound per acre) on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

(3) <u>The effect of three herbicides, used in a summerfallow crop sequence, on the seasonal trend of carbohydrate and the control of Canada thistle</u>.

Treatments D, E and F were summerfallowed until July 10. After July 10 they were allowed to recover until Aug. 23 by which time some of the plants were budding. On Aug. 23 treatment D was sprayed with MCPA amine at 16 ounces per acre, treatment E with amitrole T at 4 pounds per acre and treatment F with dicamba at 4 pounds per acre. In 1965 oats were sown and treatments D and E were sprayed with MCPA amine at 16 ounces per acre, while treatment F was sprayed with dicamba at 4 ounces per acre.

None of these treatments effectively prevented the late summer and fall buildup of carbohydrate in 1964 (Fig. 4, 5, 6). Cultivation in the early part of the season reduced the carbohydrate level to about 20 to 21 per cent by July 8. This was 9 to 10 per cent lower than the untreated weedy checks and was almost the same effect which was obtained in the summerfallow and defoliation treatments (treatments B and C). In addition the low point in carbohydrate level was delayed until Aug. 1 in these three treatments. However, these apparent advantages were completely lost by allowing the plants to grow from July 10 to Aug. 23 before spraying, since by the spray date of Aug. 23 the carbohydrate in all three treatments was again equal to the level in the untreated weedy check.

These results further substantiate an apparent difference in the efficiency of net carbohydrate production in the plant at different periods in its growth. The growth of shoots prior to the flowering stage appears to cause a considerable net decrease in the percentage carbohydrate when this growth occurs during the May to July period. However in these three treatments (D, E, F) the same amount of growth, occurring after July 10, produces a substantial net accumulation of carbohydrate. This appears to be due to more efficient production of carbohydrate by photosynthesis in the months of July and August. More efficient production of carbohydrate could result from physiological or morphological changes in the plant, a higher intensity and duration of sunlight during these months or a combination of these factors.

The effect of the three chemicals on the carbohydrate level of the roots in 1964 was indicative of their different modes of action as well as of the degree of control obtained.

Amitrole T is a chlorophyll inhibitor which affects only new growth (36). Since there was little active growth after its application on Aug. 23 there was very little visible effect on the top growth and no apparent effect on the carbohydrate level which continued to increase and closely paralleled that in the weedy check. However amitrole T caused a substantial reduction in the thistle stand the following year as the roots collected in 1965 were not sufficient to construct a reliable carbohydrate curve. Following spraying of MCPA in the oat crop in 1965, the control rating for the treatment was 8.5 (Appendix 3). Thus amitrole T was a fairly effective treatment even though these results showed no reduction in carbohydrate percentage as a direct result of its application.

MCPA amine and dicamba sprayed in the summerfallow year caused a substantial reduction in the carbohydrate level (Fig. 4,6). The greater reduction resulted from the dicamba treatment which reduced the carbohydrate by 18 per cent from the date of spraying to Oct. 23 as compared to 12 per cent for MCPA amine. The dicamba treatment also gave the highest control rating of 9.3 as compared to 8.5 for MCPA. It does not seem likely however that the better control rating for dicamba was the direct result of a greater lowering of the carbohydrate level. Field notes taken during the 1964 growing season indicate that top growth control was almost complete by the middle of September although the carbohydrate level was still 34 per cent at that time. This indicates that, in the dicamba treatment, the lowered carbohydrate level was probably a symptom rather than the cause of the death of the plants.

The yield of oats in 1965 was 90.9 bushels per acre in the MCPA amine treatment and 92.8 bushels per acre in the amitrole T treatment (Appendix 3). However the average yield of the dicamba treated plots was only 69.6 bushels per acre as a result of severe crop injury caused by the residue of the extremely high rate of dicamba used the previous year.

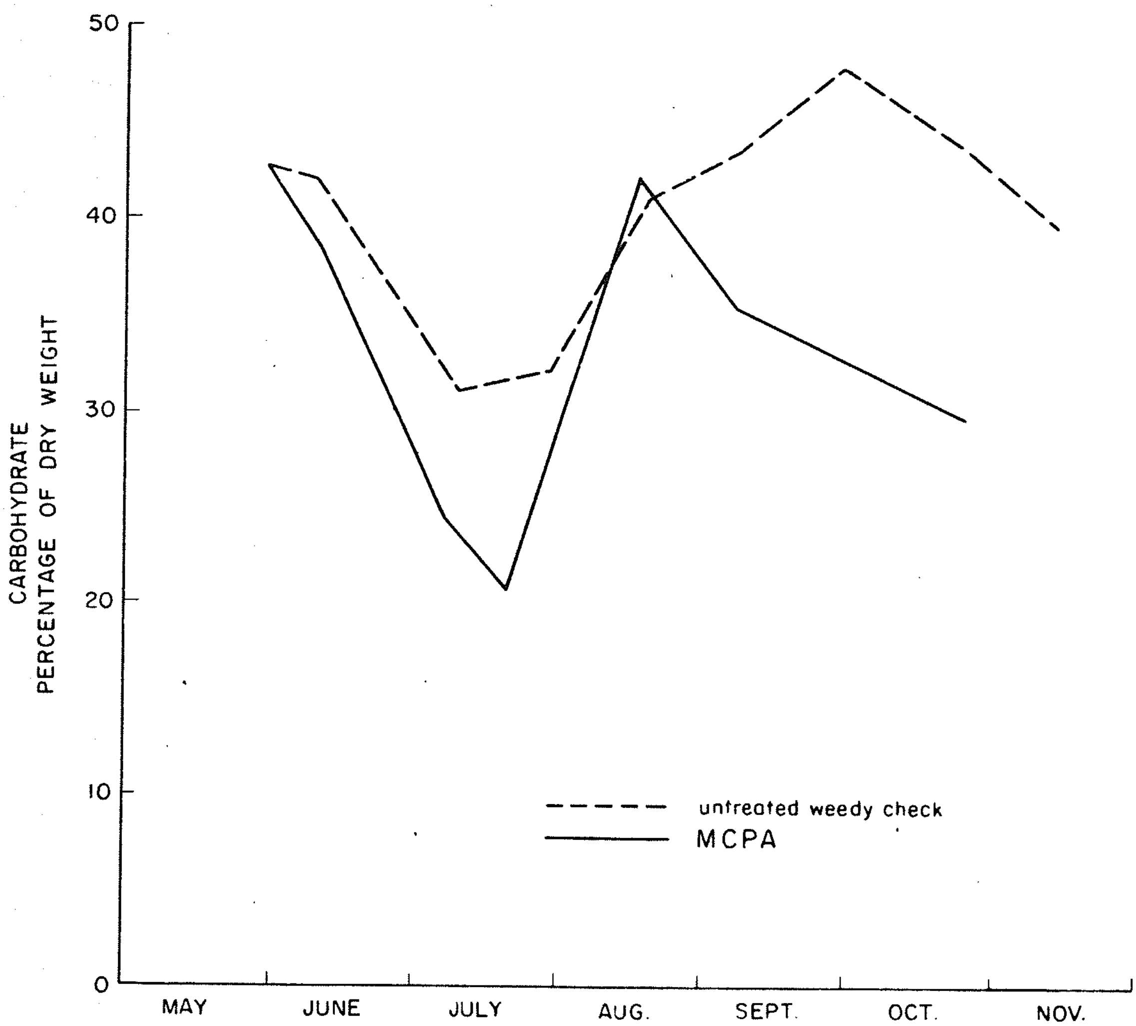

FIG. 4

TREATMENT "D"

The effect of MCPA amine (16 ounces per acre) applied during a summerfallow year on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

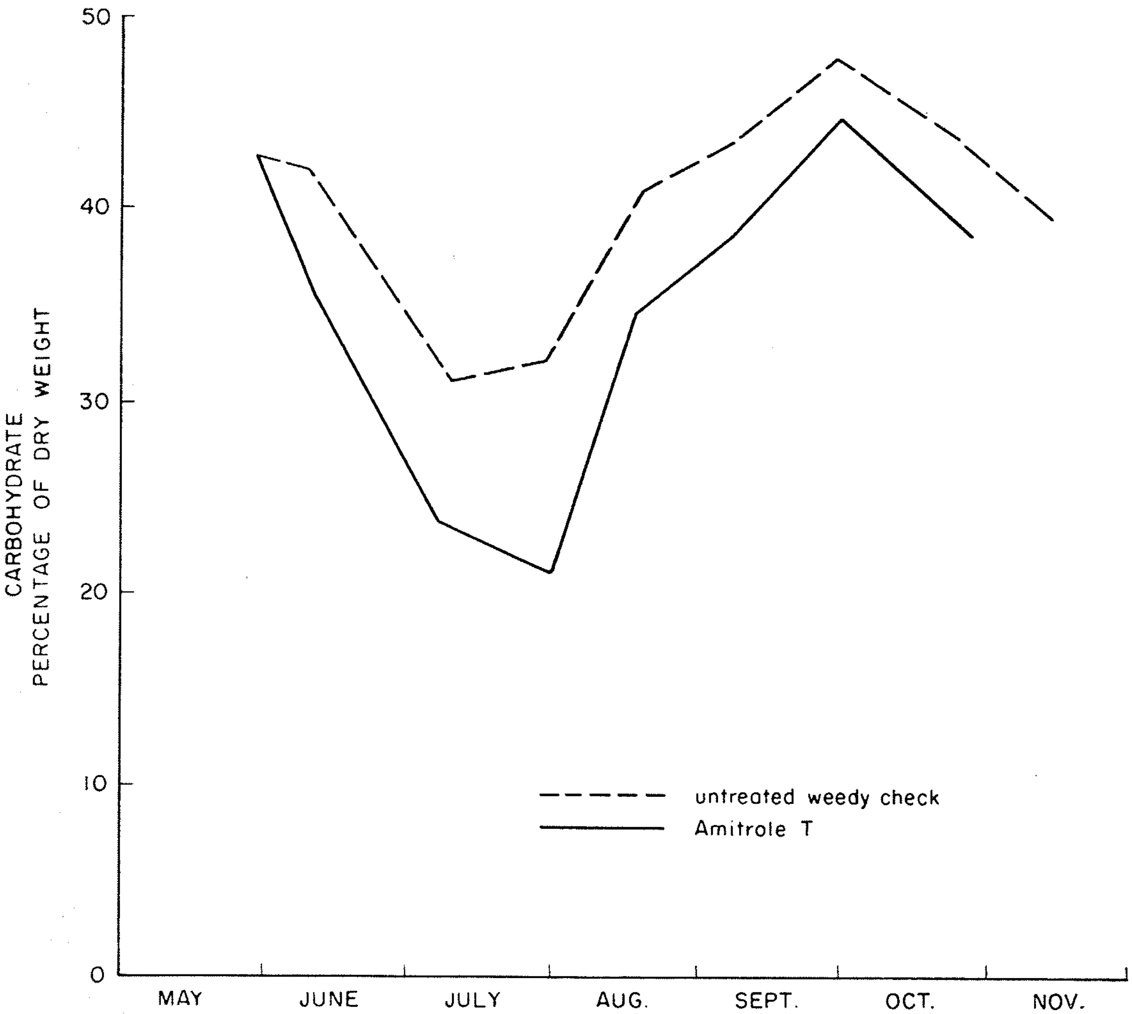

FIG. 5

TREATMENT "E"

The effect of Amitrole T (4 pounds per acre) applied during a summerfallow year on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

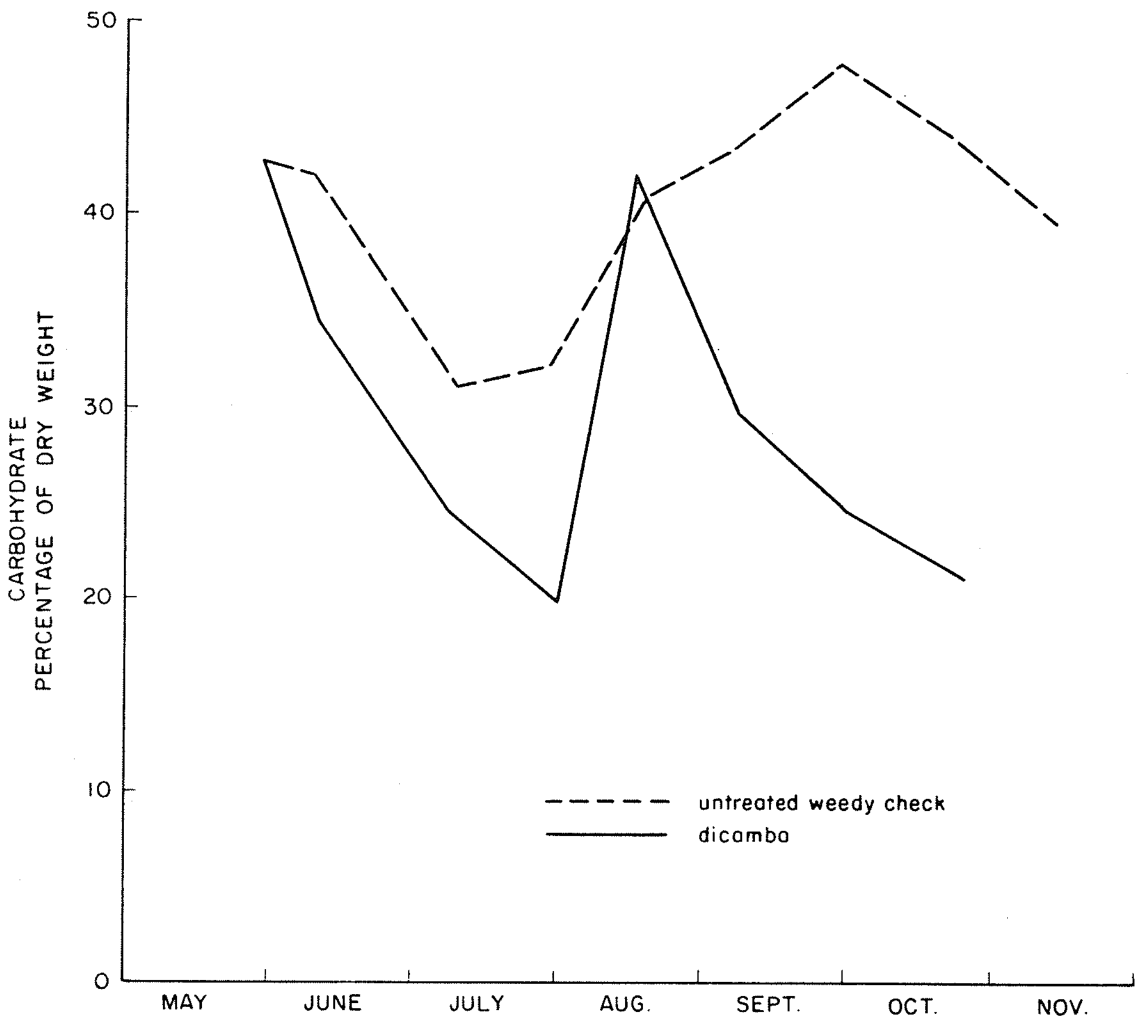

FIG. 6

TREATMENT "F"

The effect of dicamba (4 pounds per acre) applied during the summerfallow year on the seasonal trend of carbohydrate in Canada thiste roots, 1964.

(4) The effect of crop competition on the carbohydrate level and control of Canada thistle.

The competition afforded by a crop of oats (treatment G) did not cause any lasting effects on the seasonal carbohydrate levels in either year.

In 1964 crop competition resulted in a slight decrease in the carbohydrate level during the early part of the season (Fig. 7). After the flowering stage, carbohydrate percentage began to increase normally and this increase was apparently accelerated as the oats reached maturity in August and September. As a result the carbohydrate level was equal to the normal seasonal level by early September and thereafter followed the seasonal trend.

In 1965 the carbohydrate levels in treatment G remained slightly higher than the untreated levels (Fig. 8). However by early September the carbohydrate level was again the same as the level in the untreated check.

Visual estimates of control (Appendix 3) indicated a slight reduction in Canada thistle stand (control rating 2.3) as a result of two years of thistle-crop competition.

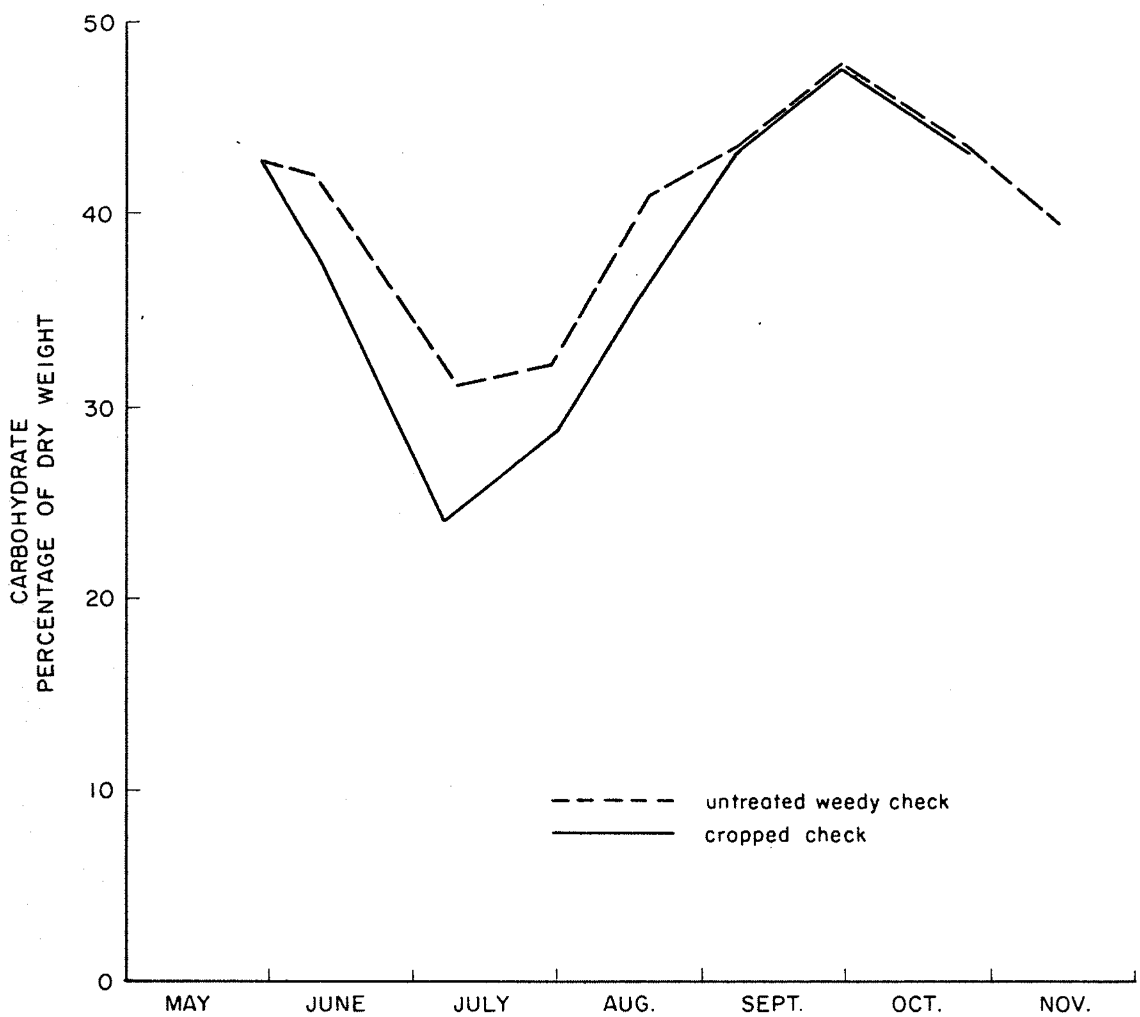

FIG. 7

TREATMENT "G"

The effect of crop competition on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

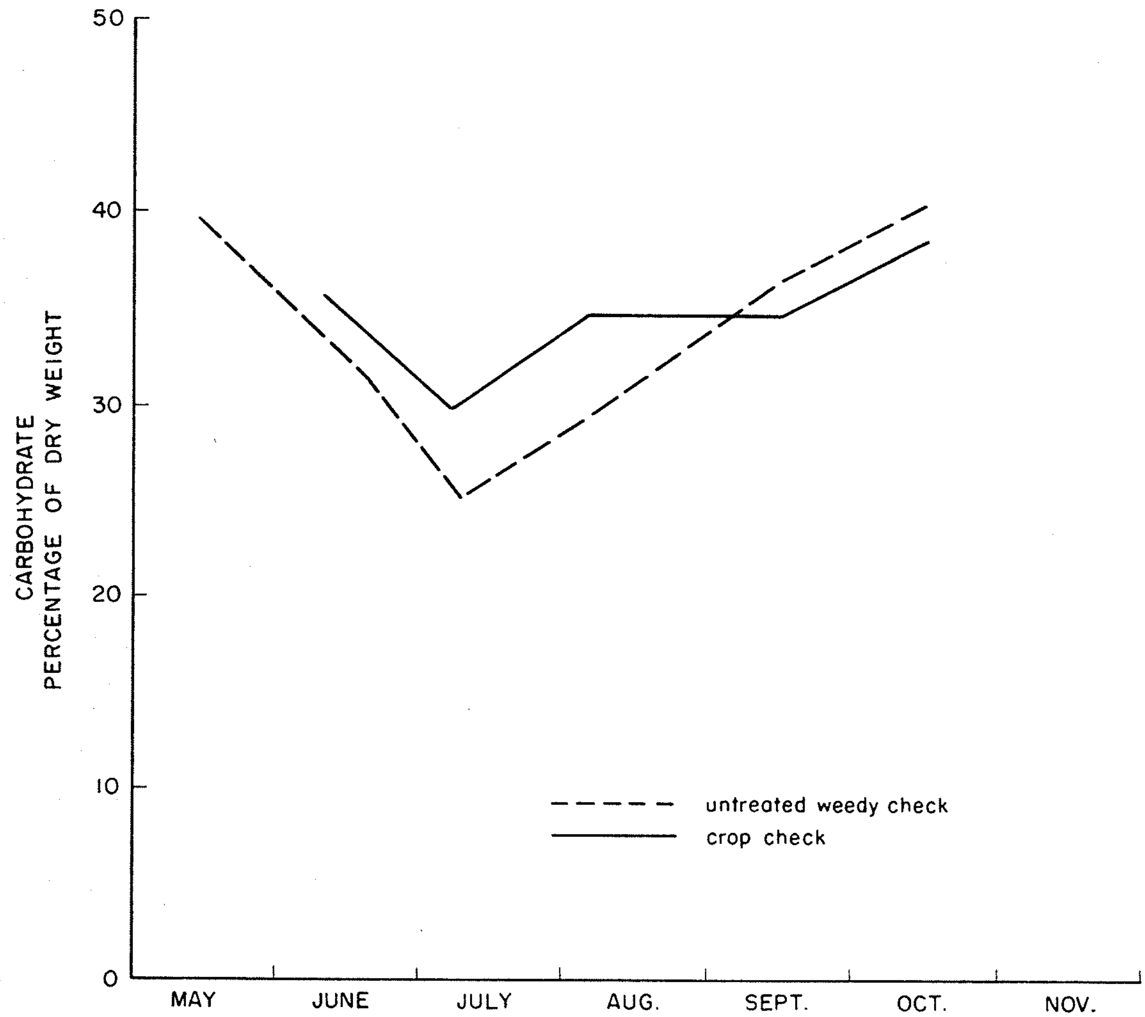

FIG 8

TREATMENT "G"

The effect of crop competition on the seasonal trend of carbohydrate in Canada thistle roots, 1965.

(5) <u>The effect of a two year sequence involving chemical herbicides in oats on the seasonal level of carbohydrate in Canada thistle roots</u>.

Treatments H, I and J were sown to oats in both seasons. Treatment H was sprayed with MCPA amine (16 ounces per acre in both seasons), treatment I with dicamba (4 pounds per acre in 1964 and 4 ounces in 1965) and treatment J with picloram (2 pounds per acre in 1964, 2 ounces in 1965).

The carbohydrate trend in the MCPA treatment was very similar to that of the cropped check until Sept. 8, 1964 (Fig. 9). After Sept. 8 it became slightly higher than the cropped check and remained higher until the end of the season. It is not immediately apparent why the MCPA should have caused an increase in carbohydrate level over the level of the cropped check. However, the effect is very slight, the MCPA treatment never exceeding the check by more than 5 per cent, and may have been due to a change in the competitive ability of the crop as a result of herbicide treatment.

Both dicamba and picloram treatments resulted in large depressions of the carbohydrate level in the roots. Immediately after spraying, the carbohydrate level in both treatments dropped to 17 per cent, the lowest level recorded in any treatment. Carbohydrate levels in the dicamba treated plots remained at 17 per cent until Aug. 1, then increased to 24 per cent by Aug. 18. After Aug. 18 top growth control was virtually complete and it was no longer possible to obtain root samples large enough for carbohydrate analysis.

Similarly in the picloram treated plots carbohydrate levels declined to 17 per cent and remained at that level until Aug. 1 after which sampling was discontinued because roots could not be found.

Whenever herbicidal eradication of a weed is accompanied by a large decrease in the carbohydrate percentage the question arises as to whether this decrease is the cause of death or merely a symptom. In the dicamba and picloram treatments (I and J), although the levels were the lowest of any of the treatments, they had stopped decreasing or were increasing by the time top growth control became complete. This seems to indicate that the low carbohydrate levels were a symptom rather than the cause of death.

The heavy rates of dicamba and picloram applied in 1964 caused a severe crop injury. As a result of herbicide residues from these heavy applications, injury was also evident in these plots in 1965. However in spite of this severe injury, crop yields were higher in these treatments in both years than they were in the untreated cropped checks. This increase was due to the removal of thistle competition in these plots and consequently the yields tended to be related to the degree of control obtained. The picloram and dicamba treatments gave the best control (9.8 and 9.5) and also gave the highest yields (Appendix 3). The MCPA treatment resulted in lower control (7.8) and somewhat lower yields.

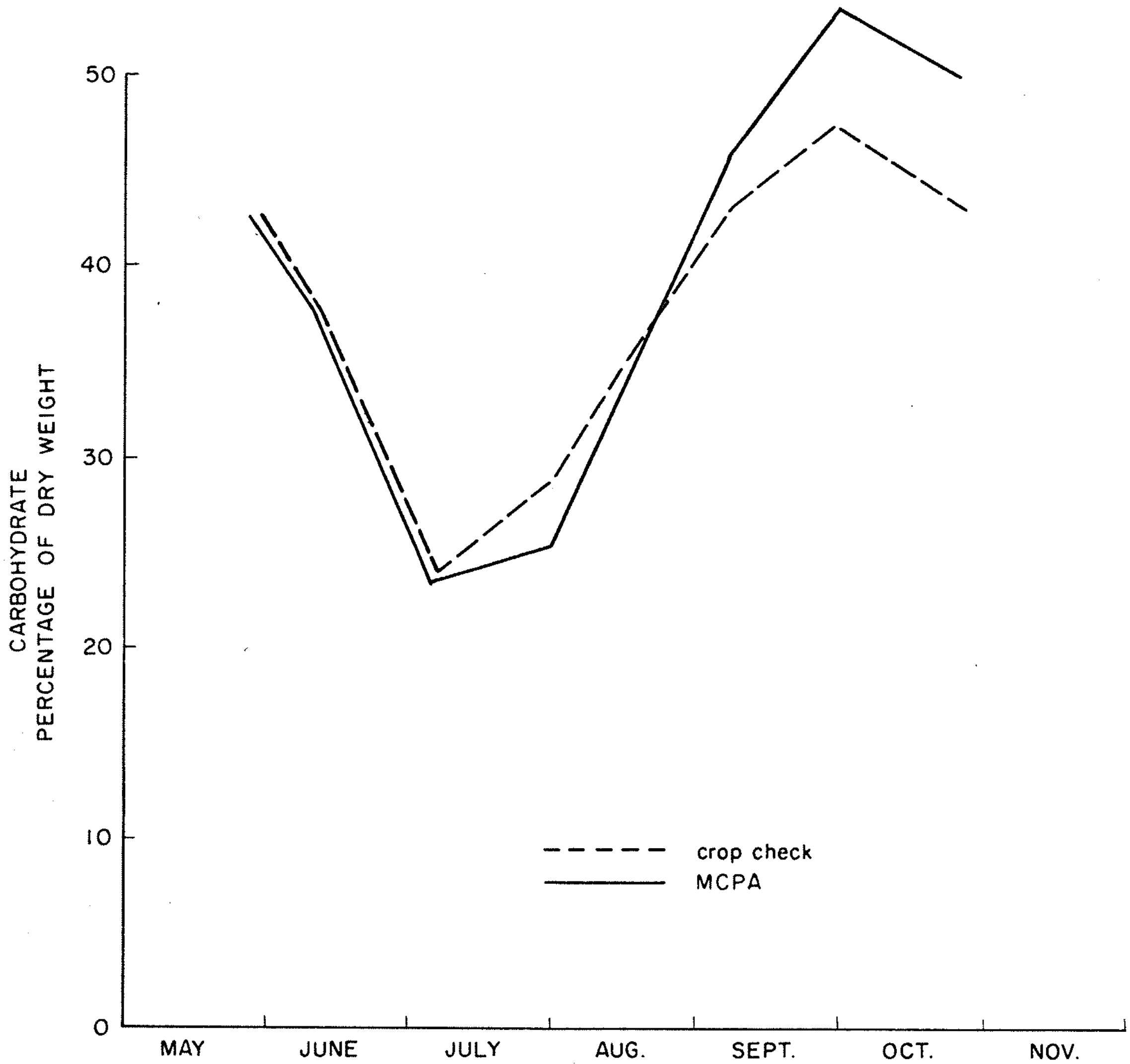

FIG. 9

TREATMENT "H"

The effect of MCPA amine (16 ounces per acre) applied in crop on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

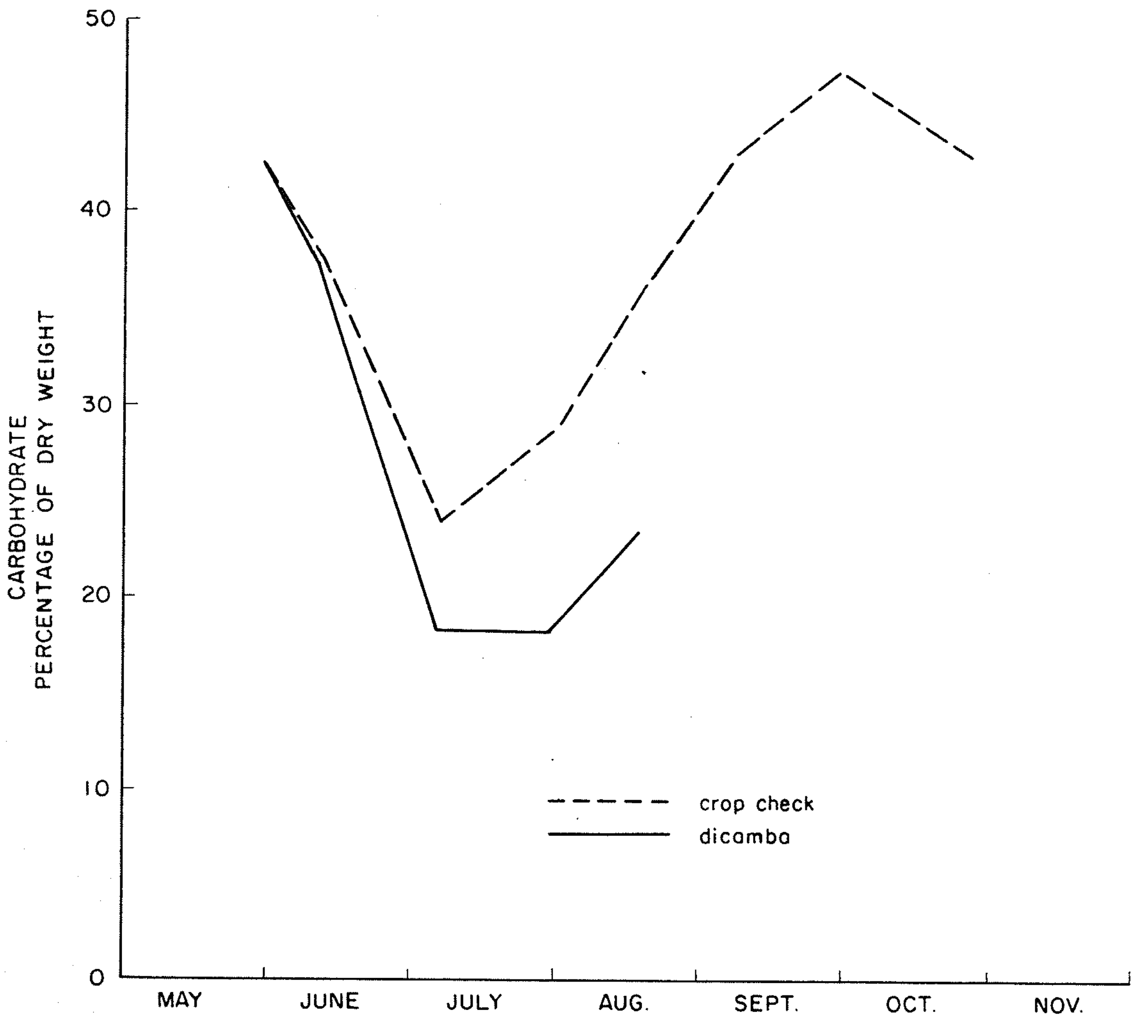

FIG. 10

TREATMENT "I"

The effect of dicamba (4 pounds per acre) applied in crop on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

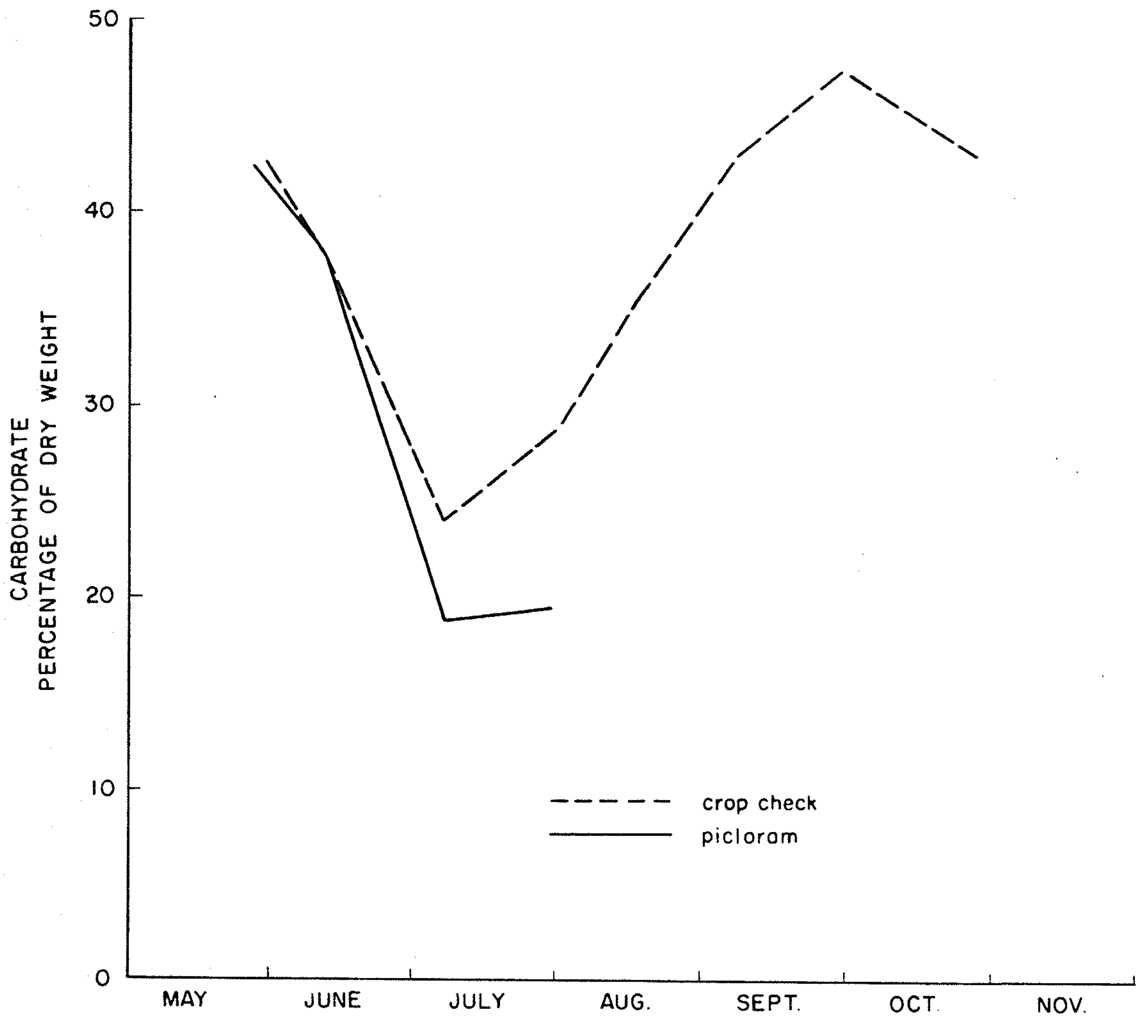

FIG. 11
TREATMENT "J"

The effect of picloram (2 pounds per acre) applied in crop on the seasonal trend of carbohydrate in Canada thistle roots, 1964.

SUMMARY AND CONCLUSIONS

The ability of Canada thistle to recover after cultivation depends on the supply of carbohydrate in the perennial root. Consequently a knowledge of the relative level of this carbohydrate supply at various seasons and stage of growth is important to farmers and others interested in Canada thistle control. This study was therefore intended to determine the level of carbohydrate under Manitoba conditions, both seasonally and after various cultural and chemical treatments.

A two year field experiment consisting of ten treatments including a check was laid out in a randomized block design in an established infestation of Canada thistle near Rosser, Manitoba. Samples of lateral roots were taken from the first foot of soil in each plot at approximately three week intervals. Sampling was continued for two growing seasons although several of the treatments reduced the infestation enough that samples could not be obtained in 1965. These samples were taken into the laboratory and after drying and grinding were analysed for carbohydrate content by a combination of acid hydrolysis and a colorimetric estimation of the reducing sugars.

Oat yields and Canada thistle control ratings were determined as supplementary data. This data was found useful in interpreting and assessing the changes in carbohydrate level which were observed.

The results of this study indicate that:

(1) Under Manitoba conditions the carbohydrate levels in Canada thistle roots exhibit a fairly definite seasonal fluctuation. This consists of a rapid decrease from the beginning of growth in the spring until flowering time and then a rapid increase until growth is stopped by fall frosts. A slight decrease in carbohydrate percentage occurs after the fall frosts.

These results are in agreement with the findings of other workers if different dates of flowering at different locations are taken into account.

(2) During the early part of the season the carbohydrate level could be reduced below the seasonal level by regular destruction of the top growth. However the later summer build-up of carbohydrate was not prevented by the treatment interval used because of the unexpectedly high photosynthetic efficiency of thistle shoots 2 to 3 inches in height.

(3) The efficiency of photosynthesis appeared to be much higher during July and August than it was either before or after this period. It was not immediately evident whether this results from the increase in sunlight intensity and duration during these months, or from possible physiological and morphological changes in the plant.

(4) Because a large increase in percentage carbohydrate coincided with excellent control in the regularly defoliated plots it was suggested that total dry weight of the root system

may be a better indicator of the vigor of a stand of Canada thistle than the carbohydrate percentage.

(5) A competing crop of oats caused only a temporary reduction in carbohydrate level which was rapidly replaced as the crop matured. Some reduction in the stand density occurred as a result of competition however.

(6) The reduction in carbohydrate resulting from summer-fallowing was quickly and completely replaced if cultivation was suspended during July and August.

(7) It was possible to completely eradicate Canada thistle with high rates of dicamba and picloram. When these herbicides were sprayed in crop, the elimination of Canada thistle competition resulted in a yield increase even though severe crop injury resulted from the high herbicide rates. These treatments caused reductions in carbohydrate percentage which appeared to be a symptom rather than a cause of the death of the plant.

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APPENDIX

Appendix 1. Carbohydrate levels in Canada thistle roots (per cent of dry weight). Treatments A to J, 1964.

Treatment	Sampling Dates								
	May 27	June 10	July 7	July 29	Aug.18	Sept.8	Oct.1	Oct.23	Nov.12
A	42.6	41.9	30.9	32.3	40.7	43.5	47.8	43.8	39.6
B		38.2	22.3	22.3	35.0	37.8	-	-	
C		36.7	22.7	23.7	23.9	38.7	45.9	-	
D		38.9	24.6	20.9	42.3	35.1	30.8	29.62	
E		35.7	23.8	21.2	34.4	38.5	44.6	38.6	
F		34.4	24.6	20.0	42.1	29.1	24.5	23.7	
G		37.8	23.4	28.9	35.6	43.8	48.2	43.8	
H		37.4	23.3	25.0	36.5	45.4	53.1	49.8	
I		37.6	17.6	17.6	23.4	-	-	-	
J		38.3	18.8	19.5	-	-	-	-	

Appendix 2. Carbohydrate levels in Canada thistle roots (per cent of dry weight). Treatments A - J 1965.

Treatment	Sampling Dates					
	May 5	June 10	July 8	Aug.8	Sept.14	Oct.15
A	39.7	31.4	25.1	29.9	36.6	40.1
B	47.2	50.2	17.3	29.4	61.7	
C		32.0		24.9		
D	29.6				41.8	
E	34.0	41.9	22.1		44.1	
F						
G		35.7	29.8	34.9	34.8	38.6
H					49.08	
I						
J						

Appendix 3. Yield of oats in treatments A to J in 1964 and 1965 and Canada thistle control ratings (0-10) at the end of the two year study.

Treatment	Oat Yield (bus./Ac.)		Control 0 = no control 10 = complete control
	1964	1965	
A	-	-	-
B	-	-	8.5
C	-	-	8.3
D	-	90.9	8.5
E	-	92.8	8.5
F	-	69.6	9.3
G	12.7	37.5	2.3
H	23.0	50.6	7.8
I	30.3	55.6	9.5
J	38.5	62.9	9.8