# FACTORS AFFECTING THE GROWTH OF HORSENETTLE (SOLANUM CAROLINENSE L.) AND ITS RESPONSE TO GLYPHOSATE AND TERBACIL

Dissertation for the Degree of Ph. D.
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ABSTRACT

FACTORS AFFECTING THE GROWTH OF HORSENETTLE (*SOLANUM CAROLINENSE* L.) AND ITS RESPONSE TO GLYPHOSATE AND TERBACIL.

By

Gregory Paul Pagano

Horsenettle (*Solanum carolinense* L.) is a persistent perennial weed in many orchards, vineyards and cultivated fields in Michigan. The major problem in controlling this weed is the regrowth potential of the storage roots which can repeatedly produce new shoots as tops are removed. The objective of this study was to determine some factors which influence growth and reproduction of horsenettle and to define parameters affecting the toxicity of a soil sterilant and translocated herbicide on this weed.

High light and low night temperatures were found the most conducive to root formation and early flowering. Young plants (61 days from seed) were capable of producing new shoots from root sections within 28 days from sectioning. Plants with the root system left intact and the tops removed produced new shoots within 18 days.

Horsenettle seed is susceptible to freezing. A temperature of -18.9 C for 40 days killed all seed. Seed from berries overwintered in the field had a 30% germination.

The starch content of the storage roots was about 36% prior to emergence in the spring, declined to 13% at flowering and increased by late summer to the original level.

Horsenettle regrowth was controlled for the season with 2.2 kg/ha terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) independent of the time of application in both a cultivated field and an orchard. Storage roots of plants receiving terbacil were almost completely depleted of starch by the end of the season. Glyphosate [N-(phosphonomethyl)glycine] provided effective control if applied at 4.5 kg/ha when the plants were in full flower. Glyphosate at 2.2 hg/ha applied during bloom did not control regrowth in the cultivated field. Glyphosate applied at the prebud stage only slightly reduced starch levels in the storage roots at the end of the growing season, whereas applications at other times caused no apparent starch depletion.

Both $^{14}C$-labeled glyphosate and terbacil were applied to the leaves of horsenettle at four stages of growth. Labeled terbacil was also applied to the roots via nutrient solution. The $^{14}C$-methyl labeled glyphosate was readily absorbed and translocated to meristematic regions. Glyphosate translocation in older plants was over ten-fold that which occurred in younger plants although immature leaves of the younger plants initially absorbed more herbicide. Translocation of glyphosate follows a source to sink pattern typical of phloem transported chemicals.

Plants treated with 2-$^{14}$C-terbacil were uniformly labeled after uptake from nutrient solution indicating xylem transport. Only 0.5 to 1.0% of the applied terbacil was taken up by the leaves. There was limited translocation of $^{14}$C after foliar terbacil applications.

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By

Gregory Paul Pagano

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# INTRODUCTION

With the increase in the world population, the burden placed on agriculture to produce both food and fiber is one that must be carried well enough to insure an adequate supply of essentials. Anything that would impair the productivity of our farm lands must be controlled or abated.

It has been estimated that in the United States weeds cause greater crop losses than either insects or plant diseases (16). Weeds are the major barrier to food production and economic development in many regions of the world. Our ability to control weeds which compete with crop plants for light (18), water (52) and nutrients (29, 33) and thereby reduce all important crop yields may be a determining factor in the condition of the human health and well being for years to come.

Weeds are often grouped into three general classifications; perennial, annual and biennial. A perennial plant is one that lives at least 3 or more years. Simple perennials such as dandelion (*Taraxacum officinale* Weber), curly dock (*Rumex crispus* L.) and broadleaf plantain (*Plantago major* L.) reproduce primarily by seed but can also reproduce vegetatively. Creeping perennials may reproduce by tubers, such as purple nutsedge (*Cyperus rotundus* L.), creeping roots as with field bindweed (*Convolvulus arvensis* L.), stolens as with bermudagrass (*Cynodon dactylon* (L.) Pers.) or by rhizomes as in quackgrass (*Agropyron repens* (L.) Beauv.) and spread rapidly through a field. They may also be introduced to another field by seed.

Creeping perennials are probably the most difficult weeds to control, because of their extensive storage organs and capacity for vegetative reproduction.

Horsenettle (*Solanum carolinense* L.) is a member of this class of weeds. In Michigan it is often found in perennial crops such as orchards or asparagus grown on coarse textured soils, where perennial grass competition is minimal. Being a member of the Solanaceae, it is often found in fields where Solanaceous crops such as tomato (*Lycopersicon esculentum* Mill.) have been grown for a number of years and where herbicides were selective for horsenettle along with tomatoes. Horsenettle is also a host for several insects and diseases of solanaceous crops, compounding the problems it causes in the field. Herbicides and cultivation have not provided adequate horsenettle control in these crops. The results of some workers (12, 23) indicated that cultivation actually enhanced the problem since the weed is capable of reproducing from small root sections.

The purpose of this investigation was to study the factors which affect the growth and reproduction of horsenettle and to determine if several new herbicides would provide effective control. Factors affecting herbicide uptake and translocation and effect on storage reserve depletion in the horsenettle roots were also investigated.

## CHAPTER 1

## LITERATURE REVIEW

### Horsenettle (*Solanum carolinense* L.)

*Distribution*. Horsenettle (*Solanum carolinense* L.) is one of the numerous members of the nightshade family, Solanaceae. The plant is a perennial weed (17) often found in waste places where grass competition is minimal (12).

Originally it was native to the southeastern states, but is not considered a troublesome weed there (1, 19). Currently, it is established as a weed as far north as Vermont, Pennsylvania, Ontario, Michigan and Minnesota and west to Washington and California (11, 17, 19, 27).

*Shoot morphology*. Horsenettle is an erect stemmed and branched plant up to 1 m. The main stem, branches, petioles, calyx, midribs and lateral veins of leaves have rigid, sharply pointed, yellowish spines and four to eight rayed hairs. The leaves are alternate, simple, 5 to 9 cm long, oblong to ovate in outline with unevenly lobed, toothed or deeply cut margins (23).

The flowers, on spiny petioles are perfect, complete and open indeterminant racemes. The spiny peduncles are irregularly spaced along the sides of the stems. The five lobed carolla varies in color from violet to white and is about 2.5 cm in diameter. It closely resembles the flower of the common potato (*Solanum tuberosum* L.). There are five fused yellow stamens surrounding the two-celled ovary (17).

The fruit or berry is tomato-like, smooth, round, one to two cm in diameter, orange yellow in color when mature and subtended but not enclosed by the calyx (17).

The seeds of horsenettle resemble those of the common potato in size, shape, color, and internal structure (23). It is yellow brown in color, orbicular in outline and flattened in cross section. The surface has a slightly pebbled appearance.

*Root morphology*. Horsenettle is considered to have a taproot and creeping rootstock system (49). The roots range in color from light brown to almost white. They are thick and somewhat fleshy. The lack of agreement in the literature on whether horsenettle is spread by means of rhizomes or creeping horizontal roots or rootstocks (3, 25, 31, 49) prompted Ilnicki *et al*. (23) to investigate the nature of the tissue. Using phloroglucinal stain which is specific for lignins it was determined that in no instance did an underground part of the plant produce the characteristic staining which would have classified it as a stem like structure. Ilnicki concluded that all underground portions are roots and not shoots.

*Economic importance of horsenettle*. Horsenettle along with other weeds compete for water, nutrients, light and space with crop species. This alone would be reason enough to attempt total eradication of the weed. Perennial weeds are particularly competitive and cause not only yield reductions, but may also reduce the stand of perennial crops like asparagus.

Horsenettle has been shown to be an important host for the potato psyllid (*Protrioza cockerelli* Sulc.) which transmits psyllid yellows disease to the potato and tomato (51). Other important crop insects reported to be harbored by horsenettle are the potato flea beetles (*Epitrix fuscula* Crotch) (14), Colorado potato beetle (*Leptinotarsa decemlineata* Say) (29), potato stalk borer (*Trichobares thinotata* Say) (23), onion thrips (*Thrips tobaci* Lind.) (23), and the greenhouse redspider mite (*Tetranychus telaruis* L.) (31).

Horsenettle is also a host for the fungi (*Septoria lycopersici* Speg.) which causes a leafspot in tomato (36), the disease organism (*Verticillium albo-atrum* Reinke and Berth) which causes Verticillium wilt of tomato (23), and to the mosaic virus which causes potato and tomato mosaic (31).

Weed control methods requiring considerable hand labor or fuel may represent a large portion of the input required to produce a crop. This input is reflected in higher food costs (26). Horsenettle, a perennial without any acceptable long time chemical means of control, is often spot treated with a contact herbicide or repeatedly cultivated. This adds to the time requirement and the cost of production. Horsenettle presents another problem when present in crops such as asparagus and tomatoes where hand harvesting is a common practice. The spines present on the leaves and stems make hand picking of the crops slow and difficult and labor may actually refuse to enter the field. Heavily infested fields may actually have to be taken out of crop production causing great economic loss.

Horsenettle berries have been reported as being poisonous (32) and when present in forage, they have caused losses in cattle and horses (27).

The vegetative development and reproduction of horsenettle. Bradbury (12) observed differences in growth patterns between disturbed and undisturbed infestations of the weed. The older undisturbed areas were less thickly populated with horsenettle than were cultivated areas. Thicker stands on cropland were attributed to propagation from pieces of root stalks mixed in the soil by seedbed preparation and other cultural practices.

Kiltz (25) showed that the roots grow to depths of 2.4 m in heavy soils and that creeping roots found in the upper 45 cm of soil extended 0.9 to 1.2 m from the main root. Bradbury's (12) excavation of horsenettle revealed a penetration of at least 2 m in depth. He excavated a single intact creeping root 5.8 m in length having numerous shoots emerging from it.

Most of the work completed on the reproduction of horsenettle was done as a joint effort of the New Jersey Agricultural

Experiment Station and New York Agricultural Experiment Station under Ilnicki and Fertig respectively (23). Working with seedlings, it was found that the maximum increase in plant height occurred during the middle of the growing season (August) whereas the maximum increase in root length occurred during the latter part of the season (September). Plants started from seed become firmly established early in the growth period due to rapid and extensive root development which occurred at the expense of foliage growth. They concluded seedling control is necessary to prevent firm establishment of plants from seed.

A study on the influence of size of root cutting on reproduction showed that cuttings over 2.5 cm in length and 1 cm in diameter would produce new plants within 42 days. Thus cultivation could play an important role in the dessimation of the weed by root cuttings. Bradbury (12) reported that older, undisturbed areas were less thickly populated, most likely because of the roots being contiguous and poor reproduction by seed.

In another test, several plants which had been grown for one year from seed in wooden flats were dug up and the roots cut into sections 15 cm in length. These were planted outdoors at depths of 7.5, 15, 30 and 45 cm. All cuttings produced at least one shoot each from depths down to 30 cm. Four out of six cuttings produced plants from the 45 cm depth 67 days after planting. Based on this observation the authors did not expect an appreciable reduction in stand from deep tillage that is normally practiced in the Northeast. They speculated that fallowing may gradually reduce the stand by depletion of the root reserve.

The effect of polarity on asexual reproduction from horsenettle roots has also been studied. Segments were taken from the vertical taproot developed shoots at the morphological apex in approximately 80% of the cases. The lateral root sections produced very few shoots.

Explanations offered for this phenomenon include a concentration differential of nutrients or other growth factors from one end of these segments to the other end. However, it was also shown that the lower portions of the taproot are higher in starch than the upper portion suggesting that a slight starch differential exists in the taproot section. The morphological apex being somewhat lower in starch content than the other end could affect a relative increase in the amount of protein available for new shoot growth.

Apparently, segments are capable of producing shoots from the morphological apex regardless of the planting position or size of the cutting. Furthermore, the distance removed from the top of the vertical taproot did not affect the production of the shoots.

The findings have both practical and fundamental applications. A growth controlling mechanism exists and if determined, could give greater insight to the nature of regrowth of perennial weeds. Furthermore, practical control of horsenettle might be achieved by adjusting conditions to take advantage of this growth controlling mechanism either by stimulating shoot growth in an attempt of systematically exhaust the reserves in the root or total suppression of shoot growth.

Development of Horsenettle from Seed. Ilnicki and Fertig (23) also performed a number of tests on seed germination and seedling growth.

There was considerable variation in the number of seeds per berry. They found that numbers of seeds varied from 161 for large berries to 13 for small berries. The average number of seeds for all berries was 86.

In a study to determine the effect of light on seed germination it was found that light was not an important factor affecting total germination, but that exposure to light increased the germination rate.

Pre-germination cold treatments had no effect on germination. The condition most favorable to germination of mature seed overwintered in the field was alternating the temperatures from 20 to 30 C in the dark and moistening the seed with a 0.2 percent potassium nitrate solution.

Bradbury (17) indicated that emergence of horsenettle plants from seed under field conditions was a rare occurrence. Ilnicki (23) studied the dissemation of seed under natural conditions. On May 15th about .04 ha of a 1.6 ha field near

Newfield, New York was surveyed. Fifteen seedling locations were found and marked for future observations. About 200 seedlings were found in this area. Observations in September, 1959, showed that 50 percent of the seedlings had survived the summer. No data was reported on the number of seedlings that survived the winter, although the author concluded that emergence form seed was an important factor to be considered in the dissemination of the weed. It is important to note however, that this work was conducted on non crop land (ie. land that received no tillage or herbicide applications).

Tests were conducted to determine the effects of soil type on the germination of horsenettle seed planted at various depths (23). The most rapid rate of emergence was at the 2.5 cm depth and occurred during the first 3 weeks after planting. Seed planted at the 7.5 cm depth showed practically no emergence until 4 weeks after planting. Emergence of seedlings from the 2.5 cm depth was four to ten times greater than from the 7.5 cm depth in the well drained soils. Percent emergence in the fine textured soils was less than one half that in the well drained soils at the 2.5 cm depth. No seedlings emerged from the 7.5 cm depth in the fine textured soils. It appears that the chances of horsenettle infestations starting from seed are considerable greater on coarse textured well drained soils than on heavy, somewhat poorly drained soils.

Reports indicate that seedlings can emerge from mature, undried whole berries located within 2.5 cm of the soil surface. Seedlings emerged more rapidly from broken berries than from unbroken ones (23). The emergence from berries may indicate why the horsenettle seedlings found growing in the field described earlier were generally found to be clustered with about 15 to 20 seedlings at a location.

The effect of over-wintering on young horsenettle seedlings has been studied. On August 9, 120 seeds were placed in frames at a depth of 2 cm. Sixty eight percent emergence was obtained within 37 days. Of the 82 plants produced from seed, eight plants (10%) survived the winter.

## Control of Horsenettle

Effect of management practices on control. Ilnicki (23) studied the influence of the stage of seedling development on regrowth after top removal. Top growth was cut from seedlings at the soil surface at intervals of 15 to 30 days after emergence of the seedling. Regrowth was produced when shoots were removed even early in the seedling stage. With increased age of horsenettle plants, more rapid regrowth of shoots occurred following temporary setbacks due to top removal.

Ilnicki *et al*. (2) also studied the effect of cutting frequency on subsequent growth. A cutting height of 6.3 cm was used to approximate the height of cut of the conventional farm mowing equipment. After planting root cuttings on April 28, shoots began emerging by May 30 and of the 92 root cuttings, 91 produced at least one shoot. All plants receiving the cutting treatment at 6.3 cm every 5 to 8 days survived the treatment. These plants over-wintered with no mortality and were growing vigorously in the following spring. Two of the 12 plants receiving this treatment produced berries below the cutting height. These plants, subjected to a vigorous cutting schedule produced a relatively large number of leaves due to the greatly shortened internodes on the main stem. This compact, rosette-type appearance was a characteristic change in the habit of growth. The large leaf area provided sufficient photosythetic surface to maintain a daily growth rate comparable to plants cut at less frequent intervals.

Plants cut more than once during the season maintained a greater daily growth rate than those cut at less frequent intervals or not at all possibly because they remained in the actively growing vegetative stage for a longer period of time. In plants that were alowered to flower and seed, the growth rate declined sharply. The data indicated that frequent clipping is not a reliable method of reducing a horsenettle infestation.

<u>Herbicides for the control of horsenettle</u>. Neville (34) studied horsenettle control in corn and obtained moderate control with 2.2 and 3.4 kg/ha of 2,4-D (2,4-dichlorophenoxyacetic acid) and a 2,4-D plus 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) mixture produced moderate injury to the plant. Bradbury (12) in New Jersey reported a 100 percent reduction in a horsenettle stand with a fall application of 35.8 kg/ha

of 2,4-D. He also found that 4.5 kg/ha of amitrole (3-amino-1,2,4-triazole) applied during flowering produced good control. It was shown that a mixture of 2,4-D and 2,4,5-T was more effective than either chemical used alone. Hemphill (20) in Missouri obtained control of horsenettle with amitrole at 2.2 kg/ha applied to the foliage in August. Fair control was obtained with 2,3,6-TBA (2,3,6-trichlorobenzoic acid) at 3.4 kg/ha per acre.

<u>Control of perennial weeds with glyphosate [N-(phosphonomethyl) glycine] and terbacil (3-tert-butyl-5-chloro-6-methyluracil)</u>. Glyphosate, introduced in 1971, was shown by numerous workers to have a wide range of activity on both annual and perennial weeds. Tweedy *et al*. (48) reported effective yellow nutsedge (*Cyperus esculentus* L.) and purple nutsedge (*Cyperus rotundus* L.) control in the greenhouse at 2.2 kg/ha when applied when the plants were at the 3rd, 6th, 9th, and 12th leaf stage. Stoller (47) also reported good yellow nutsedge control with 2.2 kg/ha glyphosate. His ratings, taken 4 weeks after application, indicated that younger plants were more susceptible to glyphosate than older more established plants.

Arnold (4) reported that a single fall application of glyphosate was more effective than spring applications for Canada thistle [*Cirsium arvense* (L.) Scop.], however, a fall application at 2.2 kg/ha following by 1.1 kg/ha in the spring was the best treatment. Messersmith (30) also reported good Canada thistle and field bindweed (*Convolvulus arvensis* L.)

control with a single 2.2 kg/ha application of glyphosate in the fall. Jorgenson (24) reported that glyphosate at 1.1 and 2.4 kg/ha applied twice in the fall showed good field bindweed control the following year.

Glyphosate has given excellent control of the perennial grasses, quackgrass [*Agropyron repens* (L.) Beauv.] (44) and johnsongrass [*Sorghum halepense* (L.) Pers.] (46). Rates of 1.1 to 3.4 kg/ha gave effective control of quackgrass the season after treatment (6, 7, 10, 45). Herbicide efficiency also appeared to be related to the stage of growth of the quackgrass. Older plants appear to be more susceptible than younger ones (6, 13, 50).

Terbacil at rates of 0.5 to 3.5 kg/ha gave good annual weed control whereas rates up to 8.5 kg/ha provided effective control of bermudagrass [*Cynodon dactylon* (L.) Pers.], quackgrass, horsenettle and red sorrel (*Rumex acetosella* L.) (5, 38, 39, 40). In the papers reviewed, the time of application did not appear to be a factor in control. This may be related to the fact that uracils are less firmly absorbed by soils than many herbicides and will move into the deeper soil horizons following rain (42).

# CHAPTER 2

# SOME FACTORS AFFECTING VEGETATIVE GROWTH AND REPRODUCTION OF HORSENETTLE.

## ABSTRACT

Horsenettle (*Solanum carolinense* L.) is a persistent perennial weed found in many areas of Michigan. High light and low night temperatures were found the most conducive to root formation and early flowering. Young plants (61 days from seed) were capable of producing new shoots from root sections within 28 days from sectioning. Plants with the root system left intact and the tops removed produced new shoots within 18 days. Horsenettle seed is susceptible to freezing. A temperature of -18.9 C for 40 days killed all seed. Seed from berries overwintered in the field had a 30% germination.

# INTRODUCTION

The ability of horsenettle (_Solanum_ _carolinense_ L.) to produce new plants from intact or cut sections of roots is the major factor in the spread of this perennial weed. When undisturbed, horsenettle produces a formidable storage root system seemingly out of proportion to its limited shoot growth. Little is known about the influence environmental factors have on the production of shoots, flowers and roots. If shoot and flower production is at the expense of root growth, the manipulation of the plant to decrease storage root production would aid control of this perennial weed.

Experiments conducted in the northeast demonstrated the high reproductive capacity of horsenettle roots (2). Reproduction from seed was also considered to occur on non-crop areas.

The objective of this study was to determine the effects of light intensity and temperature on production of shoot and root growth and to ascertain the reproduction capabilities of Michigan clones by root cuttings and seed.

# MATERIALS AND METHODS

Effect of Light Intensity and Temperature on Shoot and Root Growth. Root sections were collected from a field in central Michigan, cut into segments 2 cm in length and planted in a soil peat moss mixture (4:1, v:v). After emergence they were taken out of the greenhouse and placed in growth chambers. One chamber was maintained with a night temperature of 23.8 C and the other 15.5 C. Both had a day temperature of 32.2 C (14 hours). Light intensity was regulated by varying the distance the plants were from the light source. The two light intensities studied were 17.8 and 6.5 klux.

Flower counts were made 30 days after treatments were imposed. Forty-five days after the plants were first placed in the growth chamber they were harvested, separated into shoot and roots, dryed (47 C) and weighed.

Reproductive Capability from Root Sections. Twenty-five day old seedlings were planted in 10 cm pots and allowed to grow for 5 or 7 weeks. After initial growth the roots were measured and sectioned into four equal sections: based on morphological position; ie. nearest the root apex or nearest the crown. In another test only the shoot was removed and the root left intact.

The root sections were incubated in a growth chamber with a 14 hour day (17.2 klux) with a day temperature of 30.0 C and a night temperature of 18 C. The growth medium was horticultural vermiculite and a half strength Hoagland's solution (pH 6.3) (1) was supplied as needed.

Germination Capability of Frozen and Refrigerated Seed. Horsenettle berries were harvested on October 27 from a field in central Michigan. They were separated into nine categories, based on berry size and ripeness. They were then split into two groups. Half of the berries were frozen at -18.9 C and the rest were refrigerated at 2.8 C for forty days. After the cold treatment the berries were split on one side and seeds were placed on filter paper and dryed for 24 hours at 33 C. Ten seeds from each treatment were then placed on filter paper in petri dishes to which 8 ml of distilled water was added. The seeds were incubated in the dark at 29 C for 8 days.

In another test berries were collected on March 15 at a field in southwestern Michigan and were dryed and incubated as before to determine the viability of seed after exposure to a Michigan winter.

## RESULTS AND DISCUSSIONS

Effect of Light Intensity and Temperature on Shoot and Root Growth. Plants growing under the higher light intensity flowered earlier than those grown with lower light (Table 1). The combination of high light and low night temperature had the earliest flowers. In a later experiment it was noted that

Table 1.--Horsenettle growth as affected by light intensity and temperature. [a]

| Temperature ( C) | | Light intensity | | | |
|---|---|---|---|---|---|
| | | 17.8 klux | | 6.5 klux | |
| Day | Night | Flowers/ Plant | Root/ Shoot | Flowers/ Plant | Root/ Shoot |
| 32.2 | 15.5 | 5.3 | 0.52 | 0.9 | 0.36 |
| 32.2 | 23.8 | 1.4 | 0.41 | 0.4 | 0.38 |

[a] Each observation is the average for nine plants.

horsenettle growing in an open field flowered earlier than that under the canopy of trees in an orchard. The delay in flowering maybe related to light intensity as demonstrated in the growth chambers.

Higher light and low night temperatures also produced the highest root:shoot ratio. Apparently, with the higher light more carbohydrates are produced and in turn, less is respired if the night temperature is low. This could provide more storage root as opposed to production of foliage.

Reproduction Capability from Root Sections and Intact Roots. The time required of horsenettle to obtain the perennial habit was less than 61 days from seed (Table 2). Although root sections at the morphological apex of the root did not have the same regrowth potential as sections from the other

Table 2.--The development of new horsenettle plants from roots.

| Days from seed | Days to new shoots from root sections [a/] 1 | 2 | 3 | 4 | Days to new shoots from intact roots |
|---|---|---|---|---|---|
| 61 | 28.0[b/] | 20.8 | 20.5 | 19.3 | 17.3 |
| 75 | 24.3 | 19.5 | 20.0 | 27.8 | 17.5 |

[a/] 1-section nearest the root apex
4-section nearest the plant crown
2 and 3 intermediary.

[b/] 50% regrowth all others had 100% regrowth.

parts of the root, the perennial habit of the plant even at early stages of growth is evident since over all 95% of the root sections produced shoots. When only the shoot was removed and the root left intact, new shoots were visible within 18 days.

It is evident from this data that control of horsenettle seedlings is important to prevent introductions to a non-infested field. Disking of horsenettle would encourage spread of the weed problem and not be an effective means of control. The use of a contact, non-translocated herbicide would apparently give little season long control.

Germination Capability of Frozen and Refrigerated Seed. The exposure of the berries to -18.9 C killed all the seed. With the refrigerated seed the germination percent over all berry sizes and stages averaged 22.8% with extremely variable results (Table 3).

Table 3.--The germination of horsenettle seed from berries of various size and stages of ripeness following temperature treatments.

| Stage | Berry Diameter (cm) | %Germination | |
|---|---|---|---|
| | | 2.8 C | -18.9 C |
| Green | 1.7 | 30.0 | 0.0 |
| Green | 1.3 | 36.7 | 0.0 |
| Green | 0.8 | 46.7 | 0.0 |
| Yellow | 1.7 | 23.6 | 0.0 |
| Yellow | 1.4 | 23.6 | 0.0 |
| Yellow | 1.2 | 10.0 | 0.0 |
| Ripe | 1.7 | 33.6 | 0.0 |
| Ripe | 1.4 | 0.0 | 0.0 |
| Ripe | 0.8 | 3.0 | 0.0 |
| | Average | 22.8 | 0.0 |

Seed from unripe berries had the highest percent germination (37.8%). The berries overwintered in the field had a 30.0% germination.

The data indicates that germination of seed is variable and that extreme prolonged freezing would kill the seed.

In Michigan horsenettle seedlings are rarely seen. The most likely location for infestations by seed would appear to be fields where Solanceous crops are produced and herbicides used are selective for horsenettle along with the weed. In fields where effective pre-emergence herbicides are used and non-Solanaceous crops are planted, infestation from seed would not be likely to occur.

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CHAPTER 3

# THE CONTROL OF HORSENETTLE WITH GLYPHOSATE AND TERBACIL AND THEIR EFFECT ON ROOT RESERVES

## ABSTRACT

Horsenettle (Solanum carolinense L.) regrowth was controlled with 2.2 kg/ha terbacil (3-tert-butyl-5-chloro-6-methyluracil) in both a cultivated field and an orchard situation independent of the time of application. Glyphosate [N-(phosphonomethyl)glycine] provided effective control if applied at 4.5 kg/ha when the plants were in full flower. Glyphosate at 2.2 kg/ha applied during bloom did not control regrowth in the cultivated field. All terbacil treatments in the cultivated field caused 82-98% reductions in starch content in the roots late in the season. Glyphosate applied prior to flowering only slightly reduced starch levels in storage roots at the end of the growing season whereas the other glyphosate treatments did not reduce the starch content in the remaining viable roots.

# INTRODUCTION

Horsenettle (*Solanum carolinense* L.) has become a persistant perennial weed in many orchards and cultivated fields in Michigan. The use of herbicides which control annual weeds and quackgrass [*Agropyron repens* (L.) Beauv.] has reduced competition and are believed to have favored the spread of perennial weeds in many fields.

The substituted uracil, terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) controls a wide range of both perennial and annual weeds (12, 13). The uptake of terbacil is primarily through plant roots and movement is apoplastic in the xylem. It is relatively soluble in water (710 ppm) and hence will leach into the deeper soil horizons when irrigated or moistened by rainfall (11, 14). Its mobility in soil may be advantageous in controlling deep rooted perennials.

Glyphosate [N-(phosphonomethyl)glycine] has been reported to be a non-selective translocated herbicide which controls perennial weeds (2, 4, 9). Numerous workers reported that time of application was an important factor in the control achieved with glyphosate (2, 3, 5, 9).

Often the concept of "root or storage reserve" (10) is mentioned as the determinant factor in eradication of creeping perennial weeds. Ilnicki *et al*. (7) analyzed horsenettle roots for starch content as an estimate of this food reserve and found that root starch levels fluctuated from 34% at emergence to 13% at flower in the upper 25 cm of root whereas the lower 25 cm of roots remained at about 40% throughout the growing season. Starch estimations are justified since an available carbohydrate source is necessary in the spring for early growth and tissue production when carbohydrate demand exceeds photosynthetic capability (1, 15).

The purpose of this investigation was to determine the optimum time to apply either glyphosate or terbacil for horsenettle control in an orchard and a cultivated field, and if these herbicides would effectively deplete the starch reserve of storage roots.

## MATERIALS AND METHODS

Herbicide application. Preliminary experiments to select the most effective herbicides for the control of horsenettle were conducted in a cherry orchard in northwestern Michigan and in an asparagus field in southwestern Michigan in 1972. Since glyphosate and terbacil were the most effective herbicides in these experiments, tests were set up in 1973 to determine the optimum time of application for these herbicides.

Two locations in southwestern Michigan were chosen as test sites because of their severe and relatively uniform infestations. One was a young pear orchard where grass was not a serious weed problem under the trees and horsenettle was the predominant perennial weed. This orchard was maintained with sod strips between the rows of trees, a common practice in

Michigan orchards. Treatments were applied four times during the season. The time of application was based on the morphological stage of the horsenettle, i.e., emergence, full leaf, bloom and late bloom.

The other location was a field which had been planted to tomatoes for several years. Horsenettle was the predominant weed in the area since the herbicides and cultivation practices used for tomato production had encouraged its development. Pertinent data regarding application at both locations are presented in Table 1.

The treatments at both locations were applied in volumes equivalent to 337 L/ha, with a plot size of 3.7 by 7.6 m in the cultivated field and 2.4 by 7.6 m in the orchard. The experimental design was a randomized complete block with three replications. The date of application at both locations did not coincide since the horsenettle in the orchard emerged and flowered later than that in the cultivated field.

The 80% wettable powder formulation of terbacil and the 0.35 kg/L formulation containing the isopropylamine salt of glyphosate were applied with a small plot sprayer using carbon dioxide as a pressure source.

Weed control ratings were based on a 1 to 9 scale with 1 representing no injury and 9 indicating complete foliage kill. A rating of 6.5 indicated commercially acceptable horsenettle control.

All data were subjected to analysis of varience and means were compared using Tukey's H.S.D test.

Table 1.--Description of conditions for four herbicide applications on horsenettle in a pear orchard and a cultivated field.

| Site Description | Application | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Cultivated field<br>Soil - loamy sand | | | | |
| Application date | May 23 | June 12 | June 20 | July 20 |
| Stage of growth | Emergence | Full leaf | Early bloom | Late bloom |
| Plant height (cm) | 0-8 | 20-35 | 35-60 | 60-75 |
| Air temperature (C) | 14 | 30 | 30 | 31 |
| Soil temperature (C) | 15 | 31 | 34 | 30 |
| Rain within 24 hours (cm) | 0.9 | — | 1.6 | — |
| Pear orchard<br>Soil - sandy loam | | | | |
| Application date | May 23 | June 12 | July 5 | July 25 |
| Stage of growth | Emergence | Full leaf | Bloom | Late bloom |
| Plant height (cm) | 0-8 | 10-20 | 20-35 | 35-45 |
| Air temperature (C) | 14 | 30 | 34 | 29 |
| Soil temperature (C) | 16 | 31 | 33 | 28 |
| Rain within 24 hours (cm) | 4.4 | — | — | — |

Root collection and preparation for analysis. Seven times throughout the season, storage roots were collected in the cultivated field for starch analysis. Roots used for analysis were only those which had shoots growing from them, (i.e. viable roots in the upper 30 cm of the soil). Shoots arising from the roots were discarded and only fleshy storage roots were placed in plastic bags in ice for transport to the laboratory.

Roots were kept frozen until they were prepared for lypholyzation. Frozen roots were cut into 2 cm sections and washed under distilled water to remove any soil. After being placed in a covered Petri dish and covered with crushed dry ice for 45 minutes, the roots were then freeze-dried for 24 hours. The dried roots were then ground through both a 20 and 60 mesh screen with a Wiley Mill. The powder was collected in plastic vials with tight caps and stored frozen until starch levels were determined.

Starch Analysis. The procedure was similar to that of McCready *et al*. (8) and Yemm and Willis (16) with the following modifications:

To remove any sugars which would react with the anthrone reagent used for starch determinations an ethanol cleaning procedure was utilized. Into a round bottom plastic centrifuge tube, 20 ml of 80% ethanol was added to 50 mg of ground root tissue, stirred, and placed into a hot water bath (70 C) for 10 minutes. The samples were then centrifuged at 1290 g for 10 minutes, after which the supernatant was discarded. This was repeated a total of three times. To the residue 5 ml of water and then 6.5 ml of 52% perchloric acid was added. Samples were stirred for 10 minutes and 5.0 ml of water added to the tubes prior to centrifugation for 10 minutes at 1290 g.

The supernatant was transferred to a 50 ml test tube and the residue retreated as before with 5 ml water and 6.5 ml 52% perchloric acid and stirred. After 5 minutes, 5 ml water was again added, the sample was shaken, and quickly poured into the graduated test tube through a suction apparatus equipped with Buchner funnel and Whatman no. 1 filter paper. The samples were brought up to a volume of 50 ml with distilled water prior to the anthrone test.

The anthrone solution (0.2 anthrone per 100 ml 75% $H_2SO_4$) was prepared daily. One tenth of the solublized starch solution was placed in a test tube. Ten ml of the anthrone solution was then added and the tube shaken vigorously for 15 seconds. The tubes were placed in a boiling water bath for 7.5 minutes and placed in an ice bath to stop the reaction. Absorbance at 620 nm was determined with a Beckman Grating Spectrophotometer Model DB-G. A conversion factor of 0.92 was used to convert the solublized starch levels to starch. The glucose standard was made fresh daily. Starch levels are expressed as milligrams per gram dry weight. All starch data were subject to analysis of varience and means were compared to control using Tukey's H.S.D. test.

# RESULTS AND DISCUSSION

Horsenettle control in the orchard. At the earliest application date, May 23, only terbacil was applied to the plots since few horsenettle shoots had emerged and glyphosate was believed to have no soil activity. Terbacil provided excellent control of horsenettle 90 days after a single application of 3.4 kg/ha at the time shoots were just emerging through the soil (Table 2). It appeared that terbacil was absorbed by the roots of horsenettle, and either prevented the formation of new shoots or killed them soon after they were formed.

By June 12 the horsenettle was 10 to 20 cm high and in full leaf, however, no flowers or flower buds were apparent on the plants. Terbacil applied at that time gave excellent control for 75 days after a single application of 3.4 kg/ha or when combined with glyphosate at 2.2 plus 2.2 kg/ha or with paraquat at 2.2 plus 1.1 kg/ha. Glyphosate applied alone on this date at 4.5 kg/ha did not give acceptable season-long control although it initially burned off the foliage. Apparently this herbicide was not translocated to the roots in sufficient quantity to control regrowth from the roots.

At the third treatment date, July 5, the horsenettle in the orchard was 20 to 35 cm high and in full bloom. Glyphosate at 4.5 kg/ha applied on this date gave excellent control of horsenettle 45 days after the single application. As with the earlier treatments, terbacil when applied either alone or in

Table 2.--Control ratings on horsenettle in a pear orchard with herbicides applied at several stages of growth. [a]

| Herbicide | Rate (kg/ha) | | Stage of growth [b] A [c] | B | C | D |
|---|---|---|---|---|---|---|
| None | — | | 1.0 | 1.0 | 1.0 | 1.0 |
| Terbacil | 3.4 | | 7.7 | 8.0 | 8.0 | 6.7 |
| Glyphosate | 4.5 | | — | 3.0 | 8.3 | 2.7 |
| Glyphosate + Terbacil | 2.2+2.2 | | — | 7.3 | 8.7 | 8.7 |
| Terbacil + Paraquat | 2.2+1.1 | | — | 8.7 | 8.7 | 8.7 |
| | | H.S.D. 5% | | 1.0 | 1.5 | 2.4 |

[a] Rating date: August 25.

[b] Rating system where 1 = no control and 9 = complete regrowth inhibition.

[c] Time of application for A was May 23 (emergence); B was June 12 (full leaf); C was July 5 (bloom) and D was July 25 (late bloom).

combination with the other herbicides gave excellent control. The effectiveness of the glyphosate applied at bloom indicates that the herbicide was translocated, most likely symplastically to the roots and accumulated at a high enough concentration to give control of newly emerging shoots.

The latest applications were made when the horsenettle was in late bloom stage and the plants were 35 to 45 cm high. Again, terbacil applied either alone or in combination with the other herbicides gave good control of horsenettle. Glyphosate applied on this date failed to give the desired control, perhaps because the herbicide wasn't translocated to the roots as occurred with glyphosate applications on June 12. It was apparent that to control horsenettle in the orchard with glyphosate, the time of application was of extreme importance. With the soil sterilant, terbacil, control was independent of the time of application.

Horsenettle control in a cultivated field. In contrast to the previous experiment in the orchard, glyphosate was applied at 2.2 and 4.5 kg/ha and terbacil at 2.2 and 3.4 kg/ha. Paraquat was included at the 1.1 kg/ha rate and in combination with terbacil at 3.4 plus 1.1 kg/ha paraquat.

At the earliest application date in the field (Table 3) only terbacil was applied. A single application of terbacil at either 2.2 or 3.4 kg/ha gave excellent horsenettle control 105 days after treatment.

Table 3.--Control ratings on horsenettle in a cultivated field with herbicides applied at several stages of growth.[a]

| Herbicide | Rate (kg/ha) | | Stage of growth [b] A | B | C | D [c] |
|---|---|---|---|---|---|---|
| None | — | | 1.0 | 1.0 | 1.0 | 1.0 |
| Terbacil | 2.2 | | 8.0 | 7.3 | 7.7 | 7.0 |
| Terbacil | 3.4 | | 8.0 | 8.0 | 8.0 | 6.6 |
| Glyphosate | 2.2 | | — | 3.3 | 3.3 | 3.0 |
| Glyphosate | 4.5 | | — | 2.3 | 1.3 | 7.3 |
| Glyphosate + Terbacil | 2.2+2.2 | | — | 8.0 | 7.3 | 7.3 |
| Paraquat | 1.1 | | — | 1.3 | 1.0 | 1.0 |
| Terbacil + Paraquat | 3.4+1.1 | | — | 7.7 | 8.0 | 7.0 |
| | | H.S.D 5% | | 1.3 | 1.2 | 1.3 |

[a]Rating date: September 6

[b]Rating system where 1 = no control and 9 = complete regrowth inhibition.

[c]Time of application for A was May 23 (emergence); B was June 12 (full leaf); C was June 20 (early bloom) and D was July 5 (late bloom).

By June 12, the horsenettle was 20 to 35 cm high, with fully expanded leaves but no flowers or flower buds were present. Terbacil applied at either rate or in combination with the other herbicides tested gave excellent control 85 days after the single herbicide application. Glyphosate applied at this time at either 2.2 or 4.5 kg/ha gave only short term control, as did paraquat when used alone.

On the third treatment date, June 20, the horsenettle was 35 to 60 cm high and in the early bloom stage. Terbacil at either 2.2 or 3.4 kg/ha or in combination with either glyphosate or paraquat gave excellent control of horsenettle 77 days after the single application of the herbicides. Glyphosate at either 2.2 or 4.5 kg/ha did not provide effective season-long control although it was somewhat better than paraquat.

On July 5, the horsenettle was 60 to 75 cm high and in the late bloom stage. Terbacil at either 2.2 or 3.4 kg/ha gave good control when applied either alone or in combination with the other herbicides tested. Glyphosate, at 4.5 kg/ha provided good control 62 days after application while 2.2 kg/ha was not adequate. Paraquat at 1.1 kg/ha was identical to the check non-treated plot.

It is evident that season long control of horsenettle is possible with a single application of terbacil at 2.2 kg/ha regardless of the time of application. To achieve optimum control with glyphosate, however, the time of application was of paramount importance. Horsenettle was most susceptible to glyphosate when in the flowering stage. This may be related to the carbohydrate reserve in the roots being at a seasonal low, hindering the ability of the plant to regenerate shoots from below ground portions. It may also be related to the amount and primary direction of translocation of sugars during this period. During flowering, sugars may be translocated from leaves or storage roots to active sinks in meristematic areas.

The effect of glyphosate and terbacil on root starch reserves. The early reduction of starch reserves in the roots indicates hydrolysis and utilization during the initial plant growth phase whereas starch accumulates during subsequent plant development (Figure 1A). With an increase in shoot growth the first 3 weeks after emergence the reserves dropped from 360 mg/g to 140 mg/g. Afterward there was a gradual rise in root starch levels even as the plants were going through flower and fruit set reaching 353 mg/g by late August. The plant had in effect completed the cycle or reserve depletion caused by early growth and refurnished the starch during the subsequent 10 weeks.

Terbacil at 2.2 kg/ha applied on either May 23, June 12, June 30, or July 5 reduced the starch level 82 to 98% by August 23. The May 23 treatment, applied while the plants were still emerging, had 146 mg/g starch on June 12, approximately the same as the control (140 mg/g). However, as the starch reserves began increasing after this date in the control it continued to decrease in the treated plants. Roots from plants treated with terbacil at 2.2 kg/ha on June 12 had starch levels of 115.0 mg/g eight days after treatment. This indicates the beginning of a rapid drop in root starch while concurrently it was increasing in the non-treated check. Eventually the root reserves dropped to 11 mg/g starch on August 23. The depletion of starch caused by terbacil appeared to be independent of the date of treatment. Roots from all plants receiving 2.2 kg/ha terbacil average 31 mg/g starch by August 23, a 92% decrease when compared to the control on the same date.

Terbacil, when applied at 3.4 kg/ha (Figure 1B) gave results similar to that of the 2.2 kg/ha rate. A slight rise in root starch was noted at the first determination date after application for both the May 23 and the June 12 treatment. By July 5, however, both were significantly lower than the control. Similarly a slight rise was noted in root starch levels on July 25 after the June 12 treatment. However one month later, both the May 23 and the June 12 treatments had reduced the reserves to about 12 mg/g starch. The average for all the 3.4 kg/ha treatments was 38 mg/g root starch compared to 353 mg/g starch in the control roots on August 23.

Since glyphosate did not exhibit any soil activity, it was not used at the earliest date. The June 12 application of 2.2 kg/ha glyphosate appeared to cause a rise in root starch reserves by June 20 (Figure 2A). However, on July 5 the root starch content was about the same inroots of glyphosate

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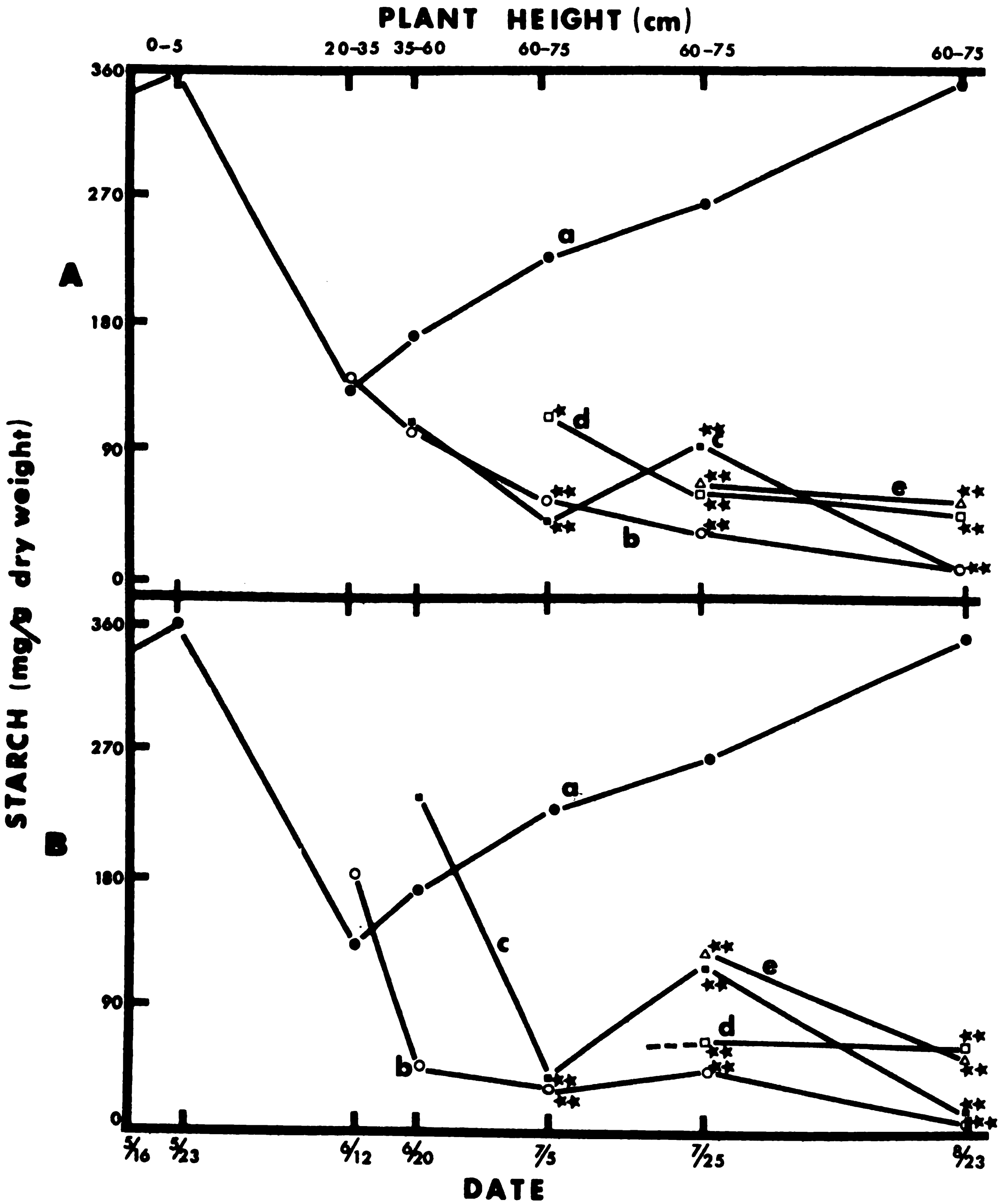
Figure 1A.--Effect of terbacil at 2.2 kg/ha on the starch reserve in horsenettle roots; a, control; b, applied May 23; c, applied June 12; d, applied June 20; e, applied July 5.

B.--Effect of terbacil at 3.4 kg/ha on the starch reserve in horsenettle roots; a, control; b, applied May 23; c, applied June 12; d, applied June 20; e, applied July 5.

**Means differ significantly from control at 1% level.


PLANT HEIGHT (cm)
0–5
20–35
35–60
60–75
60–75
60–75
STARCH (mg/g dry weight)
360
270
180
90
0
A
B
a
b
c
d
e
5/16
5/23
6/12
6/20
7/5
7/25
8/23
DATE

treated and check plants. The glyphosate applied on June 12, apparently inhibited an accumulation of starch after the initial drop between June 20 and July 5. This early treatment gave a significant decrease in root starch at the last harvest date, August 23. When compared to the terbacil however, the 2.2 kg/ha glyphosate was not nearly as effective in reducing starch reserves. Applications at later dates did not significantly reduce the starch levels in the roots.

Glyphosate at 4.5 kg/ha applied on June 12 significantly reduced the starch levels present on August 23 (Figure 2B). The same rate applied on later dates did not reduce the starch levels significantly late in the season. The application of 4.5 kg/ha on July 5 caused a significant increase in root starch reserve by July 25 when compared to the control at the same date.

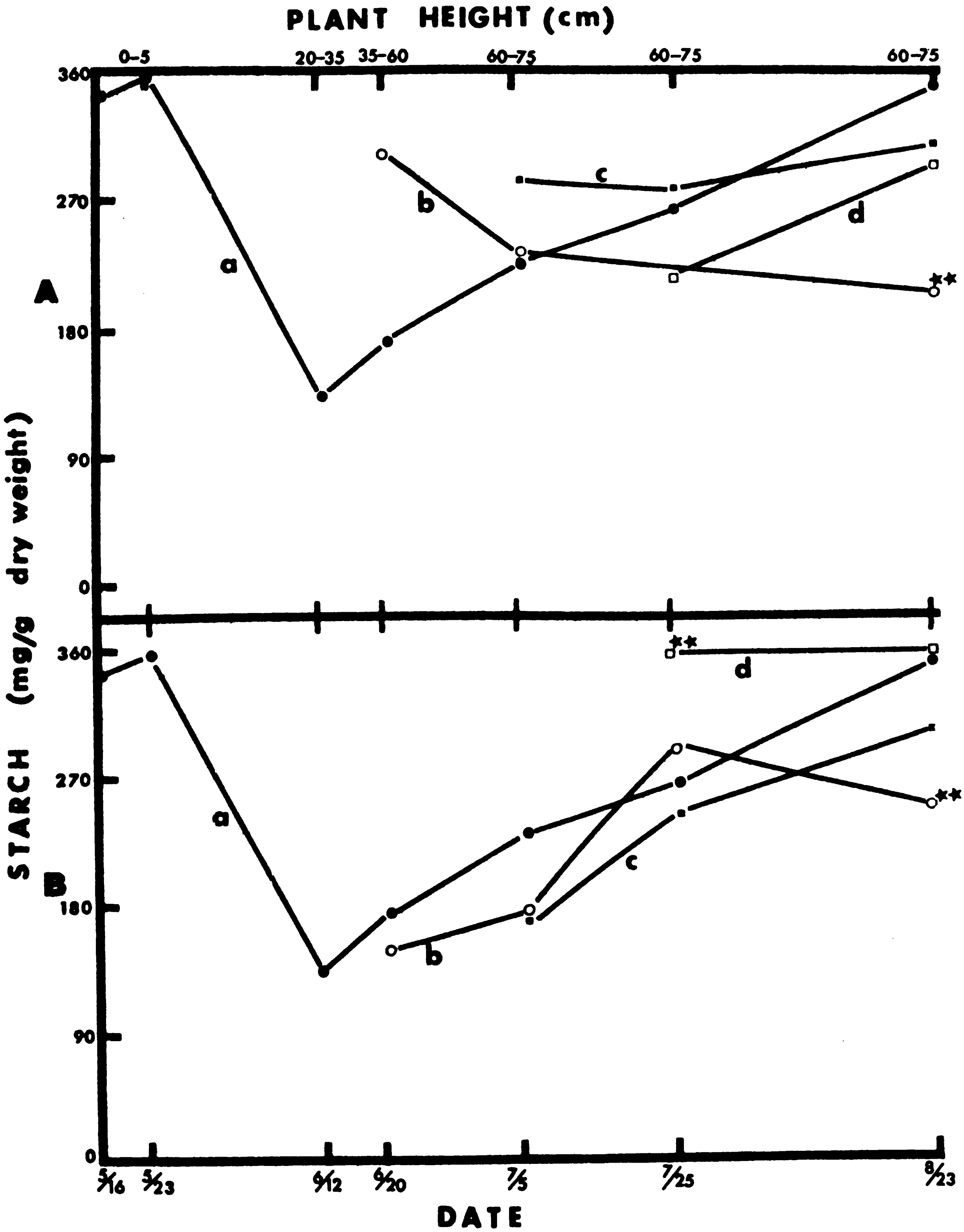
Paraquat reduced the root starch content late in the season only if applied while the plants were in early bloom (Table 4). Paraquat or glyphosate when in combination with terbacil, caused a significant decrease in root starch levels late in the season. Based on the data previously presented in Figure 1, the major part of this decrease can be attributed to terbacil.

Ilnicki et al. (7) conducted an investigation on root starch reserves of horsenettle in an abandoned field in New Jersey. They reported starch data from root sections in the upper 25 cm of the taproot and from the lower 25 cm. In the lower 25 cm of taproot, the starch reserve remained fairly

Figure 2A.--Effect of glyphosate at 2.2 kg/ha on the starch reserve in horsenettle roots; a, control; b, applied June 12; c, applied June 20; d, applied July 5.

B.--Effect of glyphosate at 4.5 kg/ha on the starch reserve in horsenettle roots; a, control; b, applied June 12; c, applied June 20; d, applied July 5.

**Means differ significantly from control at 1% level.



PLANT HEIGHT (cm)
0-5
20-35
35-60
60-75
60-75
60-75
360
270
180
90
0
A
B
a
b
c
d
**
**
STARCH (mg/g dry weight)
5/16
5/23
6/12
6/20
7/5
7/25
8/23
DATE

Table 4.--The effect of herbicides and herbicide combination on the root starch reserves in horsenettle roots on August 23.

| Herbicide | Rate (kg/ha) | Application date | Plant stage | Plant height (cm) | Root [a] starch (mg/g) Aug. 23 |
|---|---|---|---|---|---|
| None | —— | —— | —— | —— | 352.6 a |
| Paraquat | 1.1 | 6/12 | Pre-bud | 25-35 | 345.3 a |
| Paraquat + terbacil | 1.1+3.4 | 6/12 | Pre-bud | 25-35 | 129.6 c |
| Glyphosate + terbacil | 2.2+2.2 | 6/12 | Pre-bud | 25-35 | 57.0 d |
| Paraquat | 1.1 | 6/20 | Early bloom | 35-60 | 257.3 b |
| Paraquat + terbacil | 1.1+3.4 | 6/20 | Early bloom | 35-60 | 35.6 d |
| Glyphosate + terbacil | 2.2+2.2 | 6/20 | Early bloom | 35-60 | 63.6 cd |
| Paraquat | 1.1 | 7/5 | Late bloom | 60-75 | 369.6 a |
| Paraquat + terbacil | 1.1+3.4 | 7/5 | Late bloom | 60-75 | 28.6 d |
| Glyphosate + terbacil | 2.2+2.2 | 7/5 | Late bloom | 60-75 | 42.0 d |

[a] Means followed by similar letters not significantly different at the 5% level by Tukey's H.S.D. test.

constant at about 40% starch. In the upper 25 cm of root however, the reserve dropped to 13% starch during early growth but within 8 weeks after emergence the starch content in both sections of root were comparable. Data obtained from the control plants in this study approximate those obtained in New Jersey.

Terbacil, a soil sterilant, was effective in reducing root starch and regrowth independent of the time of application or rate applied. Being relatively soluble in water (710 ppm) it will move deep into the soil horizon after sufficient rainfall (13). The persistance of this herbicide and its movement into the deeper horizon of the soil most likely was the predominant factor in the starch reductions obtained. The herbicide was absorbed by roots and moved via the xylem. Being a strong photosynthetic inhibitor (6) it killed new shoots as they emerged. The plants continued to hydrolize starch from deeper roots which in turn were depleted of starch.

Glyphosate on the other hand has no soil activity and to control regrowth must translocate symplastically at toxic levels. Applications at 4.5 kg/ha, on July 5 was the only glyphosate treatment that adequately suppressed regrowth although defoliation was initially obtained with all the treatments. Three weeks after application there was a significant increase in the root starch level (104 mg/g) with this treatment. Since the increase could not have come via sugar production from photosynthetic activity it may be explained by the transport of reserves from deeper root horizons. Glyphosate may have killed the active metabolic portions of roots, causing a decrease in the total root weight of the plants, hence an increase in starch present on a percentage basis in the remaining living roots. This would require a redistribution of the reserves in the roots.

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## CHAPTER 4

## THE UPTAKE AND MOVEMENT OF $^{14}C$-LABELED GLYPHOSATE AND TERBACIL IN HORSENETTLE


### ABSTRACT

Both $^{14}C$-labeled glyphosate [N-(phosphonomethyl)glycine] and terbacil (3-_tert_-butyl-5-chloro-6-methyluracil) were applied to the leaves of horsenettle (_Solanum_ _carolinense_ L.) at four stages of growth. Labeled terbacil was also applied to the roots via nutrient solution. The $^{14}C$-methyl labeled glyphosate was readily absorbed and translocated to meristematic regions. Glyphosate translocation in older plants was over ten-fold that which occurred in younger plants although immature leaves of the younger plants initially abosrbed more herbicide. Translocation of glyphosate followed a source to sink pattern typical of phloem transported chemicals.

Plants treated with 2-$^{14}C$-terbacil were uniformly labeled after uptake from nutrient solution indicating xylem transport. Only 0.5 to 1.0% of the applied terbacil was taken up by the leaves. There was limited translocation of $^{14}C$ after foliar terbacil applications.

# INTRODUCTION

Horsenettle (*Solanum carolinense* L.) is a persistant perennial weed in many orchards and fields in Michigan. The plant reproduces asexually by root sections which can develop shoots within 20 days after cutting (5). During a growing season, intact plants may produce several shoots along the fleshy storage root.

Glyphosate (N-(phosphonomethyl)glycine) has been shown in our previous work to provide excellent season-long control when applied at 4.5 kg/ha while the plants were flowering. Applications at 2.2 kg/ha were not adequate regardless of timing. Other workers have reported that the time of application is crucial for control of perennial weeds with glyphosate. Stoller (8) found yellow nutsedge (*Cyperus esculentus* L.) was more susceptible if young plants were treated. Arnold (1) working with Canada thistle (*Cirsium arvensis* (L.) Scop.) and Messersmith (6) with both Canada thistle and field bindwind (*Convolvulus arvensis* L.) found that fall applications were superior to those made in the spring. The control of quackgrass (*Agropyron repens* (L.) Beauv.) was improved if applications were delayed until the plants were 25 to 30 cm tall (2,7).

In our earlier studies terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) controlled horsenettle effectively independent of the time of application. Terbacil also caused a depletion of the root starch reserves late in the season.

This test was conducted to relate the control of horsenettle with glyphosate and terbacil to the movement of these compounds within the plant and to determine if translocation was influenced by the stage of growth of the plant.

## MATERIALS AND METHODS

Roots were collected March 15 in a field heavily infested with horsenettle in southwestern Michigan. The roots were excavated, covered with soil and transported to the laboratory where they were refrigerated at 2.8 C. At intervals the roots were removed from the soil, washed with distilled water, cut into 2 to 3 cm sections and planted in vermiculite. Root sections were maintained in a growth chamber (32 C day for 14 hours at 17.2 klux and 24 C night) and allowed to develop new shoots. After shoots had emerged the plants were removed from the vermiculite and placed in cups containing 120 ml of one-half strength Hoagland's solution (4) containing 8 mM $NO_3$ at pH 6.3. By successive plantings, plants were obtained that ranged from recently emerged to full flower.

The $^{14}C$-methyl-labeled glyphosate was converted from the acid to the isopropylamine salt (formulated compound) by the addition of an equimolar amount of isopropylamine to 6.1 mg (66 μCi) of the acid in 3 ml of deonized water containing 0.8% Mon 0818 surfactant.[1] One 10 μl drop containing 0.2 μCi of

[1]Mon 0818 is a non-ionic surfactant produced by Monsanto Company, St. Louis, Missouri.

$^{14}C$-glyphosate was applied over the midrib of the third most recently expanded leaf. On the youngest plants, the most fully expanded leaf was treated. After treatment, the plants were placed in the growth chamber for 4 days under conditions previously described.

The 5.9 mg (30 μCi) of 2-$^{14}C$-terbacil was initially dissolved in 4 ml of acetone. A 0.54 ml aliquot of this stock solution was then placed into another vial and evaporated to dryness. Formulated terbacil (200 μl containing 3.7 g/l) was then added to the dried 2-$^{14}C$-terbacil and resuspended with an Aerograph Ultrasonic Cleaner. Three successive 10 μl drops were then applied to the leaf giving a total of 0.15 μCi per treated leaf. The plants were then maintained in the growth chamber for 4 days prior to assay.

Labeled nutrient solution (5 μCi/l) was prepared by adding 1 ml of the stock 2-$^{14}C$-terbacil to 1500 ml of half strength Hoagland's solution. Plants were put in cups containing 120 ml of this solution and placed in the growth chamber for 12 hours. Each treatment was replicated 3 times on plants of 4 different stages of growth.

At harvest, the plants were cut into five sections; 1, treated leaf; 2, acropetal to the treated leaf; 3, between the treated leaf and the storage root; 4, the storage root; 5, fibrous roots. The treated leaf was washed with five successive 50-ml washes (glyphosate treated leaves were washed with water and the terbacil treated leaf with water : ethanol, 1:1 v:v) to remove the herbicide not absorbed.

Plants treated with labeled terbacil in nutrient solution were plotted dry and quickly frozen with crushed dry ice. All of the plant parts were frozen and lyophilized for 24 hours.

One plant from each treatment was not sectioned. It was rehydrated, mounted and radioautographed for 5 or 14 days as described by Yamaguchi and Crafts (10). To show movement within the storage root, this organ was cut lengthwise prior to mounting.

Each sample was then combusted with a Nuclear Chicago Model 3151 Oxidizer unit equipped with magnetic stirrers. If the dry weight of the sample was more than 50 mg, the sample was ground through a 20 mesh screen with a Wiley Mill and only 50 mg of the sample was combusted. Total dpm absorbed and translocated to other parts of the plant were based on the total weight of the plant section.

One liter flasks were purged with oxygen and stoppered with rubber septum cups prior to combustion. Upon cooling of the flask, 10 ml of ethanol:ethanolamine (2:1 v:v) was injected and they were stirred for 15 minutes. One ml of the $CO_2$ trapping solution was removed for liquid scintillation counting. A scintillation solution was prepared by dissolving 4 grams of BBOT [2,5,-bis-5-*tert*-butly-1-bensozexolyl (2') thiopen] in one liter of toluene.

The scintillation fluid had 63% efficiency as determined by External Standardization on a Packard Tricarb Scintillation

Spectrometer. All cpm (counts per minute) data were converted to dpm (disintegrations per minute).

## RESULTS AND DISCUSSION

Uptake and translocation of $^{14}C$-glyphosate in horsenettle. Glyphosate was readily absorbed by both immature and fully expanded leaves of horsenettle. Although the expanding leaves of the younger plants absorbed the most $^{14}C$, the fully expanded leaves on older plants exported more of the label to other plant parts (Table 1). Translocation increased with plant age reaching 76% of that absorbed on plants in the late flowering stage. Rate of export of glyphosate from leaves appears to be related to their capacity to produce and export sugars. Thrower (9) working with soybean showed that translocation of photosynthates into and out of a leaf was a function of stage of development. During early expansion, translocation was into the young leaf. As the leaf expanded transport became export. A similar phenomenon is apparently true with horsenettle.

Bidirectional transport of $^{14}C$ was indicated even while the plants were extremely young (Figure 1A). The label moved from the treated leaf via the phloem through the storage root and to another shoot on the same root section. It was noted however, that in the storage root, the $^{14}C$ remained in the conductive tissue and did not appear to move out into the cortex.

Figure 1.--Translocation of $^{14}C$-glyphosate and/or metabolites in horsenettle. The treated plant (left) and the corresponding radioautograph (right).

A. Emergence
B. Pre-Bud
C. Early Flower
D. Late Flower

A

B


C


D

Table 1.-Uptake and translocation of $^{14}C$-glyphosate in horsenettle plants at four stages of growth.

| Plant Stage | Percent Absorbed [a] | DPM/Plant Section: Treated Leaf | DPM/Plant Section: Acropetal Shoot Portion | DPM/Plant Section: Basipetal Shoot Portion | DPM/Plant Section: Storage Root | DPM/Plant Section: Fibrous Roots | Translocation out of Treated Leaf (%) [b] |
|---|---|---|---|---|---|---|---|
| Emergence | 16.7 a | 70464 | 1950 | 831 | 950 | — | 5 |
| Pre Bud | 7.7 b | 24418 | 3264 | 1081 | 2531 | 2845 | 28 |
| Early Flower | 5.5 b | 13522 | 1472 | 7348 | 2196 | 3060 | 51 |
| Late Flower | 13.9 a | 15000 | 5376 | 33062 | 2520 | 5623 | 76 |

a/ Means followed by similar letters not significantly different at the 5% level by L.S.D. test.

b/ F value for linear increase with plant stage significant at 5% level.

In all the $^{14}C$-glyphosate treated plants the label appeared to move by the classic phloem transport mechanism described by Crafts (3). Figures 1-B,C, and D all indicate that $^{14}C$ moved preferentially to the meristems, young leaves acropetal to the treated leaf, and fibrous roots. Only slight transport was noted to the older leaves basipetal to the treated leaf and above the storage root indicating little xylem transport following a basipetal phloem movement. However, transport occurred through branches leading to other active sinks (Figure 1D).

Our previous work indicated that both time of application and the rate of glyphosate are critical for control of horsenettle regrowth following initial defoliation. Regrowth occurred if the plants were young with no flower buds present at the time of application. It appears that this may be related to differential transport of the glyphosate and/or metabolites in younger and older plants. Young leaves, alLthrough they readily absorb the herbicide do not export it as well as older leaves. Also on young plants, there is a relatively small leaf area to capture the glyphosate at treatment. These two factors in combination would decrease the effectiveness of the glyphosate and account for poor chronic toxicity at early stages of growth.

The variable control of horsenettle at later stages of the plants development could also be related to two factors. The first would be differential metabolism of the glyphosate based on the age of the plant. Horsenettle, when it is most susceptible to the herbicide may not metabolize it as rapidly as when it is either a much younger or much older plant. Another factor is a delay in shoot development following the herbicide application. The storage organs of horsenettle are roots (5) with few buds already present. Buds will develop however, if the roots are sectioned as was done in this experiment or if the shoots are killed by a contact herbicide. The production of new shoots is not a rapid process, taking a minimum of 20 days for new shoots to emerge. When glyphosate is applied to actively growing plants, it is evident from the radioautographs that it will quickly translocate to active meristems. These meristems along with the rest of the shoot may be killed within 2 weeks. In the storage root however, the active meristems are either not present at the time of application or can develop long after the treatment is applied. The storage root in this instance acts as a conduit and not as a sink for the herbicide. Here the glyphosate, although present, has no place to exert a herbicidal effect since there are no meristems present. Shoots will develop later on the storage organ, but by that time the herbicide may be inactivated or diluted to the point that the concentration is not high enough to control regrowth. This may explain the rate responses as related to chronic toxicity.

<u>Translocation of 2-$^{14}$C-terbacil in horsenettle</u>. In contrast to the variable control of horsenettle with glyphosate, our field test demonstrated that terbacil inhibits regrowth inde-

pendent of the time of application at 2.2 kg/ha. Terbacil is not readily absorbed by the leaves of horsenettle as is glyphosate (Table 2). Only 0.5 to 1.0% of that applied was absorbed by the treated leaves. There was some movement of $^{14}C$ when applied to the leaf (Figure 2A). Much of that absorbed moved acropetally in the treated leaf although there was slight transport of $^{14}C$ down into the fibrous roots.

The primary transport of 2-$^{14}C$-terbacil when applied to the roots appears to be through the xylem (Table 3). $^{14}C$ was uniformly translocated to all plant parts following a 12-hour exposure to the labeled terbacil in nutrient solution. As expected, more radioactivity was present in older larger plants. The control of horsenettle with terbacil is related to its being continually available to the roots, killing new shoots as they develop and causing a depletion of the starch reserves in the storage roots as was noted in our previous work. Foliar uptake probably does not contribute greatly to the toxicity obtained.

Table 2.--Uptake and translocation of $^{14}C$-terbacil and/or metabolites by leaves of horsenettle at four stages of growth.

| Plant Stage | Percent [a/] Absorbed | DPM/Plant Section | | | | | |
|---|---|---|---|---|---|---|---|
| | | Treated Leaf | Acropetal Shoot Portion | Basipetal Shoot Portion | Storage Root | Fibrous Roots | Translocation [a/] out of Treated Leaf (%) |
| Emergence | 0.67 a | 888 | 223 | 200 | 797 | 128 | 61 a |
| Pre Bud | 0.53 a | 969 | 149 | 207 | 302 | 144 | 45 a |
| Early Flower | 1.03 b | 442 | 741 | 857 | 50 | 1358 | 87 b |
| Late Flower | 1.05 b | 335 | 65 | 1254 | 494 | 1352 | 92 b |

[a/] Means followed by similar letters not significantly different at the 5% level by L.S.D. test.

Figure 2.--Translocation of $^{14}$C-terbacil and/or metabolites in horsenettle. Treated plants (left) and corresponding radioautographs (right).
A. Leaf treated.
B. Roots treated.

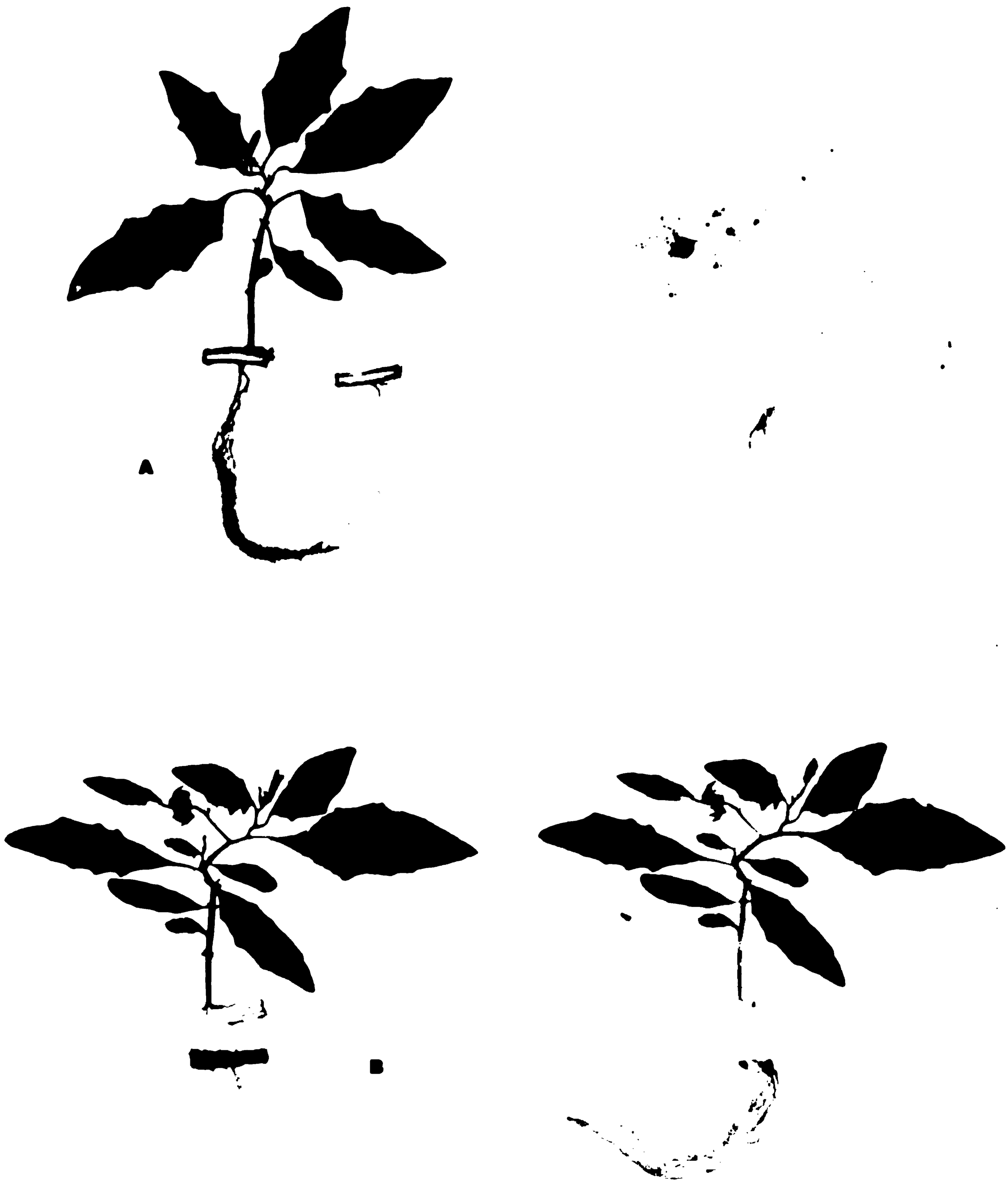
A
B

Table 3.--Uptake and translocation of $^{14}C$-terbacil in horse-nettle when applied in nutrient solution.

| Plant Stage | DPM/Section | | |
|---|---|---|---|
| | Fibrous Roots | Storage Roots | Shoots |
| Emergence | 895 | 353 | 1,769 |
| Pre Bud | 1087 | 679 | 11,519 |

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# APPENDICES

# APPENDIX A

## Hoagland's Solution

| Chemical | Stock solution (g/l) | Final solution (ml stock/l $H_2O$) |
|---|---|---|
| A Fe (Chelate 12%) | 16.6 | 2.5 |
| B $KH_2PO_4$ | 27.2 | 2.5 |
| C $MgSO_4 \cdot 7H_2O$ | 98.6 | 2.5 |
| D $K_2SO_4$ | 87 | 1.0 |
| E Minor elements: | | 1.25 |
| $ZnSO_4 \cdot 7H_2O$ | 0.088 | |
| $H_3BO_4$ | 1.144 | |
| $MnCl_2 \cdot 4H_2O$ | 0.724 | |
| $CuSO_4 \cdot 5H_2O$ | 0.032 | |
| $H_2MO_4 \cdot H_2O$ | 0.008 | |
| F $KNO_3$ | 101.11 | |
| G $Ca(NO_3)_2 \cdot 4H_2O$ | 236.16 | |
| H $CaCl_2 \cdot 2H_2O$ | 147 | 1.5 |

Equal quantities of F and G for the following $NO_3$ concentrations:

| | 2 mM | 3 mM | 4 mM | 8 mM | 12 mM | 16 mM |
|---|---|---|---|---|---|---|
| ml stock/l $H_2O$: | 0.66 | 0.99 | 1.33 | 2.66 | 3.99 | 5.32 |

# APPENDIX B

## Perennial Weed Control on Non-Crop Land

Location: Sodus Experimental Farm
Soil type: Genesee sand clay loam
Application date: June 11, 1971 (terbacil), July 14, 1971 (glyphosate)
Conditions: Plot area was plowed, dragged and disked 4 weeks prior to spraying.
Plot size: 6 x 25 Replications: 4 GPA: 36
Weeds present: Quackgrass, Canada thistle, Horsenettle, Common milkweed, Hedge bindweed, Swamp smartweed.

| Herbicide | Rate (kg/ha) | Weed Control Ratings (June 3) | |
|---|---|---|---|
| | | Broadleaf | Quackgrass |
| None | — | 1.0 | 1.0 |
| Terbacil | 1.1 | 5.0 | 3.3 |
| Terbacil | 2.2 | 6.0 | 3.8 |
| Glyphosate | 2.2 | 7.3 | 7.3 |
| Glyphosate | 4.5 | 8.5 | 9.0 |
| Glyphosate + Terbacil | 2.2+2.2 | 8.8 | 8.8 |

# APPENDIX C

## Horsenettle Control in a 2 Year-Old Asparagus Planting

| | |
|---|---|
| Location: | Decatur, MI |
| Soil type: | Oshtemo loamy sand |
| Application dates: | June 20, 1972, soil dry - 80°, air temp. - 88°, horsenettle size - 20 to 30 cm, August 22, 1972, soil - 82°, air temp. - 90°. |
| Plot size: | 3 x 25 Replications: 3 GPA: 72 |
| Note: | Applications were directed on each side of the asparagus row with a single nozzle boom to prevent as much spray as possible from reaching the asparagus fern. |

| Treatment | Rate (kg/ha) | Surfactant | Ratings | | |
|---|---|---|---|---|---|
| | | | Horse-nettle 8-8-72 | Horse-nettle 8-22-72 | Horse-nettle 9-21-72 |
| Glyphosate* | 2.2 | None | 6.0 | 6.3 | 8.0 |
| Glyphosate* | 4.5 | None | 7.0 | 6.7 | 8.0 |
| Terbacil | 2.2 | X-77 (.5%) | 7.7 | 8.3 | 8.0 |
| Glyphosate* | 2.2 | Fomex (.75%) | 3.7 | 5.0 | 7.0 |
| Glyphosate* | 4.5 | Fomex (.75%) | 4.7 | 6.3 | 8.0 |
| Terbacil | 2.2 | Fomex (.75%) | 7.7 | 8.0 | 8.0 |
| LSD at 5% level | | | 3.2 | 4.2 | 2.9 |
| Coefficient of variation (%) | | | 19 | 23 | 13 |

*Received a second application 8-22-72.

# APPENDIX D

## Horsenettle and Quackgrass Control in a Tart Cherry Orchard

| | |
|---|---|
| Location: | Honor, MI |
| Soil type: | Plainfield sand |
| Tree age: | 12 years |
| Application date: | June 13, 1972 |
| Conditions: | Soil - 81° - dry, air temp. - 87°,<br>Weeds were 10 to 15 cm high when sprayed. |
| Plot size: | 8 x 8 Replications: 3 GPA: 72 |

| Treatment | Rate (kg/ha) | Surfactant | Ratings | |
|---|---|---|---|---|
| | | | Horsenettle | Horsenettle |
| | | | 7-13-72 | 8-10-72 |
| Glyphosate | 2.2 | None | 6.3 | 6.0 |
| Glyphosate | 4.5 | None | 7.7 | 7.0 |
| Terbacil | 2.2 | X-77 (.5%) | 4.3 | 6.3 |
| Glyphosate | 2.2 | Fomex (.75%) | 7.0 | 5.3 |
| Glyphosate | 4.5 | Fomex (.75%) | 5.7 | 6.0 |
| Terbacil | 2.2 | Fomex (.75%) | 5.3 | 7.0 |
| LSD at 5% level | | | 3.6 | 4.2 |
| Coefficient of variation (%) | | | 30 | 29 |

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