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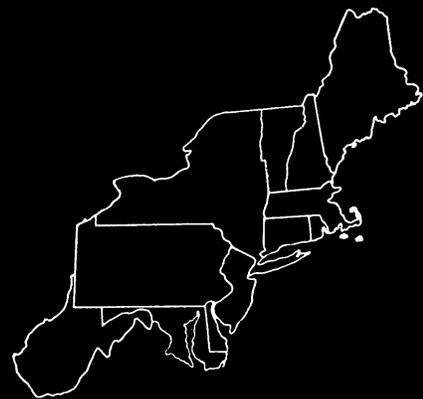
**LIFE HISTORY
STUDIES AS
RELATED TO
WEED CONTROL
IN THE
NORTHEAST**

3 -- Horse Nettle



Northeast Regional Publication

**Agricultural Experiment Station
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The research reported in this bulletin was conducted as part of the investigations of the regional weed research technical committee under Northeast Regional Project NE-42 "Weed Life Cycles, Soil Micro-organisms and Light as Factors in the Control of Weeds in the Northeast," a cooperative study involving the experiment stations in the Northeastern region. The work concerned with soil micro-organisms and light was initiated on July 1, 1954 under Northeast Regional Project NE-12 "Influence of Environmental Factors on the Effectiveness of Herbicides," and was continued under NE-42.

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LIFE HISTORY STUDIES AS RELATED TO WEED CONTROL

IN THE NORTHEAST

III. Horse Nettle

INTRODUCTION

Horse nettle (*Solanum carolinense* L.) is a persistent perennial weed having a very extensive and deeply penetrating root system which permits storage of large food reserves. It is normally disseminated by means of seeds, creeping roots, and root cuttings.

Some species, such as (*Physalls spp.*), produce edible fruits and others, such as (*Solanum nigrum* L.), are poisonous (65)¹. Horse nettle berries have been reported as being poisonous. When present in forage, they have caused losses among horses and cattle (59). Two members of the family that are economically important as a source of food are, the common potato (*Solanum tuberosum* L.) and tomato (*Lycopersicum esculentum* Mill.).

Because of its extensive root system this weed is quite resistant to most control attempts. Some success has been achieved with high rates of phenoxy herbicides applied in the fall (15). Generally, herbicides only provide control of the current season's topgrowth.

Horse nettle is native to the southern part of the United States but does not appear to be very troublesome in its native environment (1, 40). It has spread as far north as Ontario (59) and west to California (11). It is firmly established in practically all parts of Pennsylvania (40) and throughout the state of New Jersey (15). By 1961, 37 states (77) listed horse nettle as a noxious weed.

In New Jersey, horse nettle was found to have a relatively short growing season. New shoots usually did not emerge before the middle of May in the southern part of the state and early June in the northern sections. Bradbury (15) observed that the plant matured rapidly during the hot summer months and set berries from August through September.

Horse nettle appears to thrive best on sandy or gravelly soils (15, 40). However, it will grow in any type of soil (15). The plant was noticed most frequently in corn, followed by pastures, alfalfa, potatoes, and tomatoes with the most extensive infestations occurring in fields on which corn had been grown for several years (15).

Economic Importance:

Horse nettle acts as an important host for insects and diseases of econom-

¹Numbers refer to publications cited in the Bibliography.

ically important crop plants. Pritchard and Porte (72) reported that horse nettle is a very susceptible host for the fungus (*Septoria lycopersici*) which causes leafspot of tomato. The weed is also host for the disease organism (*Verticillium alboctrum*) which causes Verticillium wilt of egg plant. According to Muenscher (64) the weed is host to mosaic viruses which cause potato and tomato mosaic.

Wallis (90) showed that horse nettle is an important host for the insect called the Potato Psyllid (*Paratrioza cockerelli* (Sulc.)) which transmits Psyllid Yellows disease to potatoes and tomatoes. Other important crop insects harbored by this plant are as follows: potato flea beetles (*Epitrix fuscula* Crotch) and (*Epitrix cucumeris* (Harr.)) (25); Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) (60, 64) potato stalk borer (*Trichobaris trinotata* (Say)); onion thrips (*Thrips tabaci* Lind.); greenhouse redspider mite (*Tetranychus telarius* (L.)) (64).

PREVIOUS WORK

Growth Habits:

Kiltz (48) showed that the roots would grow to depths of eight feet in heavy alluvial soils and that creeping roots found in the upper eighteen inches of soil extended three to four feet from the main root. Bradbury's (15) excavations of horse nettle roots revealed a penetration of at least six feet in depth. He excavated a single intact rhizome 18.9 feet in length having numerous shoots emerging from it.

Bradbury (15) observed differences in growth patterns between disturbed and undisturbed infestations of the weed. The older undisturbed areas were less thickly populated with horse nettle- than were cultivated areas. Thicker stands on cropland were believed due to propagation from pieces of root stalks mixed in the soils by seedbed preparation and other cultural operations. He further stated that new plants could be formed from rootstock sections less than one inch in length.

Several investigators agree that berries may be eaten by farm animals and the undigested seeds scattered over large areas by means of the droppings (11, 12, 40, 74, 75). Robbins, Bellue and Ball (74) reported that sheep were the only animals that grazed the weed, feeding primarily on the berries. However, cattle will also eat the mature berries (75). Robbins, Crafts, and Raynor (75) reported that spread by undigested seeds in animal droppings has been shown.

Although the above investigators presented no definite evidence or data to show that horse nettle is propagated by seeds, the probability that it is spread by seed is good.

CHARACTERISTICS OF HORSE NETTLE PLANTS

Stems and Leaves:

Horse nettle is an erect, simple or branching plant, from one to three feet tall, with rather stout stems. The entire plant is pubescent. The main stem, branches, petioles, calyx, midribs, and lateral veins of the leaves have ridged, sharply pointed, yellowish spines and short, stiff, four to eight rayed hairs. The leaves are alternate, simple, two to six inches long, oblong to ovate in outline with unevenly lobed toothed or deeply cut margins (Figure 1).



Figure 1. The horse nettle plant develops a branching stem. The stem, petioles, and leaves are covered with yellow spines. Flower buds, open flowers and nearly mature fruit may all be present at the same time.

Flowers, Berries and Seeds:

The flowers, on spiny petioles, are perfect, complete, and in open indeterminate racemes. The spiny peduncles are irregularly spaced along the sides of the stems. The five-lobed corolla varies in color from violet to white and is about one inch in diameter. It closely resembles the flower of the common potato. There are five united yellow stamens surrounding the two-celled ovary (Figure 2).

The fruit or berry is tomato-like, smooth, round, one-half to three-quarters of an inch in diameter, orange yellow in color when mature, with a moist interior having a very disagreeable odor (Figure 3).

The seeds of horse nettle resemble those of the common potato in size, shape, color, and internal structure. The seed is about two millimeters in diameter and a half millimeter in thickness. It is yellowish to light brown in color, orbicular in outline, and flattened in cross section. The surface has a slightly pebbled appearance.



Figure 2. The flower, buds and spiny petioles of the horse nettle plant. The five stamens surrounding the ovary are clearly visible.

CONTROL STUDIES

The primary objective in any perennial weed research program is eradication or control. The control of horse nettle has been studied by many workers, but as yet no satisfactory control measure is available.

Neville (67) studied the control of horse nettle in field corn. It was found that two and three pounds per acre of 2,4-dichlorophenoxyacetic acid (2,4-D) and a 2,4-D-2,4,5-trichlorophenoxyacetic acid (2,4,5-T) mixture produced some injury to these plants. Bradbury (15) in New Jersey reported a 100 per cent reduction in a horse nettle stand with a fall application of 32 pounds per acre of 2,4-D. He also found that four pounds per acre of 3-amino-1,2,4-triazole (amitrole) applied during flowering produced good control. It was shown that a mixture of 2,4-D and 2,4,5-T was more ef-

fective than either chemical used alone. Hemphill (42) in Missouri obtained control of horse nettle with amitrole at two pounds per acre applied as a wetting spray in August. Fair control was obtained with 2,3,6-trichlorobenzoic acid (2,3,6-TBA) at three pounds per acre.

Organization of the Horse Nettle Project:

The horse nettle work conducted at New York and New Jersey was a contributing project for NE-42 (Weed Life Cycles As They Relate To Control Methods).

Many facets of the life history of horse nettle were studied at both stations. These investigations included seed work, root studies, and observations of the growth habit of the plant.

The experimental work reported was conducted between 1958 and 1961.

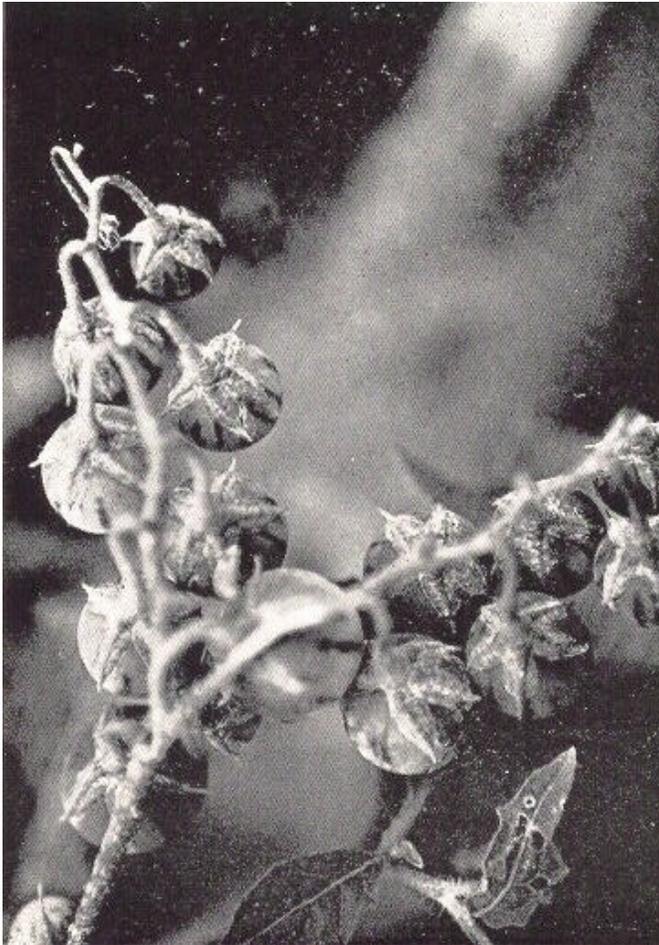


Figure 3. The fruit or berry of the horse nettle plant is tomato-like, smooth round and one-half to three-quarters of an inch in diameter. A single plant may produce as many as 100 fruits.

SEED INVESTIGATIONS

Number of Seeds Produced:

Materials and Methods:

On October 14, 1958, mature horse nettle berries were collected from a corn stubble area in a field near Newfield, Tompkins County, New York. This field had been in corn for three years (1956 through 1958). The soil was classified as Palinyra Gravelly Silt Loam, well drained to droughty, derived from glacial outwash. A sample of the soil tested by the Cornell University Soil Testing Laboratory showed the following analysis: organic matter-3.2 per cent; pH - 5.4; P - 8 pounds per acre; K - 150 pounds per acre; Mg -- 115 pounds per acre. Interpretation of the test showed a medium level of organic matter, very low pH, and high levels of P, K, and Mg.

Seeds and vegetative parts of the horse nettle plant were collected from this area for various phases of the investigations to be reported. Also, the area was used as a field laboratory to observe development of the horse nettle plant. For identification in subsequent discussions, this area will be referred to as the "Lackner Field."

From the berries collected on October 14, sixteen berries were placed in four different groups. Selections were made from a large number to get four berries in each group as near alike in size and shape as possible. All berries were fairly uniform in maturity. The selected berries were measured, cut open, and the seeds counted.

Results and Discussion:

There was considerable variation in the numbers of seeds per berry within each size classification. The average number of seeds in berries of each size group were: (a) large-161, (b) moderately large-117, (c) medium-54, and (d) small-13. Thus, in mature berries, as the size of berry increased, the number of seeds per berry increased. The average number of seeds per berry for all groups was 86.

Effect of Light on Seed Germination-

Bradbury (15) investigated the effect of light on germination of horse nettle seed and concluded that this species was sensitive to light. The literature indicated that alternating temperature regimes of 20 to 30°C are optimum for seed germination. Some evidence also exists that potassium nitrate may be substituted for light for seeds that require light for germination. The present studies were initiated to further explore these factors.

Materials and Methods:

The seeds used in this investigation were collected at Millstone, New Jersey on December 23, 1958. Since the berries were still moist at the time of

collection, it was assumed that they had developed during the previous growing season.

One experiment was conducted from January 5, 1959 to January 23, 1959. The following temperature regimes were studied: (1) 20°C for 16 hours and 30°C for 8 hours; and (2) 15°C for 16 hours and 30°C for 8 hours. The seeds were kept under light and dark conditions in both of these schedules. The daylight treatment was achieved by placing the seeds in a daylight germinator at the State Seed Laboratory, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey. One germinator was employed for each temperature treatment. The dark treatment was achieved by wrapping the petri dishes in aluminum foil and placing the dishes in the same germinators as the seeds receiving daylight. These daylight germinators were also on an alternating schedule with 8 hours of light and 16 hours of darkness. There were three replications in this experiment.

The second experiment was initiated on January 27, 1959 and completed on February 6. In this study only the 15°C to 30°C temperature schedule was employed. Half the seeds in this germinator were kept in the dark in the manner previously described. The remainder were exposed to daylight for 8 hours each day. Half the seeds in both the daylight and the dark treatments were initially moistened with a 0.1 per cent solution of potassium nitrate, while the others received a similar quantity of distilled water. Four replications of these treatments were used. The seeds in the wrapped dishes were not exposed to light at any time during the course of the experiment. The number of germinated seeds was recorded after ten days.

In the germination studies conducted at Cornell on the influence of temperature on germination, seeds in the dark germinators were exposed to daylight for brief periods while germination counts were being made. To investigate the influence of light, a test was initiated to include a complete dark treatment.

A seed lot harvested on April 12, 1958 from the "Lackner Field" was used. A temperature treatment of 20 to 30°C alternating (20°C for 15 hours, 30°C for 9 hours) with tap water and 0.2 per cent potassium nitrate moistening agents was used under the following conditions: (1) complete darkness and (2) light for 9 hours during the high temperature period and dark for 15 hours during the low temperature period.

For the complete dark treatment, petri dishes were wrapped in aluminum foil. Four sets of dishes were opened and counted after 10, 17, 24, and 31 days.

Results and Discussion:

Results from the first experiment are presented in Table 1. These data show that there were no differences among the light or temperature treatments.

Table 1. Effect of Light and Temperature on Germination (Experiment 1)¹

Treatment	Per Cent Germination		Average
	20 to 30°C	15 to 30°C	
Daylight	15.9	51.9	48.9
Darkness	42.5	43.9	43.2
Average	44.2	46.0	

¹Average of three replications of 50 seeds per replication.

The data obtained in the second experiment are found in Table 2. These data show no differences with the light treatments or the substrate moistening agent. Germination with the potassium nitrate substrate was slightly higher, than the water check. However, this difference was not a significant one.

Table 2. Effect of Light and Substrate Moistening Agents on Germination. (Experiment 2).¹

Treatments	Per Cent Germination		Average
	KNO ₃ 0.1 per cent	H ₂ O	
Daylight	37.1	33.8	35.3
Darkness	45.1	40.1	42.6
Average	41.1	36.8	

¹Average of four replications of 50 seeds per replication.

The data obtained in these two experiments indicate that light was not a necessary factor in the germination of this particular lot of horse nettle seed and in total agrees with the studies conducted in New York.

A Chi-square index of dispersion applied to the complete dark treatment showed that the rate of germination in the two media, tap water and potassium nitrate, was fairly constant at the four counting dates. The differences in the

Table 3. Effect of Light on Germination 20-30°C.

Time Intervals	Complete Darkness		Partial Darkness		Light for 9 hrs/day	
	H ₂ O	KNO ₃	H ₂ O	KNO ₃	H ₂ O	KNO ₃
	Per Cent Germination*					
0-10 days	5	11	1	5	13	22
0-17 days	37	57	56	53	52	58
0-24 days	41	54	66	59	61	64
0-31 days	54	71	67	60	61	66

*Figures are averages of four replications of 25 seeds each.

rate of germination were not significant. However, there was a highly significant difference at the one per cent level of probability in total germination between tap water and potassium nitrate moistening agents.

Light appeared to be a factor affecting germination when tap water was used as the moistening agent, but not when potassium nitrate was used as shown in Table 3. However, the overall comparison of dark vs. light would indicate

that light is not an important factor affecting total germination, but that exposure of seeds to light tends to increase the germination rate in the early stages of germination.

Effect of Temperature and Substrate Moistening Agents on Germination:

A series of investigations was conducted on the Germination of horse nettle seed as influenced by temperature and moistening agent.

Materials and Methods:

The first tests at Cornell were started on June 26, 1959. A single lot of seed, harvested on April 12, 1959 in the "Lackner Field," was used. The experiment was arranged as follows:

1. Pre-germination treatments: (a) no cold treatment; (b) 5°C for 5 days; and (c) 5°C for 10 days.
2. Temperature treatments (for each pre-germination treatment) : (a) 20 to 30°C alternating (20°C for 15 hours, 30°C for 9 hours) ; (b) 10 to 30°C alternating (10°C for 15 hours, 30°C for 9 hours) ; and (c) 20°C constant temperature.
3. Light conditions: seeds were exposed to daylight for short periods when germination counts were made. To get an indication of the effect of long periods of light on germination, one sub-treatment in the 20 to 30°C condition was exposed to light during the high temperature period.
4. Substrate: cotton and filter paper in glass petri dishes.
5. Substrate moistening agents: (a) tap water; and (b) 0.2 per cent potassium nitrate solution (only potassium nitrate was used in "light" treatment).
6. Replication: four replications of 25 seeds per replication.
7. Seed treatment: dusted with 50 per cent Arasan prior to placement into petri dishes to prevent seed decay.

Germination counts were made at intervals of 5 to 6 days. Seeds were considered germinated when the radicle measured one inch in length and appeared vigorous and healthy. Germinated seeds were removed from the petri dishes at the time each count was made.

Results and Discussion:

Pre-germination cold treatments had no effect on germination in this lot of seed. There was a highly significant difference at the one per cent level between the germination temperature treatments. There was also a highly significant difference due to the kind of substrate moistening agent used. The interaction between germination temperatures and moistening agent was not the same at the three different temperature treatments.

Potassium nitrate greatly increased germination at the 20°C constant temperature (Table 4). This may have been due to the effect of breaking dormancy or on increasing the permeability of the seed coat or both.

The data in Table 4 might indicate a slight inhibitory effect on germination due to exposure of seed to light. However, a later test showed little effect.

The condition most favorable for the germination of mature seed overwintered in the field was alternating temperatures of 20 to 30°C in a dark germinator using a 0.2 per cent potassium nitrate solution as the substrate moistening agent. The most rapid rate of germination was also obtained with this treatment.

Materials and Methods:

A second series of germination tests was started at Cornell on August 12-13, 1959. A personal communication from New Jersey suggested that good germination of horse nettle seed was obtained using a treatment of 30°C constant temperature with tap water as the moistening agent. Therefore, this treatment was included. Six different seed lots were tested:

Table 4. Effect of Light, Temperature and Substrate Moistening Agents on Germination of "Lackner Field" Seed.

	PER CENT GERMINATION*									
	0-10 days		0-15 days		0-20 days		0-25 days		0-40 days	
	H ² O	KNO ³	H ² O	KNO ³	H ² O	KNO ³	H ² O	KNO ³	H ² O	KNO ³
	<u>No Cold Treatment</u>									
20-30°C Dark †	28	29	51	55	59	64	61	67	62	69
10-30°C Dark	3	1	24	29	42	44	49	52	56	59
20°C Dark	0	10	0	33	0	40	0	41	0	42
20-30°C Light ‡		29		44		49		51		51
	<u>5°C FOR 5 DAYS, THEN:</u>									
									0-42 days	
20-30°C Dark	34	33	50	57	56	63	56	65	58	67
10-30°C Dark	0	1	25	28	41	40	49	54	52	60
20°C Dark	1	15	2	36	3	45	5	47	5	47
20-30°C Light	35		45		50		52		52	
	<u>5°C FOR 10 DAYS, THEN:</u>									
									0-37 days	
20-30°C Dark	25	32	47	55	56	61	56	66	58	67
10-30°C Dark	2	0	15	13	36	33	51	47	57	56
20°C Dark	0	24	0	35	3	48	3	49	3	49
20-30°C Light		33		53		57		58		58

* Figures are the averages of four replications of 25 seeds each.

† Seeds in dark germinator-counts made in daylight.

‡ Seeds exposed to light for at least 9 hours a day.

I. Seed from Somerset Co., N. J., harvested in December 1958.

II a. Seed from Tompkins Co., N. Y., harvested on October 14, 1958.

II b. Seed from Tompkins Co., N. Y., harvested on October 14, 1958, overwintered in berries on the soil surface in concrete frames.

III Seed from Dutchess Co., N. Y., harvested on September 19, 1958.

IV. Seed from Tompkins Co., N. Y., harvested on August 26, 1958.

V. Seed from Tompkins Co., N. Y., harvested on August 2, 1958.

VI. Seed from Tompkins Co., N. Y., harvested on August 3, 1959.

The experiment was established as follows:

1. Two pre-germination treatments: (a) no cold treatment and (b) 5°C for 8 and 9 days.

2. Two temperature treatments for each pre-germination treatment: (a) 20 to 30°C alternating (20°C for 15 hours, 30°C for 9 hours) and (b) 30°C constant.

The above treatments were conducted in dark germinators. The seeds were exposed to daylight for short periods when germination counts were made. In addition to the dark treatment, a separate group of the recently harvested immature lot VI seed was exposed to light during the high temperature periods of the alternating temperature treatments. The remainder of the experimental technique was the same as that used above.

Results and Discussion:

The results are shown in Table 5. There was no significant effect of pre-germination cold treatments on germination. The effect of germination temperatures was highly significant at the one per cent level of probability for lots 11 and 111, and significant at the five per cent level of probability for seed lots 1, IV, and V. The effect of the substrate moistening agent, or medium, was significant at the five per cent level of probability for lots I, IIa, III, and V.

Over the entire five lots of seed, there was a highly significant difference in germination at the one per cent level of probability due to the temperatures, lots, media, and in the interaction of these three variables.

Germination was low at all seed lots under the condition of 30°C constant temperature with tap water as the moistening agent (Table 5).

The potassium nitrate moistening agent greatly increased the germination at 30°C constant temperature except in lot IV.

The initial germination at the 30°C constant temperature, using potassium nitrate, was more rapid than at 20 to 30°C alternating temperature treatments with potassium nitrate. However, the final germination tended to be lower at 30°C constant temperature.

Seed lots IIa and IV were harvested from the same field at two different dates. The results suggest that later-harvested mature seed (lot IIa) had greater viability than earlier-harvested, somewhat immature seed (lot IV). However, length and condition of storage may have been factors contributing to this difference.

Seed from New Jersey (lot 1) reacted more favorably to the 30°C constant temperature with tap water than did the other seed lots. This suggested

Table 5. Effect of Temperature and Substrate Moistening Agent on Germination, August 12, 1959.

Seed Source	Temperature Treatment	PER CENT GERMINATION*											
		0-9 days		0-16 days		0-23 days		0-7 days		0-13 days		0-20 days	
		H ₂ O	KNO ₃	H ₂ O	KNO ₃	H ₂ O	KNO ₃	H ₂ O	KNO ₃	H ₂ O	KNO ₃	H ₂ O	KNO ₃
Lot I	20-30°C†	2	16	58	64	60	66	0	6	49	62	51	69
	30°C	24	51	30	65	30	65						
	5°C, 9 days, then 20-30°C							9	34	20	51	22	54
Lot IIa	20-30°C	0	3	58	68	80	77						
	30°C	0	31	2	55	2	59	0	0	44	57	64	72
	5°C, 9 days, then 20-30°C							1	17	3	54	3	58
Lot IIb	20-30°C												
	30°C												
	5°C, 8 days, then 20-30°C												
Lot III	20-30°C†	0	1	13	42	41	52						
	30°C	1	8	2	34	3	37	0	0	10	46	42	56
	5°C, 8 days, then 20-30°C							3	15	5	31	5	37
Lot IV	20-30°C	0	0	18	8	19	8						
	30°C	0	4	3	7	3	7	0	0	16	11	19	16
	5°C, 8 days, then 20-30°C							1	5	8	11	8	11
Lot V	20-30°C												
	30°C	1	5	56	53	66	59						
	5°C, 9 days, then 20-30°C	2	29	5	47	4	51	1	0	55	49	63	59
Lot V	20-30°C												
	30°C												
	5°C, 9 days, then 20-30°C							4	21	16	52	17	56

* Figures are an average of four replications of 25 seeds per replication.

† All seeds tested in dark germinators-counts made in daylight.

the possibility of varietal differences or differences in climatic adaptation among horse nettle plants.

The germination at the 30°C constant temperature treatment with the tap water moistening agent was similar to the results obtained in the 20°C constant treatment used in the first horse nettle germination test (Table 4). This indicated that the heat applied during germination was not responsible for the higher percentage germination obtained in the alternating temperature treatments, but rather the effect of breaking dormancy or of mechanical changes in the seed coat.

There was no difference in total germination between lot IIa seed harvested in October 1958 and stored in the laboratory and lot IIb seed, of the same harvest date but over-wintered in berries on the soil surface. However, the seed over-wintered on the soil surface in berries germinated more rapidly than the seed stored in the laboratory.

The treatment of 20 to 30°C alternating temperatures (20°C for 15 hours, 30°C for 9 hours), using either tap water or 0.2 per cent potassium nitrate moistening agents, resulted in the most favorable percentage germination.

Lot VI was not included in the table because there was no germination of this seed under any of the treatments used.

Effect of Weight of Seed on Germination:

Materials and Methods:

Seed from Tompkins County, New York, harvested on April 12, 1958 (Lot V in Table 5) was separated into three weight groups: (a) 112 milligrams per 100 seeds; (b) 157.6 mg. per 100 seeds; and (c) 186.4 mg. per 100 seeds. These seeds were tested in a dark germinator at 20 to 30°C alternating temperature with potassium nitrate as the moistening agent. The procedures used were the same as those followed in previous tests.

Results and Discussion:

The results show that the greater the average weight of seed (Table 6) the higher the percentage germination. One serious limitation of this test was that no check was made for the presence of embryos in the seeds used.

Table 6. Effect of Weight of Seed on Germination.

Seed weight in milligrams per 100 seeds	DAYS				
	0-8	0-15	0-22	0-29	0-36
	<u>Per Cent Germination</u>				
112.0	0	9	17	18	18
157.6	0	32	50	54	55
186.4	0	57	80	82	82

Emergence of Seedlings from Horse Nettle Infested Soil:

Materials and Methods:

Soil from the horse nettle infested "Lackner Field" was obtained in November 1958. Samples were taken at depths of 0 to 3, 3 to 6, and 0 to 6 inches. Two wooden flats of each of these soils were placed in the greenhouse on February 3, 1959.

Results and Discussion:

The average seedling emergence of two replications at each soil depth was as follows: 0 to 3 inches-9.2 seedlings per square foot; 3 to 6 inches-0.9 seedlings per square foot; 0 to 6 inches-zero seedlings per square foot. These results were quite variable but showed that seed in this naturally infested soil was viable and could produce vigorous seedlings under greenhouse conditions.

Emergence of Seedlings under Natural Conditions:

Materials and Methods:

Previous studies (15) indicated that emergence of horse nettle plants from seed under field conditions was a rare occurrence. A check was made to determine the dissemination of seed under natural conditions in a farm field. May 15, about 5,000 square feet of this four-acre field was surveyed.

Results and Discussion:

Fifteen seedling locations were found and marked with garden labels for future observations. The distribution of seedlings was from one to groups of 15 to 20 at each location. About 200 horse nettle seedlings were found in this area. Observations in September 1959, showed that 50 per cent of the seedlings had survived the summer.

Emergence from Seed Scattered on the Soil Surface

Materials and Methods:

An experiment was started on October 23, 1958 to determine the effect of over-wintering of seed on emergence the following spring.

The soil of four outdoor 2x2 feet concrete frames was spaded and 'raked prior to seeding. Two cultural treatments used were spring plowed and not spring plowed. Two lots of seed from "Lackner Field" were used. One lot was harvested in April 1958 and the other in October 1958. Two hundred seeds from the April 1958 harvest date were scattered on the soil surface of each of two frames. The same procedure was followed with the seed harvested in October '1958.

On April 15, 1959, one frame of each lot was spaded to a depth of 6 to 8 inches and leveled with a rake. The remaining two frames were left undisturbed.

Results and Discussion:

The earliest emergence, May 13, occurred in the unspaded treatment. The results, as shown in Table 7, indicate that seeds distributed on the soil surface in the fall were capable of producing seedlings the following spring.

Table 7. Emergence from Seed Scattered on Soil Surface.

Seed Lot	Spaded in Spring	Number of Seedlings Emerged by August 8	Per Cent Emergence of 200 Seeds
April, 1958	No	15	7
April, 1958	Yes	10	5
October, 1958	No	25	12
October, 1958	Yes	5	2

Evidence substantiating the above conclusion was obtained in connection with a germination test of seed over-wintered in berries on the soil surface.

Fifty mature berries were scattered on the soil surface of each of two concrete frames on October 23, 1958. The following April, the berries were collected. When the seeds from these berries were planted on May 6, 1959 in two replications, 54 seeds per replication, at 1/2 inch depth in concrete frames, the average seedling emergence was 53.7 per cent. However, several berries were not retrieved and others had broken open during the winter. Thus, some seeds were left in the frames. The frames were observed at intervals during the summer for seedling emergence. On August 8, the number of seedlings in the two frames were 114 and 28, respectively.

The above several experiments and observations all point to the fact that horse nettle seed is definitely an important factor to be considered in the dissemination of this weed.

Emergence from Seed Planted in Different Soil Types

Materials and Methods:

On March 14, 1959 an experiment was initiated in the greenhouse to determine the emergence from seed when planted in different soil types and at depths varying from one to three inches. The soils used were as follows:

1. *Palmyra Gravelly Silt Loam*-pH 5.4, derived from glacial outwash, well to excessively drained. This soil was used under two conditions: (a) surface 6 to 8 inches unscreened and (b) same soil layer screened through 1/2 inch mesh sieve.
2. *Lima Silt Loam*-pH 6.7, from calcareous glacial till, naturally moderately well-drained but the well-drained surface 6 to 8 inches was used. The soil was screened through 1/2 inch mesh sieve.
3. *Collamer Silty Clay Loam*-pH 6.5, a heavy lake-laid clay, somewhat poorly drained.

The seed used was harvested on October 14, 1958 from "Lackner Field." Four replications of 12 seeds per replication were planted in each soil type at each depth. A doweled board was used to insure uniform spacing and depth. Seedling counts were made at approximately seven day intervals from April 1 to May 20, 1959.

Results and Discussion:

The most rapid rate of emergence was at the one inch depth and occurred during the first three weeks after planting (Table 8). Seed planted at the three inch depth showed practically no emergence until four weeks after planting. Emergence of seed from the one inch depth was four to ten times greater than from the three inch depth in the well drained soils. Per cent emergence in the heavier Collamer soil was less than one-half that in the well drained soils at the one inch depth. No seedlings emerged from the three inch depth in the heavy soil.

Therefore, it would appear that the chances of horse nettle infestations starting from seed are considerably better on lighter textured, well drained soils than on heavy, somewhat poorly drained soils.

Table S. Emergence from Seed Planted in Different Soil Types at Two Depths.

Counting Dates and Depths of Seed	Palmyra Gravelly Silt Loam		Lima Silt Loam	Collamer Silty Clay Loam
	Screened	Unscreened	Screened	Unscreened
	<u>Per Cent Emergence*</u>			
Apr. 1 - 1 inch	35	48	44	8
Apr. 1 - 3 inch	2	0	0	0
Apr. 4 - 1 inch	65	67	69	21
Apr. 4 - 3 inch	2	0	0	0
Apr. 10-1 inch	65	71	77	27
Apr. 10-3 inch	6	4	6	0
Apr. 15-1 inch	67	73	81	29
Apr. 15-3 inch	8	6	6	0
Apr. 27-1 inch	67	75	81	29
Apr. 27-3 inch	10	6	17	0
May 20-1 inch	67	77	81	29
May 20-3 inch	10	8	21	0

*Each figure represents the average of four replications of 12 seeds per replication.

Field Seedling Emergence from Horse Nettle Berries

Materials and Methods:

This experiment was conducted to determine the extent of emergence of seedlings from horse nettle seed in the whole berries and in broken berries when planted at various depths in the soil.

On October 23, 1958, a number of mature undried berries harvested from the "Lackner Field" on October 14, 1958, was planted in soil in outdoor 2x2 feet concrete frames. The experiment was set up as follows:

1. Berry treatments: (a) whole berries; (b) berries cut in half; and (c) berries cut in quarters.
2. Depths of planting: (a) 1, (b) 3, (c) 5, and (d) 7 inches.
3. Replication: five replications of each berry treatment at each depth.

Results and Discussion:

Results of counts made on July 2, 1959, are presented in Table 9. Only the results of the one-inch depth treatment are shown. There was no emergence from the three inch depth, one seedling from the five inch depth, and four seedlings from the seven inch depth. Since there was no emergence from the three inch depth and the initial emergence in the five and seven inch depth plantings were observed at the same time as the emergence from the one inch depth (May 13th), it was assumed that the seedlings produced at the five and seven inch depths were due to accidental contamination.

It was shown that horse nettle seedlings can emerge from mature, undried whole and broken berries that are located within 1 inch of the soil surface. Seedlings emerged more readily from broken berries than from whole berries.

Table 9. Emergence of Seedlings from Whole and Sectioned Berries from the 1 Inch Depth.

Replication	No. Seedlings Emerged					Ave. No. of Seedlings: Per Berry Planted	No. of Seedlings: Emerg- ed	% of Berry Sections Planted Producing Seedlings
	1	2	3	4	5			
Whole Berries	30	19	0	0	0	9.8	24.5	40
Half Berries	3	15	5	25	6	10.8	10.8	100
Quarter Berries	16	5	18	22	8	13.8	13.8	100

Greenhouse Seedling Emergence from Horse Nettle Berries

Materials and Methods:

An experiment was started on March 18, 1959 in the greenhouse using only whole berries which were part of the same lot used in the above experiment. The test was arranged as follows:

1. Berry treatments: (a) air dried whole berries and (b) undried whole berries (berries were kept in a plastic bag after harvest to prevent drying).
2. Three depths of planting: (a) 1, (b) 3, and (c) 5 inches.
3. Replication: four replications of each treatment at each depth.
4. Substrate: silt loam soil was used.
5. Temperature: ranged from 16 to 32°C during the course of the experiment.

Results and Discussion:

Table 10 shows the results of an emergence count made on July 2, 1959.

Under greenhouse conditions, horse nettle seedlings can emerge from mature dried or undried whole berries that are located within one to three inches of the soil surface.

Table 10. Emergence of Seedlings from Whole Dried and Undried Berries in the Greenhouse.

Berry Treatment, Depth in Inches	Number of Seedlings Emerged per Replication			
	1	2	3	4
Dried 1	82	0	61	0
3	0	0	0	0
5	0	0	0	0
Undried 1	3	30	4	27
3	3	0	0	0
5	0	0	0	0

The results of the above two tests coincide with observations made in the field survey described earlier where horse nettle seedlings were found growing in clusters as well as individually.

Emergence from Seeds Planted at Various Depths

Materials and Methods:

On April '52, 1958 mature over-wintered horse nettle berries were obtained from the corn stubble area in "Lackner Field."

The harvested berries were cut open to speed drying. The seeds were then separated from the berries and stored in glass jars. Wooden flats were constructed having the following dimensions: 5½ inches deep, 15½ inches wide, and 20½ inches long.

On May 23, 1958, 60 seeds, 3 replications of 20 seeds per replication were planted at four different depths: ¼, ½, 1, and 2 inches. This was done by using a doweled board to obtain uniform spacing and depth. The seeded flats were placed outdoors and watered to keep the soil moist. Flats were handweeded to remove other weed species. The soil used was Lima Silt Loam obtained from a previously undisturbed wooded area on the Agronomy Research Farm at Aurora, N. Y.

The soil was screened through one half inch mesh sieve. A soil test showed the following analysis: organic matter-greater than 6 per cent; pH-6.7; P-2 pounds per acre; K-110 pounds per acre.

Results and Discussion -

There was no great difference in the emergence from seed planted at the ½, 1 and 2 inch depths. Germination varied from 56.7 per cent to 61.7 per cent. The low emergence at the ¼ inch depth may have been caused by heavy rains following planting which uncovered much of the seed. The average percentage emergence of the four depths of seeding was 49.4 (Table 11).

Table 11. Emergence of Horse Nettle Seed Collected from Lackner Field, April 1 1958 and Planted May 23, 1958.

Depth in Inches	Replication	Per cent Emergence		
		No. of Days After Seeding		
		25	34	57
¼	1	20	15*	15
	2	30	50	50
	3	0	0	0
	Average:	17	21	21
½	1	55	55	50*
	2	70	70	70
	3	60	60	65
	Average:	62	72	62
1	1	0	65	65
	2	60	60	60
	3	50	55	50*
	Average:	53	60	58
2	1	55	60	65
	2	45	60	60
	3	30	40	45
	Average:	43	53	57
Average of all depths		44	49	49

*Mortality

Because of the relatively high emergence obtained at the two inch depth, 60 seeds were planted on July 24 at depths of 3 and 4 inches. Fifty-seven days later 40 per cent emergence was obtained from the three inch depth and 23 per cent from the four inch depth (Table 12). There was no emergence from six and eight inch depths of planting in similar tests conducted in the greenhouse in 1959.

Table 12. Emergence of Horse Nettle Seed Collected from Lackner Field, April 1958 and Planted July 24, 1958.

Depth in Inches	Replication	Per cent Emergence		
		No. of Days After Seeding		
		25	34	57
3	1	40	40	4
	2	35	45	45
	3	10	30	35
	Average:	28	38	40
4	1	10	15	15
	2	10	25	30
	3	5	20	25
	Average:	8	20	23

Over-Wintering of Mature Seed

Numerous papers have been published on the effect of storage conditions on seed viability and germination (9, 21).

Studies were initiated to determine the effect over-wintering would have on the subsequent germination of seed.

erator at night and in the 30°C incubator during the day. The remaining dishes were kept at a constant 30°C. The germination tests on this lot of seed were terminated on March 29, 1961.

Results and Discussion:

When the seeds were tested at a constant temperature of 30°C, all the storage treatments showed very poor germination. Exposure of the seeds to alternating temperatures produced much better germination with all the treatments. There were no differences due to storage position or to the origin of the seeds (Table 14).

Table 14. Germination of Horse Nettle Seed at Two Temperature Regimes Over-wintered At Three Positions with Respect to the Soil Surface. Seed obtained from Plants Established from Two Sources.¹

Over-wintering Position	Seed Source	30°C	10-30°C
One foot above surface	New York	0	70
One foot above surface	New Jersey	4	46
On soil surface	New York	2	70
On soil surface	New Jersey	4	64
Four inches below surface	New York	-2	80
Four inches below surface	New Jersey	4	78

¹ Average of 50 seeds.

² Missing data.

It is evident from the presented data that a constant temperature does not favor the germination of horse nettle seed; however, when the seeds are subjected to alternating temperatures some physiological mechanism is activated whereby germination results.

Seed Maturity Studies

Results of previous experiments revealed that time of seed collection was a factor influencing germination. The present study was initiated in 1959 and continued in 1960. The 1959 investigation was conducted with seed from the Millstone site. Seeds for the 1960 studies were obtained near New Brunswick.

Materials and Methods:

Collections in 1959 were started in August and were continued at approximately one month intervals until March 1960. Usually six to ten berries were selected on each date. The berries used were all taken from the same position in their respective clusters. The berry that was closest to the main stem was the one collected. The seeds were removed from the berries, washed with distilled water, and allowed to dry at room temperature.

The dry seeds were counted into 10 lots of 50 seeds. Each of the 10 lots was weighed on an analytical balance. The germination tests were run with three replications of 50 seeds per replication. It was usually one or two days after collection before the germination tests were started. Seeds were moisten-

ed with tap water and held at a constant temperature of 30°C. The seeds that were not utilized for the initial germination test were held at room conditions for future studies. Germination tests were run on these seeds at monthly intervals throughout the season.

Half the berries that were sampled during 1960 were taken from plants that had been established with rootstocks obtained from New York. The remainder were collected from plants that had originated from rootstocks dug near Millstone, New Jersey, and established in New Brunswick. Consequently, the seed obtained from these plants will be referred to as New York and New Jersey seed, respectively.

In order to provide an estimate of the age of the seed used in this study, a large number of blossoms were tagged on the day of flowering. Berry collections were made on the following days: July 22, August 3, 15, September 7, 27, October 24, and November 18, 1960. The average number of days after flowering at each of the collection dates were 45, 56, 68, 91, 111, 138, and 167 days, respectively. The seeds were treated in the same manner as the 1959 seeds.

Results and Discussion:

Seed collected at various times from fall through the winter followed the same general pattern of poor initial germination and higher germination after a period of dry storage (Table 15). The lowered germination in certain instances after dry storage was possibly due to a dormant condition. The seeds that did not germinate appeared to be viable at the termination of the test period,

Table 15. Germination of Morse Nettle Seed on the Day of Collection and After Two and Four Months Storage. Seed Harvested in Millstone, N. J., 1959.¹

Time of Collection	Per cent Germination		
	Initially	2 Montbs	4 Montbs
August	0.0	0.0	0.6-
September	3.9	24.8	6.2
October	6.6	18.8	40.0
November	10.7	7.5	58.6
December	0.0	14.7	17.3
January	3.3	50.6	18.7
February	26.0	30.7	13.3
March	46.6	9.3	16.6

¹Average of three replications of 50 seeds per replication.

It seems evident that there was a dormancy associated in horse nettle seed immediately after harvest (Table 16). Since the seeds imbibed water, this dormancy was not associated with the seed coat inhibiting the penetration of water. The seed coat may have prevented the embryo from expanding, or the dormancy per se may have been involved in the embryo itself.

Table 16. Germination of Horse Nettle Seed an Day of Collection and after Storage From Plants Established with Rootstocks from Two Locations. Seed Harvested In New Brunswick, N.J., 1960.¹

Days after Flowering	Per cent Germination					
	New York			New Jersey		
	Initially	Nov.	Jan.	Initially	Nov.	Jan.
45	0	0	0	0	2	0
56	2	1	3	0	1	58
68	0	5	30	1	4	67
91	0	1	45	0	2	49
111	0	5	51	0	9	44
138	3	34	64	2	7	65
167	8	0	54	5	0	47

¹Average of two berries for initial germination determinations; subsequently, two replications of 50 seeds per replication were used.

THE INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF HORSE NETTLE

This investigation consisted of a number of studies oriented toward obtaining information on the growth and development of horse nettle plants exposed to different environmental conditions. These studies were designed to measure the effect of day length, the influence of temperature on the emergence and growth of plants from seeds and rootstocks, and the effect of top removal at various periods after seedling emergence on the subsequent re-growth.

Effect of Day Length on Growth

Materials and Methods:

The plants used in this study were started with seed obtained from New York and New Jersey. Plants were established in a nursery in New Brunswick, New Jersey. Seeds from these two plant sources were planted on March 1, 1960 in greenhouse flats. The experiment consisted of four treatments which included a 9 hour day and a 14 hour day, and New York and New Jersey seed. Single seedlings were transplanted into 5 inch plastic pots on April 8. One week later 20 uniform-sized plants were placed in a growth chamber. Ten received a 9 hour day, while the other half were exposed to a 14 hour day. These two day lengths had daily temperature fluctuations between 21 and 32°C.

Results and Discussion:

The results obtained in this experiment are listed in Table 17.

These data indicate that source of seed had little influence on plant development. Plants grown at a 14 hour day attained a height of more than three times that of the plants grown at a 9 hour day.

Table 17. Effect of Day Length on Height of Horse Nettle Plants Grown Under Two Day Lengths Established from Two Seed Sources.¹

Day Length	Height		Average
	New York	New Jersey	
9 hours	5.1	5.2	5.1
14 hours	17.6	16.6	17.1
Average	11.4	10.9	

¹Average of five plants.

Effects of Temperature and Soil Fertility on Emergence and Growth of Horse Nettle Plants Established from Seeds and Sections of Rootstocks

Materials and Methods:

This study was conducted in a growth chamber using a 14 hour day. The variables investigated were temperature, soil fertility, and plant source. Two temperature regimes of 15-24°C and 27-38°C were maintained in the controlled environment chamber. The lower temperature was the night temperature and the higher temperature was the daylight temperature. Three fertilizer levels of 0, 480, and 960 pounds per acre of a 5-10-10 fertilizer were used. Twenty-four pots containing Nixon silt loam soil were planted with rootstock sections, while an equal number was planted with seed. The experimental design was a 2x3x2 factorial with four replications.

The seed used in this study, obtained from Millstone, New Jersey, had an average germination of 30 per cent, after eight months of dry room storage. Ten seeds were planted to a depth of one-half inch in each pot. Two four inch sections of rootstocks, obtained from taproots from the nursery in New Brunswick, New Jersey, were placed to a depth of 11 inches in each pot.

Observations were made on the number and height of emerging shoots as well as the length of the main roots of the plants in the various treatments.

Results and Discussion:

The total number of shoots that had emerged, shoot height, and root length for each treatment are found in Table 18.

No shoots emerged from the root sections at the lower temperature, however, at the higher temperature a few shoots developed.

Shoots grown under the low temperatures were shorter than those grown at the higher temperatures.

Plants originating from seed were somewhat larger than those from rootstock sections under the lower temperature; however, at the higher temperatures there were no significant differences.

From these data it can also be seen that temperature had a marked effect on root growth. Furthermore, it is evident that with increasing fertility there is an increase in root development. This was more pronounced on the plants established from seed.

Table 18. The Effect of Temperature, Fertility Level, and Propagation Methods on The Emergence of Shoots, Shoot Height, and Root Length of Horse Nettle Plants.

Fertility Level lb/A 5-10-10	Propagation Method	15-25°C			27-38°C		
		No. ₁ Shoots	Height, ₂ cm.	Root ₂ length	No. ₁ Shoots	Height, ₂ CM.	Root Length ₂ ,
0	Seeds	7	5.3	9.9	25	11.6	19.5
	Roots	0	2.6	18.3	6	10.6	34.8
480	Seeds	3	5.5	20.4	26	11.2	35.8
	Roots	0	3.8	14.5	5	12.2	13.5
960	Seeds	3	5.2	12.7	21	12.2	35.3
	Roots	0	3.8	15.6	6	12.3	28.0

¹Average of four replications

²Measurements taken 45 days after planting

Shoots emerged from the rootstock sections at low temperatures but much slower than from the seeds. Shoot emergence, even from seeds, was markedly reduced compared to that at the higher temperatures. This suggests that at low temperatures enzyme systems which are necessary for shoot growth may be reduced in activity. The root length of seedlings was affected more by fertility than from the rootstock sections at either temperature. Plants originating from the rootstock sections had a larger food reserve than those developing from seed and were more independent of the soil nutrients. The larger reserve in these sections enabled the roots to develop much faster.

VEGETATIVE DEVELOPMENT AND REPRODUCTION

Root Growth Study of Seedling Plants

Materials and Methods:

On May 8, 1959 a study was started to determine the character and rate of root and top growth of plants started from seed.

A number of two foot sections of metal stove pipe, ten inches in diameter, were painted with asphalt paint to prevent rusting. The pipes were filled with a silty clay loam soil. A wooden frame was constructed to maintain the pipes in an upright position. Several seeds were planted in each stove pipe on May 8. The emerging seedlings were thinned to one plant per pipe. A second section of pipe, filled with soil, was set under each of the original pipes as soon as it was noticed that roots were emerging from the bottoms of the two foot sections.

Starting 77 days after seeding, single plants were dug up at intervals of about two weeks. The stove pipe joints were relatively easy to take apart so that entire root systems were obtained by washing the soil out of the opened pipes.

The following measurements were taken: (a) height of plants before opening pipes, (b) length of main root (taproot), and (c) total length of root and branches, excluding the fine rootlets.

Results and Discussion:

There was a fairly consistent increase in plant height, taproot length, and

in total length of root and branches throughout the measuring period (Table 19). Less consistent increase in root length and plant height within a larger plant population than was used in this study could be expected.

The ratio of tap root length to plant height was relatively constant in plants 2 to 6. However, the ratio of total root length to plant height increased rapidly (Table 19).

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).

The data indicate that plants starting from seed become firmly established early in the growth period due to rapid and extensive root development which occurs at the expense of foliage growth. Thus, to prevent firm establishment of plants from seed, seedlings must be killed soon after emergence.

Table 19. Root Growth Study of Seedling Plants, Seeds Planted May 8, 1959.

Date	Plant No.	Plant* Height	Tap Root Length	Total Length of Root and Branches	Ratio of Tap Root Length to Height	Ratio of Total Root Length to Height	Average Growth Per Day		
							Plant Height	Tap Root Length	Total Root Length
7-24	1	30	295	295	9.8:1	9.8:1	0.4	3.8	3.8
8-8	2	70	335	555	4.8:1	7.9:1	2.7	2.7	17.3
8-22	3	160	580	2170	3.6:1	13.5:1	6.4	17.5	115.0
9-3	4	130	630	2400	4.8:1	18.5:1	0.0	12.5	19.0
9-19	5	185	985	4205	5.3:1	22.6:1	3.4	22.2	112.0
10-5	6	250	1195	7685	4.8:1	30.8:1	4.0	13.1	285.0

*All measurements in millimeters.

Influence of Size of Root Cutting on Reproduction

Materials and Methods:

Roots of a plant which originated from seed planted in a wooden flat in 1958 were dug and cut into lengths of 5, 10, 20, 30, 40, and 60 millimeters on May 26, 1958. The cuttings varied from 3 to 3.5 millimeters in diameter. These cuttings were planted at one inch depth in 2x2 feet outdoor concrete frames containing a well drained silt loam soil. Eight cuttings were planted in each frame.

Results and Discussion:

Horse nettle reproduced from very small root cuttings, less than one inch long and about 3/16 inch in diameter (Table 20). This strongly suggests the importance of cultural practices as a means of control.

Vegetative Reproduction of Plants Grown from Root Cuttings and Seed

Materials and Methods

The objectives of this study were as follows: (a) to determine the extent of vegetative reproduction by plants grown from root cuttings and from seed;

Table 20. Influence of Size of Root Cutting on Reproduction-July 7, 1959.

Length of Cuttings, mm	Diameter of Cuttings, mm	Number of Plants Developed ¹
5	3.5	0
10	3.5	0
20	3.5	8
30	3.0	8
40	3.0	8
60	3.0	

¹Eight cuttings planted in each frame.

(b) to compare the extent of vegetative reproduction of plants grown in the greenhouse with plants grown outdoors; (c) to observe the effects of length of day on character of growth; and (d) to observe the recovery of growth from a dormant condition.

Wooden flats, containing horse nettle plants grown from root cuttings and from seed in 1958, were placed into four different groups on the basis of origin, previous cultural treatments, and numbers of plants per flat. The four groups were as follows: (a) 44 plants developed from root cuttings; (b) 33 plants grown from seed in 1958-not transplanted-3 plants per flat; (c) 24 plants grown from seed in 1958--transplanted two times before September 11, 1958-2 plants per flat; and (d) 15 plants grown from seed in 1958-transplanted two times before September 11, 1958-1 plant per flat.

All the above plants were subjected to a cycle of temperature treatments of hot-cold-hot to study the effect of low temperature on dormancy and recovery of growth. No artificial lighting was used during the course of this study. The plants were kept in a heated greenhouse for six weeks (October 15 to November 29). All plants were in a dormant condition at the end of this time. They were then placed in an unheated room for a period of eight weeks. Although the plants were not subjected to severe freezing during this cold storage period, about 1 to 1½ inches of the outer layer of soil was frozen. The plants were then moved into a heated greenhouse on February 3. During the period in which observations and new shoot counts were made temperatures ranged from 13-35°C. Counts were made of shoots emerging from parent plants at approximately one week intervals from February 21 to April 6, 1959.

Results and Discussion:

Results of the first and last shoot counts appear in Table 21. New shoots began to emerge 13 days after heat and water treatments were started. Near maximum shoot emergence was reached 28 days later. Thus, growth recovery from the dormant condition was rapid.

There was no great difference in the average number of shoots produced by plants grown from root cuttings and plants grown from seed and not transplanted (9.3 and 10.1 shoots respectively). Transplanted plants produced less

than half as many new shoots as undisturbed plants. This was undoubtedly due to the much smaller root systems of the transplants as observed when samples of each group were dug up and examined.

All plants grew slowly after emergence. The internodes remained abnormally shortened. This may have been due to short day length.

The roots were confined to a five inch soil depth. This restriction on depth of root development may account for the large number of new shoots produced per plant. Figure 4 shows the typical characteristics of shortened stem growth, extensive root system, and prolific shoot development of plants grown under greenhouse conditions.

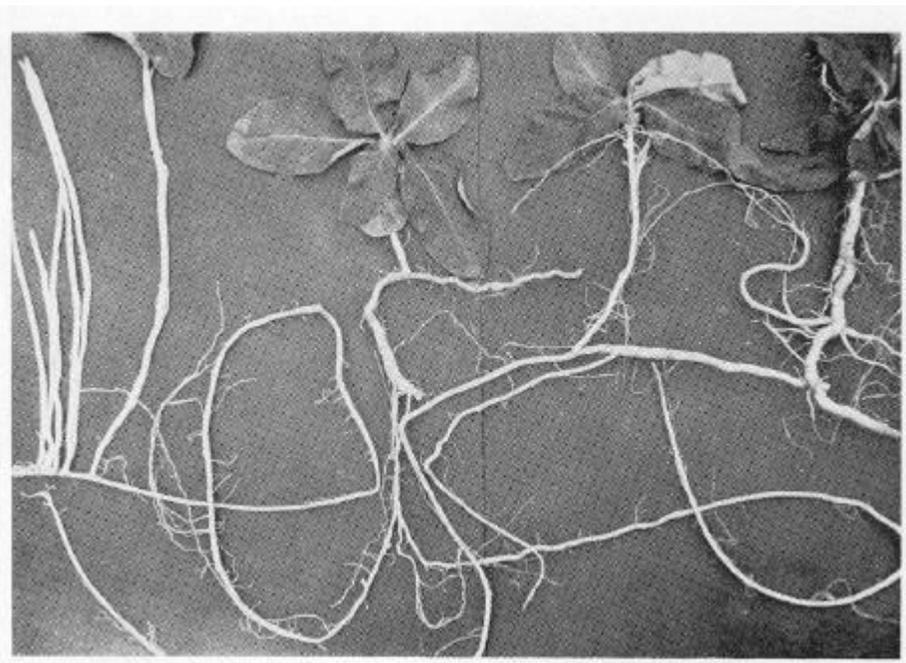


Figure 4. The development of the horse nettle plants from individual root cuttings. As many as 43 plants developed from a single root section.

A study was conducted of shoot development from plants that were grown from seed and transplanted from wooden flats into the 2x2 feet concrete frames in July 1958. These frames had no barrier to restrict the depth of root development. Shoot counts were made in June 1959. The number of new shoots that developed from 42 original plants was 104 or 2.5 shoots per plant. The range was from 0 to 8 shoots per plant. These results may be compared with those in Table 21 where plants were grown in wooden flats.

The amount of reproduction from roots under greenhouse conditions would indicate the potential reproductive capacity of the weed from vegetative

parts, whereas the amount of reproduction from roots in the outdoor concrete frames more nearly approached that level that could be expected under field conditions.

Table 21. Vegetative Reproduction of Plants Raised in Wooden Flats from Root Cuttings and Seed in 1958.

Parent Plants	First Count:			Last Count:		
	No. of Parent Plants Sprouting	No. of New Shoots	Av. No. Per Parent Plant	No. of Parent Plants Sprouting	No. of New Shoots	Av. No. Per Sprouted Parent Plant
From 17 original Root Cuttings that produced 44 plants in 1958	44	80	1.8	44	371	9.3
From 33 plants raised from seed in 1958-not transplanted -3 original plants per flat	33	84	2.5	33	334	10.1
From 24 plants raised from seed in 1958-transplanted 2 times before September 11, 1958 2 original plants per flat	16	27	1.7	24	99	3.7
From 15 plants raised from seed in 1958-transplanted 2 times before September 11, 1958-1 original plant per flat	11	18	1.6	15	62	4.1

Plants Grown in the Greenhouse from Root Cuttings from Several Locations

Materials and Methods:

On September 20, 1958, root cuttings from several locations in New York were planted in flats in the greenhouse and in outdoor concrete frames at 1½ to 2 inch depths. Two cuttings from each location were planted in wooden flats, two cuttings per flat. The same procedure was used in the outdoor concrete frames. The cuttings in flats received the same treatment as the plants discussed in the experiment above except for the period of cold treatment.

Results and Discussion

None of the cuttings planted in the outdoor frames produced shoots in the spring of 1959, because of winter killing.

All cuttings planted in flats produced shoots when placed in the heated greenhouse in February 1959. There was no great difference in the number of shoots produced among the plants from the various locations. No differences in growth habit were observed.

Influence of Depth of Planting on Reproduction

Materials and Methods:

On July 7, 1959, several plants which had been grown from seed in wooden flats in 1958 were dug up and the roots cut into sections 150 millimeters in length. These were planted in outdoor 2x2 feet concrete frames at the rate of 6 cuttings per frame and at depths of 3, 6, 12, and 18 inches.

Results and Discussion:

All cuttings produced at least one shoot each from depths down to 12 inches. Four out of six cuttings produced plants 67 days after planting (Table 22).

Table 22. Influence of Depth of Planting of Root Sections on Reproduction.

Depth of Planting, Inches	Length of Cutting, mm	Diameter of Cutting, mm	Number of Cuttings Reproducing*
3	150	2.5 - 4.0	6
6	150	2.5 - 4.0	6
12	150	3.0 - 5.0	6
18	150	3.5 - 5.0	4

*Six cuttings per frame.

Based on this observation it could not be expected that an appreciable reduction in stand would result by deep tillage that is normally practiced on farms in the Northeast. Fallowing might gradually reduce an infestation by depletion of the root reserves.

Effect of Length of Drying of Roots on Reproduction

Materials and Methods:

On July 18, 1959, a number of roots were cut into 150 millimeter lengths ranging from 3 to 5 millimeters in diameter. These root sections were placed on the soil surface to dry for 9 days. There was no precipitation during the first 4 days after planting. Daytime temperatures ranged up to 39°C.

Results and Discussion:

No shoots were produced from cuttings exposed on the soil surface for three days or longer. Thus, horse nettle roots lose their viability after being exposed to relatively short periods of drying on the soil surface.

Materials and Methods:

Rootstocks from two locations were cut into 2i, 5, and 10 centimeter segments and placed in the soil at 5, 10, 40, and 60 centimeter depths. Two of

these sections were placed in the center of each of 3x3 feet plots. The experimental design was a split-split-plot with four replications. Main plots were sources of the rootstocks, sub-plots included segment length, and the sub-sub-plots consisted of depths of planting or segment placement. The sections were planted on May 25, 1959. The soil surface in these plots was kept weed-free for the entire growing season so that natural emergence was not impaired by weed growth.

Results and Discussion:

The total number of shoots that were produced with the various treatments and the average number of days required for shoot emergence are listed in Table 23.

The 2½ and 5 centimeter segments produced shoots only at the shallow planting depths, whereas sections of 10 centimeters in length developed a few shoots from all depths. It is evident from these data that it required approximately 30 days for shoot emergence for most sections. Shoots that emerged from the 60 centimeter depth took approximately two months. Apparently, the smaller rootstock segments did not have the reserve capacity to produce shoots from the deeper plantings. However, the smaller sections were large enough to permit shoot development from a depth of four inches. This seems to indicate that no amount of plowing or disking would be enough to cut the taproot into small sections and place them deep enough to fully eliminate the species. Moreover, rainfall and temperature were favorable for shoot development during May and June, 1959.

Table 23. Number of Horse Nettle Shoots and Number of Days for Initial Shoot Emergence from Various Sized Rootstock Segments Placed at Various Soil Depths. Rootstocks Obtained from Two Sources. 1 959.

Rootstock Source	Segment size,	Depth of Planting, cm.							
		5		10		40		60	
		No. of Shoots*	Days to Emergence†	No. of Shoots*	Days to Emergence†	No. of Shoots*	Days to Emergence†	No. of Shoots*	Days to Emergence†
New York	2½	4	28.5	2	33.0
	5	4	28.7	5	35.2	...	30.3
	10	3	30.3	7	19.8	2	63.0
New Jersey	2½	1	30.0
	5	1	62.0	6	28.0
	10	2	21.0	5	24.2	1	31.0	3	73.3

*Total of four replications

† Average of four replications

A Study of the Phenomenon of Polarity in the Underground Portions of Horse Nettle Plants

Materials and Methods:

The rootstock sections used in this study, were obtained from plants that

had been growing in the horse nettle nursery since the spring of 1959. Portions of taproots and laterals were removed from the soil on October 17, 1960. This experiment consisted of a number of treatments which are listed in Table 24. There were four replications of all treatments.

The morphological apex will be used in all following discussions to designate that portion of the rootstock segments which was oriented toward the soil surface when it formed a part of the intact plant. This term when applied to segments from the lateral position of the plant refers to that end of the section that had been the closest to the main taproot.

The segments were placed, one to a pot, at a depth of one-half inch according to the schedule in Table 24.

Table 24. Treatments Used in the Polarity of Rootstocks Study.

Treatment	Type of Structure	Distance from Main Shoot Inches	Size Inches	Position Of Morphological Apex
1	Vertical	0-6	1	Normal
2	Vertical	0-6	1	Inverted
3	Vertical	0-6	3	Normal
4	Vertical	0-6	3	Inverted
5	Vertical	6-9	1	Normal
6	Vertical	6-9	1	Inverted
7	Vertical	6-9	3	Normal
8	Vertical	6-9	3	Inverted
9	Vertical	9-18	1	Normal
10	Vertical	9-18	1	Inverted
11	Vertical	9-18	3	Normal
12	Vertical	9-18	3	Inverted
13	Horizontal	0-3	1	Normal
14	Horizontal	0-3	1	Inverted
15	Horizontal	0-3	3	Normal
16	Horizontal	0-3	3	Inverted
17	Horizontal	3-6	1	Normal
18	Horizontal	3-6	1	Inverted
19	Horizontal	3-6	3	Normal
20	Horizontal	3-6	3	Inverted
21	Horizontal	6-15	1	Normal
22	Horizontal	6-15	1	Inverted
23	Horizontal	6-15	3	Normal
24	Horizontal	6-15	3	Inverted

A 1: I mixture of loam and sand was utilized as the planting medium. This experiment was conducted in the greenhouse for 5 weeks.

Results and Discussion:

The segments that were taken from the vertical taproot developed shoots at the morphological apex in approximately 80 per cent of the cases. The lateral root sections produced very few shoots. The results obtained from the 48 sections of vertical taproot are listed in Table 25. These data show that segments are capable of producing shoots from the morphological apex regardless of planting position or size of section. Furthermore, the distance re-

moved from the top of the vertical taproot did not seem to affect the production of shoots.

Table 25. Number of Shoots of the Morphological Apex an Two Sizes of Taproot Segments.

Distance from Top of Root	Position of Morphological Apex	Total Number of Shoots	
		Segment size	
		1 inch	3 inches
0-6"	Normal	3	4
0-6"	Inverted	3	4
6-9"	Normal	4	3
6-9"	Inverted	3	4
9-18"	Normal	3	4
9-18"	Inverted	3	2

From the presented data, it has been fairly well established that the new shoot growth is produced at the morphological apex on segments of the taproot. There are several possible explanations for this phenomenon. One possibility is that there is a concentration differential of nutrients or other growth factors from one end of these segments to the other end. Since the shoots are produced at the morphological apex it appears that this end contains the higher concentration of growth essentials. However ' from other studies undertaken in this investigation, it was shown that the lower portions of the taproot are higher in starch content than the upper regions of the root near the soil surface. This suggests that a slight starch concentration differential exists in the taproot sections. 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.

Another explanation which is commonly used to interpret this type of growth is that of differential auxin concentrations. It is necessary to assume that shoot growth is stimulated by relatively high auxin concentrations. If an auxin gradient from the shoot apex down through the taproot could be shown to exist, then the morphological apex of each root segment would have a higher auxin level than the rest of the section.

Leopold and Guernsey (54) have demonstrated an auxin gradient in the roots of colcus from the root apex up to the top of the root. Whether this gradient exists in horse nettle was not determined.

The findings of this experiment may have both practical and fundamental applications. Some type of growth controlling mechanism exists that, if determined, could give greater insight to the mode of action of growth regulating systems. Furthermore, practical control of horse nettle might be achieved by adjusting conditions to take advantage of this growth controlling mechanism. One possible means of accomplishing this would be to stimulate shoot growth in an attempt to systematically exhaust root reserves. Another approach could be attempts at total suppression of shoot growth.

EFFECT OF MANAGEMENT PRACTICE ON PLANT DEVELOPMENT

Influence of the Stage of Seedling Development on Regrowth after Top Removal

Materials and Methods:

This study was conducted in a growth chamber using high temperatures and a 14 hour day. Seeds at the rate of 10 per pot were planted on October 21, 1959. The pots were thinned to three plants before the treatments were started. The following four treatments were employed in this investigation: (1) clipped to soil surface 15 days after emergence; (2) clipped to soil surface 20 days after emergence; (3) clipped to soil surface 25 days after emergence; and (4) clipped to soil surface 30 days after emergence. There were four replications of each treatment.

Treatments 1, 2, 3, and 4 had average shoot heights of 20.3, 29.6, 42.2, and 53.2 millimeters, respectively, at the time of top removal. The number of shoots produced with each treatment and the number of days to develop these were recorded.

Results and Discussion:

The average number of shoots and number of days to produce these shoots after top removal are listed in Table 26. Treatment 4 produced more regrowth than treatments 1 and 2 but was no different from treatment 3. Treatment 4 also produced regrowth somewhat faster than the other treatments.

Table 26. The Effect of Clipping Schedule on the Rate and Amount of Regrowth of Horse Nettle.

Clipping, Days after Emergence	Average Number of Shoots	Average Number of Days for Regrowth
15	0.25	8.0
20	1.00	7.8
25	1.75	8.5
30	3.00	6.5
L. S. D.	1.77	

The results of this experiment show that new shoots can be produced when top growth is removed early in the seedling stage of horse nettle. With increased age of horse nettle plants the greater or more rapid the comeback of shoots occurs following temporary setbacks due to clipping.

Effects of Frequency of Cutting:

Materials and Methods:

In April 1959 an experiment was initiated to determine the effect of frequency of cutting horse nettle plants on their survival, rate of growth, seed production, character of growth. Several plants grown from seed were remov-

ed from wooden flats and the roots cut into sections ranging from 50 to 75 millimeters in length and 3 to 7 millimeters in diameter. The root cuttings were planted in outdoor 2x2 feet concrete frames, at 11 to 2 inch depths. Four cuttings were planted in each of 12 frames. The frames were identified by the numbers 71 to 82. The cuttings in each frame were numbered 1 to 4.

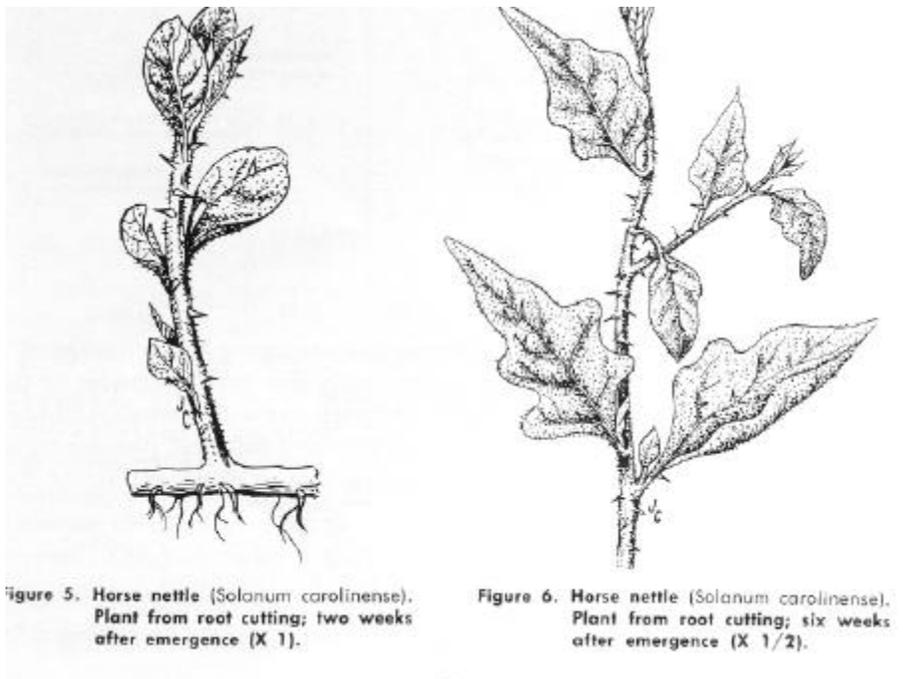
Using the same procedure, root cuttings were planted in It frames, numbers 29 to 39, on April 28. Shoots from most of the root cuttings began emerging by May 20.

On June 19, height measurements, flower and berry counts, and clipping treatments were started. Plant height measurements, counts of open flowers and numbers of berries forming were made on each plant in the experiment just prior to each clipping treatment. A cutting height of 60 millimeters was selected to approximate the height of cut of the conventional mowing machines in common use on farms.

Root cutting number 3 in frames 29 to 39 was cut every 5 to 8 days from June 19 through September 18. Root cutting number 4 in frames 71 to 82 and 29 to 39 were used as checks and were not clipped.

Results and Discussion:

Figures 5 to 7 show typical growth stages in the development of horse nettle plants started from root cuttings.

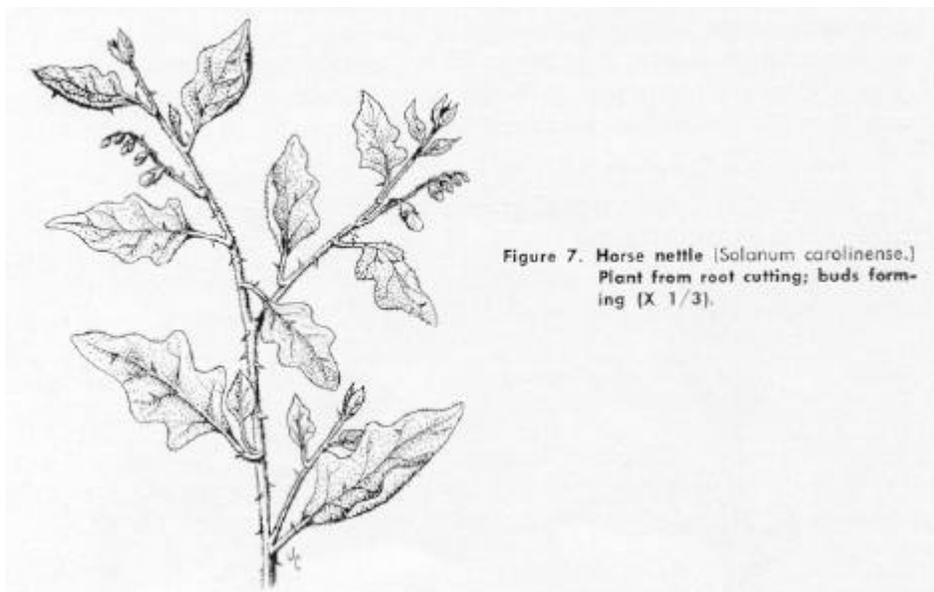


A graphic summary of the results of this experiment showing the average rate of growth per day between cutting dates is presented in Figure 8.

Of the 92 root cuttings, 91 cuttings produced at least one plant, or 98.9 per cent emergence. A total of 112 shoots were produced or 1.2 shoots per cutting.

PLANT SURVIVAL:

All 15 plants of root cutting numbers 3, cut 21 inches above ground level at 5 to 8 day intervals during the growing season, survived this treatment. These plants over-wintered with no mortality and were growing vigorously in May 1960. Two of the plants produced 4 very immature berries each below the cutting level used.



These plants, subjected to a rather 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 photosynthetic surface to maintain a daily growth rate comparable to plants cut at less frequent intervals. This is shown graphically in Figure 8.

RATE OF GROWTH:

Plants cut more than once during the season maintained a greater daily growth rate than those cut only once or not at all. This may be explained by

the fact that the more frequently cut plants remained in the actively growing vegetative stage for a long period of time. In plants that were allowed to flower and set seed, the growth rate declined sharply. These relationships are shown in Figure 8 in which the growth rates of the check with those of plants cut twice, three times, and at 5 to 8 day intervals throughout the growing season are compared.

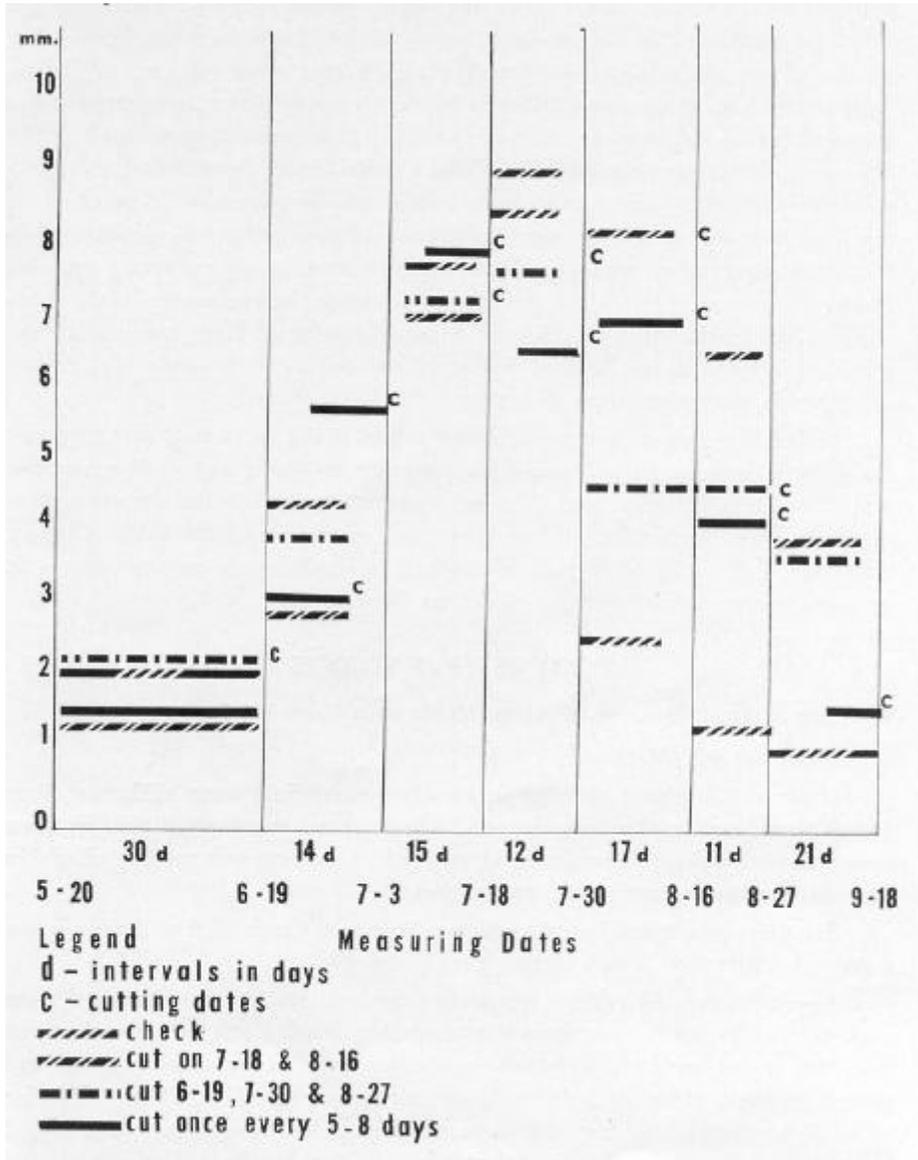


Figure 8. Average rate of growth per day in height of horse nettle between measuring dates.

FLOWERING AND BERRY PRODUCTION:

The date when flowers were first observed indicates that flowering, was delayed ten days to two weeks by clipping. However, the evidence is inconclusive because time and delay of flowering were extremely variable. The plants which were not cut, produced flowers from the latter part of June through September. Plants cut every 5 to 8 days produced almost no flowers.

The number of berries produced was a more reliable method of determining the effects of cutting on seed production. Counts made included all berries with a minimum diameter of three to four millimeters. Germination tests were made of only a single crop of berries which was harvested on July 30, 1959. No germination was observed. From the standpoint of the selection of appropriate times to clip horse nettle plants to prevent the formation of berries containing viable seed, it appears that a minimum of two cuttings made about July 15 and August 15 are necessary. However, germination tests of seed harvested during various stages of plant growth are necessary before more definite conclusions can be drawn. Variability in weather conditions from one year to another, differences in soil fertility, soil type, and competition would also strongly influence the proper time of cutting.

In brief, frequent clipping of horse nettle plants at 5 to 8 day intervals for a single growing season caused no mortality of plants and seed production was almost entirely prevented. The most desirable schedule for the prevention of viable seed production and at minimum expense appears to be clipping about mid-July and mid-August. This schedule would apply only to areas with growing seasons similar to those of Ithaca, New York.

ROOT RESERVE STUDIES

Changes in Root Reserves of Horse Nettle in a Growing Season

Materials and Methods:

Horse nettle plants growing in an abandoned field near Millstone, New Jersey were used in this investigation. These plants were established in close association with many other herbaceous weeds. Sampling was conducted within an area of approximately 1200 square feet.

Samples were taken by excavating a hole to a depth of 2 to 3 feet in the center of a group of 3 to 5 plants.

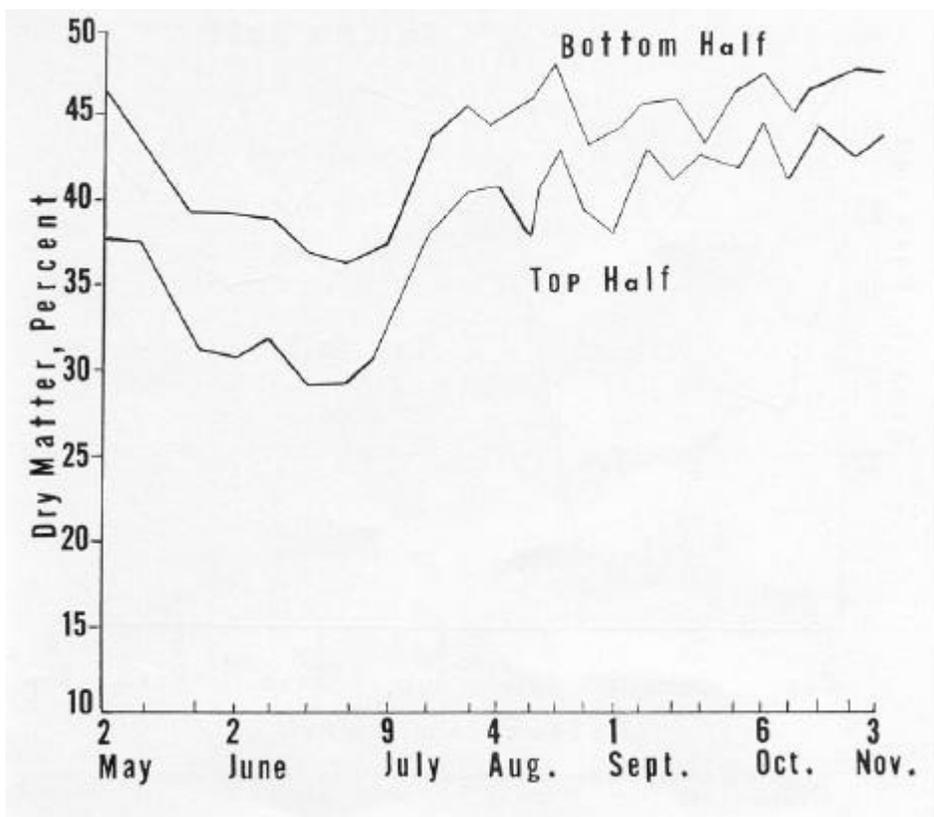
Approximately 50 centimeters of the vertical taproot were obtained from each plant. The roots were washed and surface dried. Each root was then cut into two equal sections, weighed separately, cut into smaller sections, and placed in open glass petri dishes in an oven maintained at 70 to 80°C for 12 to 16 hours. After drying, this material (still in the petri dishes) was cooled in a desiccator for 1 to 2 hours and reweighed. The dry tissue was ground in a

Wiley Mill equipped with a 40 mesh sieve. The two halves of the taproot from the individual plants were stored separately in small polyethylene vials with tight fitting caps.

The field collections for the 1960 season were initiated on May.2. Samples were taken at 9 to 10 day intervals from this date until November 3.

Previous investigations revealed that the roots contained cells with large numbers of starch grains. It was reasoned that analyses of these roots for starch could provide a fairly good estimate of the food reserves of these plants.

The method followed was a refinement of the Association of Official Agricultural Chemists procedure (4) made by Pucker, *et al.* (73). This refinement has since been incorporated into the Official Method as of 1960. The only modification of the 1960 official method was that a 40 mesh screen was employed in the initial grinding of the sample.



Collection Dates

Figure 9. Dry matter in the taproots of horse nettle plants collected during the course of a growing season, 1960.

Results and Discussion:

The per cent dry matter and starch are presented in Table 27. The dry matter values for the top and bottom halves of the taproot are illustrated in Figure 9 and the per cent starch is graphically presented in Figure 10.

It is apparent from Figures 9 and 10 that dry matter is closely related to starch content in taproot structures and that a discussion of starch reserves should also suffice for dry matter.

The per cent starch in Table 27 indicates that the bottom 25 centimeters of the taproot' sample contained more starch than the upper portion of this structure. The top 25 centimeters had an average of 32.6 per cent for the

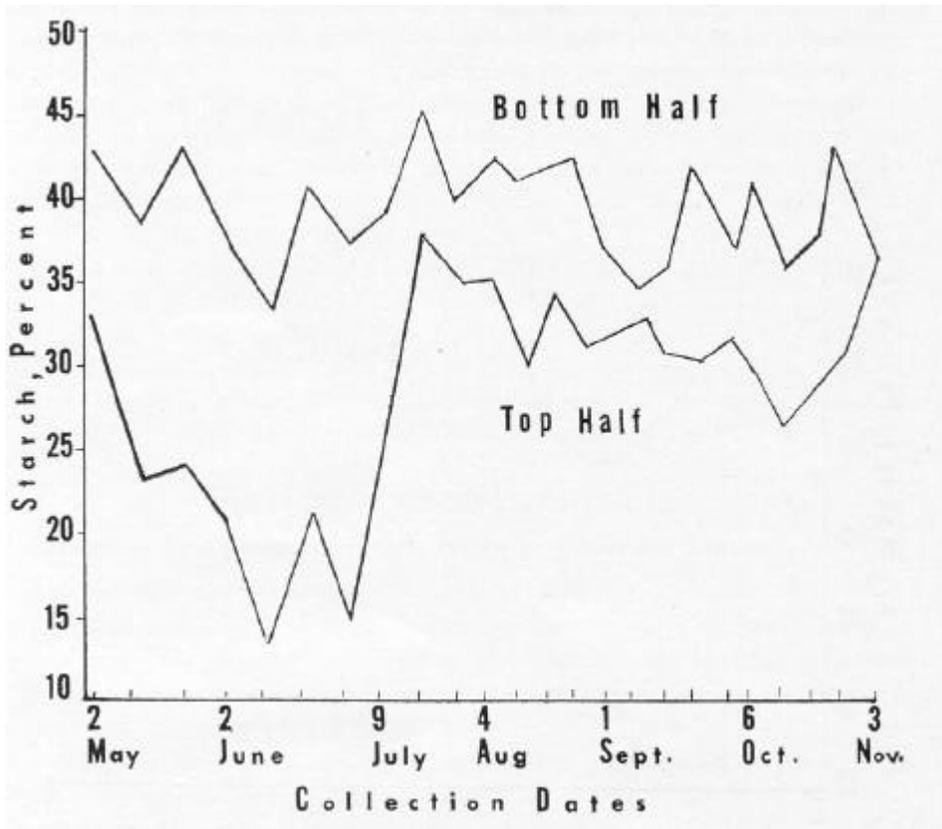


Figure 10. Starch in the taproots of horse nettle plants collected during the course of a growing season, 1960.

season while the bottom section averaged 40.1 per cent for the same period. The top 25 centimeters showed greater fluctuations in starch than the bottom portion. The top section exhibited a 20 per cent reduction in starch between May 2 and June 11 during the greatest development of above-ground struc-

tures while the bottom 25 centimeters had only a 10 per cent decrease during this same period. Furthermore, the smaller changes in starch level in the bottom 25 centimeters could be a partial explanation for the generally higher starch level in this portion of the taproot.

The sharp decline in starch between May 2 and June 11 corresponded with the rapid rate of shoot development and flowering during the period. This low level in root starch was noted approximately one month after the date of shoot emergence; however, approximately two months after the date of shoot emergence the original starch level was attained.

Two possible approaches for the elimination or control of this species are evident from these data. One would be to allow the shoots to develop to flowering then remove the top growth and repeat the process when the regrowth again flowers. The other possibility would be to permit top growth to develop until flowering then apply herbicides. At this point in the life cycle, foliar applications might have a better chance of being moved downward with translocates in the translocation stream (63).

Table 27. Per Cent Dry Matter and Starch of Horse Nettle Taproots During a Growing Season. 1960.

Collection Date		Dry Matter, per cent		Starch, per cent	
		Top Half	Bottom Half	Top Half	Bottom Half
May	2	38.5	47.8	33.9	43.9
	11	38.1	44.6	24.1	39.2
	23	31.7	39.8	25.0	44.4
June	2	31.6	39.5	20.9	38.5
	11	32.9	39.5	13.4	33.9
	20	29.6	37.8	22.5	42.3
	30	30.4	37.0	15.2	38.2
July	9	32.8	38.1	28.0	39.9
	19	39.0	44.0	38.3	46.0
	28	41.4	46.6	35.9	40.5
August	4	41.9	45.2	36.4	43.2
	11	38.8	46.4	30.8	42.0
	18	44.1	49.0	35.1	42.5
	25	40.3	44.4	32.0	43.1
September	1	38.9	45.4	24.8	37.7
	8	44.3	46.7	33.7	35.1
	15	42.4	46.8	31.9	36.7
	22	44.0	44.2	31.1	42.5
	30	43.2	47.2	32.3	37.3
October	6	45.9	48.7	30.4	41.9
	13	41.9	46.3	27.1	36.1
	20	45.2	47.9	29.8	37.9
	27	43.7	48.6	31.5	43.9
November	3	45.0	48.6	36.3	36.2

¹Average of four determinations on each collection date.

CLASSIFICATION OF ROOT SYSTEM

Root Systems:

Horse nettle is considered to have a taproot system. The roots range in color from light brown to almost white. They are thick and somewhat fleshy.

There is lack of agreement in the literature on whether or not horse nettle is spread by means of rhizomes, horizontal roots, or creeping rootstocks.

Kiltz (48) states that the plant "spreads by creeping roots," Allan (2) "spreads by means of horizontal roots," and Muenscher (64) "reproducing by seeds and by creeping rootstocks and roots."

An investigation was conducted to determine whether or not horse nettle produced rhizomes.

The stele in most roots has pith. The stems of most seed plants have a central pith which is composed largely of parenchymatous cells. The vascular tissue, of which xylem is a component, occurs in a ring surrounding the pith.

This characteristic anatomy of roots and stems provided a means of testing for the presence of root and stem structures by the use of the phloroglucinal staining technique.

Materials and Methods:

The phloroglucinal stain used, consisted of an 18 per cent concentration of hydrochloric acid saturated with phloroglucinal crystals. This solution stains lignin a purplish color. Since cells of xylem tissue are generally lignified, this stain can be used to differentiate xylem tissue from other plant tissues.

Thin slices of above-ground stems and under-ground structures of horse nettle plants were subjected to the test for lignin. Cross sections in which the central portion stained purple were classed as true root structure. Sections in which the central portion remained unstained were classed as stem structures. About 25 tests were made at various times over a one year period.

The above technique was supplemented by microscopic examinations of tissue sections.

A single plant that had been growing in the greenhouse for approximately six months was used as the source of experimental material. The vertical taproot and the lateral between shoots were the portions of the plant studied.

The roots were washed and cut into small sections and fixed in formalin acetic acid-alcohol fixative (FAA) until they were put through the normal dehydration process. The tissue was dehydrated and infiltrated with paraffin (44).

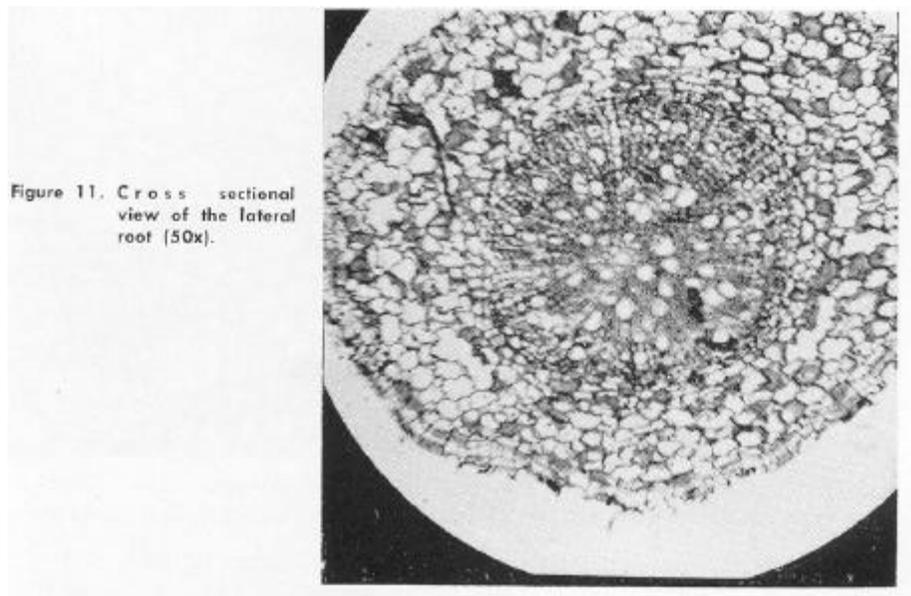
After the material was imbedded it was sliced on a rotary microtome at a thickness of 12 microns. Sections of the taproot and the lateral were placed on slides and stained with safranin and fast green (44). Microscopic examinations were carried out and photomicrographs taken.

Results and Discussion:

All above-ground stems gave the expected phloroglucinal stain for stem structure. In no instance did an underground part produce the characteristic staining which would have classified it as a stem structure.

An overall cross sectional view of the lateral structure is illustrated in Figure 11. This clearly shows the tissue characteristic of roots.

Primary xylem is noted in the center, of the section. This primary tissue resembles the usual star-shaped form in the roots. The large, thick-walled cells scattered throughout the area between the pericycle and the primary xylem are secondary xylem elements.



The pericycle has differentiated into a zone of cambial activity, producing xylem cells toward the center, the phloem to the outer side. The small cells within the region of secondary xylem are fiber cells.

Outside the pericycle is a region of parenchymatous tissue called the cortex. These parenchyma cells are irregularly shaped and contain very few starch grains. Surrounding the cortex is a layer of cork three to four cells in thickness.

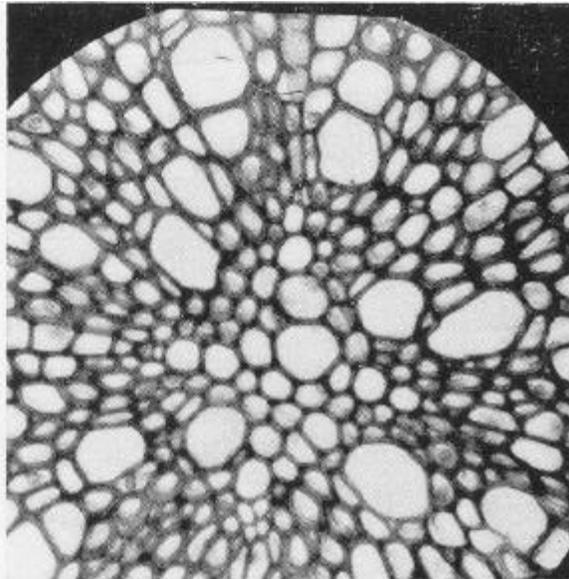
A cross section of the vertical taproot is presented in Figure 12. This photomicrograph was taken at somewhat higher magnification than the lateral photomicrographs and reveals in greater detail the central portion of this section. The large thick-walled cells that predominate in the center and radiate out toward the pericycle are xylem elements.

The elements in the immediate center are primary xylem while those that

radiate to the edges of the section are secondary xylem. The few parenchyma cells visible in this portion are limited to ray parenchyma and are not found in the exact center of the section i.e., there is no evidence of well-defined pith tissue as in a typical stem section.

Both the sections of the taproot and the lateral root have basically the same structure. Since there is little indication of any stem-like structure, it seems that the underground portions of horse nettle are roots and not rhizomes.

Figure 12. Cross sectional view of the main taproot (225x).



SUMMARY AND CONCLUSIONS

This study consisted of many experiments covering various phases in the life cycle of horse nettle. These experiments were conducted cooperatively by the New Jersey and New York Experiment Stations. Seed germination studies involved such factors as light, temperature, substrate, and age and weight of seed. Environmental factors such as day length, temperature, and soil fertility and their effects on growth of plants were explored. Factors affecting vegetative development and reproduction were studied. Management practices, such as frequency of clipping, and their effects on development and growth of plants were investigated. A survey was conducted on the changes in root reserves during an entire growing season. Finally, anatomical studies of the underground structures were also investigated.

As a result of these experiments the following observations and conclusions were made:

- (1) Horse nettle plants are capable of producing large numbers of seed.
- (2) Seed is important in the dissemination of this species.
- (3) Serious infestations may arise in the field from seed.
- (4) Seeds are capable of germinating and producing seedlings from May through August.
- (5) There is a dormancy in horse nettle seed which prevails in freshly harvested seed.
- (6) Light is not necessary for seed germination.
- (7) Alternating temperatures of 20-30°C favor germination whereas constant temperatures do not favor germination.
- (8) Heavier seeds have a higher germination percentage than lighter seeds.
- (9) Seeds are capable of germination from depths of four inches.
- (10) Seedlings were produced from whole, and broken berries located within three inches of the soil surface.
- (11) Soil type greatly influences the extent of emergence from seed. Emergence is highest in well-drained friable soils.
- (12) Long days are necessary for maximum development of seedlings. (13) Control programs should include provisions for preventing seed formation and dissemination.
- (14) Increases in root growth from seedlings and root cuttings result from increases in temperature and soil fertility.

(15) Plants may arise vegetatively from small root cuttings less than one inch long and 3/16ths of an inch in diameter.

(16) Shoots may arise from adventitious buds found on the tap root sections from depths of 12 inches.

(17) Where normal root development is restricted larger numbers of plants may arise from buds than from roots in soils permitting deep root penetration.

(18) Shoots arise from the morphological apex of root segments regardless of their position in the soil.

(19) No amount of plowing or disking would be enough to cut the tap root into small sections and place them deep enough to fully eliminate this species.

(20) New shoots can be produced when top growth is removed early in the life cycle.

(21) The most desirable schedule for the prevention of viable seed production would be to clip the top growth about mid-July and mid-August.

(22) Starch in the tap root reached a low level for the season approximately 30 days after shoot emergence.

(23) Two months after shoot emergence the starch level again approached the early spring level.

(24) The possibility exists that control of this species may result from clipping or herbicidal applications made during the period of low root reserve.

(25) Anatomical studies indicated that the vertical as well as the horizontal underground structures are roots and not rhizomes.

BIBLIOGRAPHY

1. Ahlgren, G. H., Klingman, G. C., and Wolf, D. E. 1951. *Principles of Weed Control*. John Wiley and Sons, Inc. New York.
2. Allan, H. H. 1934. Notes on recently observed exotic weeds: horse nettle and buffalo bur. *Jour. Agr. New Zealand* 48: 295-300.
3. Arny, A. C. 1932. Variations in the organic reserves in underground parts of five perennial weeds from late April to November. *Minnesota Agr. Exp. Sta. Tech. Bul.* 84: 1-28.
4. Association of Official Agricultural Chemists. 1960. *Official and Tentative Methods of Analysis*. 9th Ed., Washington, D. C.
5. Atwood, W. M. 1914. A physiological study of the germination of *Avena fatua*. *Bot. Gaz.* 57: 386-414.
6. Bakke, A. S., Gaessler, W. G., and Loomis, W. F. 1939. Relation of root reserves to control of European bindweed (*Convolvulus arvensis* L.) *Iowa Agr. Exp. Sta. Res. Bul.* 254: 115-144.
7. Barr, C. G. 1939. Applications of the ceric sulphate method in the analysis of carbohydrates in the roots of *Lepidium* and *Convolvulus*. *Plant Phys.* 14: 284-296.
8. Barr, C. G. 1940. Organic reserves in the roots of bindweeds. *Jour. Agr. Res.* 60: 391-413.
9. Barton, L. V. 1932. Effect of storage on the vitality of delphinium seed. *Contr. Boyce Thompson Inst.* 4: 141-153.
10. Barton, L. V. 1943. Effect of moisture fluctuations on the viability of seeds in storage. *Contr. Boyce Thompson Inst.* 13: 35-45.
11. Bellue, Margaret K. 1939. Carolina horse nettle, *Solanum carolinense* L., and other weedy Solanums in California. *California Dept. Agr. Bul.* 28: 471-478.
12. Bellue, Margaret K. 1946. *Weed Seed Handbook*. California Dept. Agr. Bul. 35: 13-14.
13. Bibbey, R. O. 1948. Physiological studies of weed seed germination. *Plant Phys.* 23: 467-484.
14. Bidwell, R. G. S., Kratkor, G., and Reed, G. B. 1952. Paper chromatography of sugars in plants. *Can. Jour. Bot.* 30: 291-305.
15. Bradbury, H. E. 1956. Growth of horse nettle and evaluation of control methods. Unpublished Master's Thesis, Department Farm Crops, Rutgers University, New Brunswick, N. J.
16. Bradbury, H. E. and Aldrich, R. J. 1956. A study of horse nettle (*Solanum carolinense*) and its control. *NEWCC Proc.* 10: 232-233.
17. Bradbury, H. E. and Aldrich, R. J. 1957. Survey reveals extent of horse nettle infestation. *New Jersey Agr.* 39 (4): 4-7.
18. Brandes, E. W. and van Overbeek, J. 1949. Auxin relations in hot-water treated sugar cane stems. *Jour. Agr. Reg.* 77: 223-238.
19. Brown, E. O. and Porter, R. H. 1942. The viability and germination of seeds of *Convolvulus arvensis* L. and other perennial weeds. *Iowa Agr. Exp. Sta. Res. Bul.* 294: 473-504.
20. Burt, E. O. and Willard, C. J. 1958. Control of Johnson grass (*Sorghum halepense*) by herbicides and cultural practices. *Weed Soc. America Abs.* 1-2.
21. Chandler, C. 1953. Seed germination for some species of *Plantago*. *Contr. Boyce Thompson Inst.* 17: 265-271.
22. Chepil, W. S. 1946. Germination of weed seed. 1. Longevity, periodicity of germination, and vitality of seeds in cultivated soil. *Sci. Agr.* 26: 307-346.
23. Clark, W. G. 1937. Polar transport of auxin and electrical polarity in coleoptile of *Avena*. *Plant Phys.* 12: 737-754.
24. Correll, D. S. 1952. Section tuberarium of the genus *Solanum* of North America and Central America. *USDA Agr. Monog.* 11: 243.
25. Coupland, R. T., Selleck, G. W., and Alex, J. F. 1955. The reproductive capacity of vegetative buds on the underground parts of leafy spurge. (*Eurphoria esula* L.). *Can. Jour. Agr. Sci.* 35: 477-484.
26. Crocker, W. and Barton, L. V. 1953. *Physiology of Seeds (An Introduction to the Experimental Study of Seed and Germination Problems)*. Chronica Botanica Co., Waltham, Mass.
27. Cross, Hope. 1931. Laboratory germination of weed seed. *Proc. Assoc. Off. Seed Anal.* 24: 125-126.

28. Everson, L. 1949. Germination of weed seed. Proc. Assoc. Off. Seed Anal. 39: 84-89.
29. Frazier, J. C. 1943 (a). Nature and rate of development of the root system of *Convolvulus arvensis* L. Bot. Gaz. 104: 417-425.
30. Frazier, J. C. 1943 (b). Food reserve depletion and synthesis in field bindweed (*Convolvulus arvensis* L.) cultivation. Plant Phys. 18: 315-323.
31. Frazier, J. C. 1945. Nature and rate of development of the root system of *Gonotobus laevis*. Bot. Gaz. 106: 324-332.
32. Frazier, J. C. 1948. Principal noxious perennial weeds of Kansas with emphasis upon their root systems in relation to control. Kansas Agr. Exp. Sta. Bul. 331- 1-45.
33. Fulwider, J. R. and Engel, R. E. 1959. The effect of temperature and light on germination of seed of goosegrass (*Eleusine indica*). Weeds 7 (3): 359-361.
34. Furrer, A. H., Jr. and Fertig, S. N. 1960. Life history studies of horse nettle (*Solanum carolinense*). NEWCC Proc. 14: 336-342.
35. Gill, N. T. 1938. The viability of weed seeds at various stages of maturity. Ann. Apl. Biol. 25: 447-457.
36. Gaber, L. F., Nelson, N. T., Leukel, W. A., and Albert, N. B. 1927. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wisconsin Agr. Exp. Sta. Res. Bul. 80: 1-128.
37. Grandfield, C. O. 1930. The relation of organic food reserves to the effect of cutting pasture weeds at different stages of growth. Jour. Amer. Soc. Agron. 22: 709-713.
38. Grass, E. M. 1938. Pennsylvania weeds, their control and eradication. Bul., Pennsylvania Dept. of Agr., Harrisburg, Pa. 21 (12): 20-22.
39. Gregory, F. G. and Veale, J. A. 1957. A reassessment of the problem of apical dominance. *Biological Action of Growth substances*. Sym. Soc. Exp. Biol. II: 1.
40. Hansen, A. A. 1921. Horse Nettle. Pa. Agr. Ext. Weed Leaflet 6: 4.
41. Heinze, P. H. and Murneek, A. E. 1940. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Missouri Agr. Exp. Sta. Res. Bul. 314.
42. Hemphill, D. D. 1958. Poison ivy and horse nettle control beneath bearing apple trees. Weed Sec. Amer. Abs. 14.
43. Isely, D. 1944. A study of conditions that affect the germination of *Scirpus* seed, Cornell University Agr. Exp. Sta. Memoir 257.
44. Johansen, D. A. 1940. *Plant Microtechnique*. McGraw-Hill Book Co., Inc. New York.
45. Johnson, B. G. and Buchholtz, K. P. 1958. Factors affecting the bud dormancy of quackgrass rhizomes. Weed Soc. Amer. Abs. 38-39.
46. Justice, O. L. 1957. Germination, dormancy, and viability in seeds of certain weed species of *Cyperus*. Proc. Assoc. Off. Seed Anal. 47: 167-175.
47. Kanipe, L. A. 1939. The germination of the seed of *Alsine media*. Proc. Assoc. Off. Seed. Anal. 1938.- 249-252.
48. Kiltz, B. F. 1930. Perennial weeds which spread vegetatively. Jour. Amer. Soc. Agron. 22: 216-234.
49. Kinch, R. C. and Termunde, D. 1957. Germination of perennial sow thistle and Canada thistle at various stages of maturity. Proc. Assoc. Off. Seed Anal. 41: 165-166.
50. Koller, D. 1956. Germination regulating mechanisms in some desert seeds 111. *Calligonum comasum* L. Hey. Ecology 37: 430-433.
51. Kreuson, C. F. and Behrens R. 1959. Preparation of "Double Phenoxy" compounds and preliminary evaluation of their herbicidal activity on mesquite seedlings. Jour. Agr. and Food Chem. 9(8): 551.
52. Kummer, Anna P. 1951. *Weed Seedlings*. The University of Chicago Press, Chicago.
53. Leopold, A. C. 1955. *Auxin and Plant Growth*. University of California Press. Berkeley and Los Angeles.
54. Leopold, A. C. and Guernsey, F. S. 1953. Auxin polarity in the coleus plant. Bot. Gaz. 115: 147-154.
55. Le Tourneau, D. 1956. A note on the sugars and amino acids of leafy spurge (*Euphorbia esula*). Weeds 4: 275-277,
56. Leukel, W. A. 1927. Deposition and utilization of reserve foods in alfalfa plants. Jour. Amer. Sec. Agron. 19: 597-623.
57. Lindahl, I., Davis, R. E. and Shepherd, W. O. 1949. The application of the total available carbohydrate method to the study of carbohydrate reserves of switch cane (*Arundinaria tecta*). Plant Phys. 24: 285-294.
58. Link, K. P. and Tottingham, W. E. 1923. Effects of the method of desiccation on the carbohydrates of plant tissue. Jour. Amer. Chem. Sec. 45: 439-447.

59. Little, R. D. and McMurray, R. S. 1940. *Solanum carolinense* Linne. Pharmaceut. Arch. 11 (2): 23-28.
60. McIndoo, N. E. 1935. The relative attractiveness of certain solanaceous plants to the Colorado Potato beetle *Leptinotarsa decemlineata* Say. Proc. Ent. Soc. Washington 37 (2): 36-42.
61. Meggitt, W. F. and Tisdell, T. F. 1959. Weed life cycles, soil microorganisms and light as factors in the control of weeds in the Northeast. *Regional Research Project NE-42*. Dept. of Farm Crops, Rutgers-The State University and the New Jersey Agr. Exp. Sta.
62. Meyer, B. S. and Anderson, D. B. 1952. *Plant Physiology*. 2nd ed. D. Van Nostrand Co., New York.
63. Mitchell, J. W. and Brown, J. W. 1946. Movement of 2, 4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. Bot. Gaz. 107: 393-407.
64. Muenscher, W. C. 1955. *Weeds*, 2nd ed. The MacMillan Co., New York.
65. Muenscher, W. C. and Winne, W. T. 1942. Common poisonous plants. Cornell Ext. Bul. 539: 26-37.
66. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Jour. Biol. Chem. 153: 375-380.
67. Neville, H. W. 1950. A comparison of the effectiveness of certain chemicals in controlling horse nettle and other weeds in corn. NEWCC Proc. 4: 217-219.
68. Oyer, E. B., Gries, G. A., and Rogers, B. J. 1959. The seasonal development of Johnson grass plants. (*Sorghum halepense*). Weeds 7 (1): 13-19.
69. Pammel, L. H. 1911. *Weeds of the Farm and Garden*. Orange Judd Co., New York.
70. Pammel, L. H. and King, Charlotte M. 1926. The weed flora of Iowa. Iowa Geological Survey (Revised Ed.) Bulletin 4. DeMoines. The State of Iowa. 575.
71. Phillips, W. M. 1960. The chloro-substituted benzoic acids for control of field bindweed. Weeds 8(1): 63-70.
72. Pritchard, F. J. and Porte, W. S. 1921. Relation of horse nettle (*Solanum carolinense*) to leafspot (*Septoria lycopersic*). Jour Agr. Res. 21: 501-506.
73. Pucker, G. W., Leavenworth, C. S., and Vickery, H. B. 1948. Determination of starch in plant tissues. Anal. Chem. 20: 850-853.
74. Robbins, W. W., Bellue, Margaret K., and Ball, W. S. 1951. *Weeds of California*. Sacramento Printing Division (Document Section).
75. Robbins, W. W., Crafts, A. S., and Raynor, R. N. 1952. *Weed Control*, 2nd ed. McGraw-Hill Book Co., Inc., New York.
76. Ruelke, O. C. and Smith, D. 1956. Overwintering trends of cold resistance and carbohydrates in medium red, Ladino and common white clover. Plant Phys. 31: 364.
77. *Seed Trade Buyers Guide*. 1961. Seed World Publications 45. Chicago, Illinois.
78. Smith, D. 1930. The occurrence of adventitious shoots on several alfalfa plants. Jour. Amer. Soc. Agron. 42: 398.
79. Smith, F. G., Hamner, C. F., and Carlson, R. F. 1947. Changes in food reserves and respiratory capacity of bindweed tissues accompanying herbicidal action of 2, 4-dichlorophenoxyacetic acid. Plant Phys. 22: 58-66.
80. Somes, M. P. 1916. Insects of *Solanum carolinense* and their economic relations. Jour. Econ. Ent. 9: 39-44.
81. Somogyi, M. 1945. A new reagent for the determination of sugars. Jour. Bio. Chem. 160: 61-73.
82. Somogyi, M. 1952. Notes on sugar determination. Jour. Bio. Chem. 195: 19-23.
83. Steinhauer G. P. and Grigsby, B. H. 1957. Interaction of temperature, light, and moistening agent in germination of weed seeds. Weeds 5(2): 175-182.
84. Steinhauer, G. P. and Grigsby, B. H. 1959. Methods of obtaining field and laboratory germination of seeds of bindweed, lady's thumb and velvetleaf. Weeds 7(1): 41-46.
85. Steinhauer, G. P., Grigsby, B. H., Correa, L., and Frank, P. 1955. A study of methods for obtaining laboratory germination of certain weed seeds. Proc. Assoc. Off. Seed Anal. 48-52.
86. Sturkie, P. G. 1930. The influence of various topcutting treatments on root stocks of Johnson Grass. Jour. Amer. Soc. Agron. 22: 82-93.
87. Swank W. G. 1944. Germination of seeds after ingestion by ringnecked pheasants. Jour. Wild Life Management 8 (3): 223-231.
88. Tisdell, T. F. 1959. Personal communication.

89. Ugolini, F. C. 1960. Soil Development on the Red Beds of New Jersey. Ph.D. Thesis, Rutgers-The State University, New Brunswick, New Jersey.
90. Wallis, R. L. 1951. Potato psyllid selection of host plants. *jour. Econ. Ent.* 44: 815-817.
91. Weinmann, H. 1944. Semi-micro estimation of reducing sugars. *Plant Phys.* 19-148-156.
92. Wellon, F. A., Morris, V. N., and Hartzler, A. J. 1929. Organic food reserves in relation to the eradication of Canada thistle. *Ohio Agr. Exp. Sta. Bul.* 441: 1-25.
93. Went, F. W. 1941. The polarity of auxin transport in inverted tagetes cuttings. *Bot. Gaz.* 103: 386-390.
94. Willard, C. J. 1927. An experimental study of sweet clover. *Ohio Agr. Exp. Sta. Bul.* 405: 1-84.
95. Wood, G. M. and Sprague, M. A. 1952. Relation of organic food reserves to cold hardiness of ladino clover. *Jour. Amer. Soc. Agron.* 44: 318-325.