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C. J. Scifres

M. K. McCarty

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by
C. J. Scifres
M. K. McCarty

University of Nebraska College of Agriculture
and Home Economics
The Agricultural Experiment Station
E. F. Frolik, Dean; H. W. Ottoson, Director

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Some Factors Affecting Germination and Seedling Growth of Scotch Thistle

C. J. Scifres and M. K. McCarty[1]

INTRODUCTION

Scotch thistle (*Onopordum acanthium* L.) is a native of Eurasia. Little is apparently known as to date and method of introduction into the United States. Taxonomic manuals list scattered occurrence through the Eastern part of the United States and in the intermountain region of the West. It is listed as a noxious weed in California where a concerted control effort has been made.

Scotch thistle is found in the grasslands of central Nebraska, with heaviest infestations in Gosper, Dawson, and Valley Counties and limited occurrence in 18 additional counties. The Nebraska Unicameral declared it a noxious weed in 1965. Scotch thistle usually behaves as a biennial under the climatic conditions in Nebraska, but may act as a winter annual (10). It spreads by wind-borne achenes.[2] The mature plant is 3 to 6 ft. tall (Figure 1). It forms large, attractive, violet to red flowers.

The achenes of scotch thistle are formed in an oval receptacle which is pitted or honeycombed in appearance. The pappus is not plumose, but short and stiff with small barbs (10). The ratio of pappus length to achene size is much lower than in *Cirsium* spp. and *Carduus* spp. Therefore, it is assumed that scotch thistle achenes are carried relatively short distances by wind. The achenes are approximately 5 mm. long and 2 mm. wide. Scotch thistle achenes are brown

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[1] C. J. Scifres, formerly an Assistant Research Agronomist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, conducted research on weed control in pastures at the Nebraska Agricultural Experiment Station and was Instructor of Agronomy, University of Nebraska. He is now Assistant Professor, Brush Research, Texas A & M University Agricultural Research and Extension Center at Lubbock, Texas. M. K. McCarty, Research Agronomist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, is in charge of weed control research in pastures at the Nebraska Agricultural Experiment Station and is Associate Professor of Agronomy, University of Nebraska. Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and the Nebraska Agricultural Experiment Station, Lincoln.

[2] The small, dry, hard fruit of scotch thistle is often referred to as the "seed." The fruit (achene) consists of one seed enclosed by a thin outer covering that does not burst when ripe.

Figure 1. Mature scotch thistle plants growing in the thistle nursery established near the East Campus, University of Nebraska. The photograph was taken on June 14, 1967.

to mottled black in color with conspicuous wrinkles on the coat at right angles to the longitudinal axis (Figure 2).

Achenes used in the study weighed 1.0 g./100 achenes and had a volume of approximately 0.8 ml./100 achenes as derived by water displacement. This can be compared with welted thistle (*Carduus crispus* L.) at 0.16 g./100, tall thistle (*Cirsium altissimum* (L.) Spreng.) at 0.55 g./100 and wavyleaf thistle (*Cirsium undulatum* (Nutt.) Spreng.) at 0.53 g./100 as reported by Stevens (16).

According to Toole *et al.* (17) "A seed contains an embryonic plant in an inactive condition, and germination is its resumption of growth." A seed disseminated into a new area requires certain environmental conditions, or at least certain levels of given conditions, for successful invasion. Germination requirements do not necessarily coincide with tolerance limits for seedling establishment or growth and development of juvenile and mature plants. However, clarification of germination response to certain enviromental factors may give rise to a better understanding of the ecological range of a plant.

Environmental factors that may affect seed germination are temperature, moisture, and light. Inherent characteristics such as seed coat impermeability or embryo dormancy may limit germination. In some cases, the effects of these germination inhibitors may be additive (9). This study measures the effects of some environmental

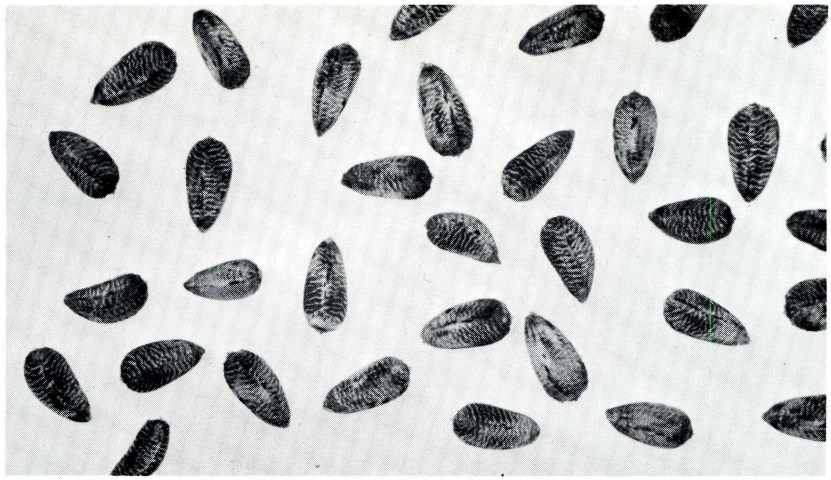

Figure 2. Example of the scotch thistle achenes used in these studies. They are approximately 5 mm. long and 2 mm. wide.

factors on scotch thistle germination and seedling growth, and clarifies some of the interactions of these with inherent characteristics of the achene. The effect of achene coat, stratification, light, temperature, growth regulators, pH, depth of planting, salinity, germination inhibitors, and moisture stress were studied.

MATERIALS AND METHODS

Achenes collected from scotch thistle plants in Dawson County, Nebraska, in late August, 1966 were used for these studies. Part of the achenes collected in 1966 were planted in gallon cans in the greenhouse and transplanted into a nursery in late April, 1967. When the rosettes were transplanted, several rows were seeded in the nursery with achenes from the same source.

Achenes were collected from transplanted nursery stock in late August, 1967. Since seed size may be a confounding factor in germination tests, the scotch thistle achenes were threshed from the heads, cleaned, and separated into size classes by weight with a "South Dakota" seed blower[3]. Only plump, well-filled achenes of uniform size were used in these studies. This insured physical uniformity throughout germination tests which presumably eliminated some of the inherent variability in the reaction of achenes to treatment prior to or during germination.

[3] Manufactured by B. L. Erickson Products, Brookings, South Dakota. (Trade name, cited for identification of implement used, is not necessarily endorsed by the USDA over similar products.)

Unless otherwise stated, germination tests were conducted with 50 or 100 achenes per 9-cm. petri dishes. Three No. 2 (coarse) filter papers wetted with 7.5 ml. of solution constituted the germination substrate. Five petri dishes (replicates) were used per achene treatment. All experiments except the one on effect of light quality were conducted twice and most of them were repeated three, four, or five times.

Treatment effect was evaluated by counting germination at given time intervals and measuring fresh-weight production and lengths of individual seedlings. Total fresh-weight production was measured by removing the seedlings from the petri dishes and blotting them dry with tissue paper before weighing. An achene was considered germinated when 2 mm. of the radicle was visible. Seedling growth in the greenhouse under various treatments was evaluated by measuring oven-dry top-growth production.

All experiments were designed as completely random unless they would not fit onto a single germinator tray; in which case they were designed as randomized complete blocks with 4 or 5 replications and blocked by shelf location. Uniformity trials showed the temperature to vary no more than ± 1 C. in the germinator. All experiments, except the effect of constant temperatures, were conducted under alternating temperatures of 16 hours of 20 C. and 8 hours of 30 C. The low temperature was used in the dark for 16 hours. All greenhouse studies were designed as randomized complete blocks with 5 or 6 replications. Specific details of each particular experiment are given in the following sections.

Prechilling

Two experiments, each 160 days in duration, were conducted to evaluate the response of scotch thistle achenes to wet or dry storage conditions at two temperatures, 0 and 5 C.

The first study was started on November 4, 1966 and ended April 10, 1967. Cold, wet conditions were accomplished by soaking germination towels in distilled water before putting them in storage. Each towel contained 100 achenes which were allowed to imbibe moisture at room temperature for 8 hours before they were placed in cold storage. Twenty towels containing achenes were then placed in cold storage at 0, and 20 at 5 C. Dry achenes were stored at the same temperature in open beakers.

At each sampling period, 5 moist germination towels containing 100 achenes each and 500 dry achenes were removed from cold storage. The dry achenes, along wth 500 achenes stored at room temperature, were placed in 100 unit lots in germination towels, moistened with distilled water, and placed in the germinator. Achenes were

sampled from cold storage at approximately weekly intervals for 4 weeks, then at monthly intervals for 4 months.

A second study was started April 15, 1967, and ended September 16, 1967. In this experiment, achenes were placed in cold storage at monthly intervals for 4 months, then at weekly intervals for 3 weeks. A week after placing the last (7-day) treatment in storage, all achenes were brought out of cold treatment and placed in the germinator along with a set of achenes stored at room temperature.

The reaction of the achenes to cold storage was essentially the same in both experiments so the data were averaged for final inspection.

Ambient Temperature

Six constant temperature chambers were used in these experiments. Achenes were placed in 0, 5, 15, 25, 28, or 35 C. in the dark or in light to evaluate germination under a range of constant temperatures. The light source was incandescent bulbs. Germination was recorded after 5, 10, 15, and 20 days. At the end of the 10-day period, achenes in the 0 and 15 C. chambers were transferred to 28 C. and left for 10 days. This experiment was repeated 3 times.

Light Requirement

The effect of light was determined by comparing germination in petri dishes wrapped in aluminum foil with that in unwrapped dishes. The effect of 30 p.p.m.w. gibberellic (GA) or indoleacetic (IAA) acids on achenes germinating in the light or dark was compared to germination of achenes under light or dark in distilled water. Achenes left in "light" received 8 hours exposure to fluorescent lights in the germinator, then 16 hours in the dark. The experiment was repeated 3 times.

Depth of Planting

In three experiments, Sharpsburg silty clay loam soil was mixed at a 3:1 ratio with sand to prevent crusting. Containers were filled and packed so that a bulk density of 1.1 was obtained. Achenes were planted on the surface and at 0.5 cm. increments to a depth of 6 cm.

In the first and third experiments, 20 achenes were planted at the appropriate depth in each 3-inch diameter by 3-inch deep plastic pot. In the second experiment, 20 achenes were planted at the appropriate depth in each row spaced 4 inches apart in 4- by 18- by 24-inch flats. Emergence was recorded periodically after planting. At the end of the third experiment when emergence appeared complete, soil from the pots was spread about 1 to 2 cm. deep in flats. The flats were sprinkler irrigated and the additional germination recorded.

Growth Regulators

The germination response of scotch thistle to gibberellic acid (GA), indoleacetic acid (IAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) was studied. Concentrations of 0, 8, 15, 31, 63, 125, or 250 p.p.m.w. of GA or IAA and concentrations of 0, 2, 3, 6, 12.5, 25, 50, 75, or 100 p.p.m.w. of 2,4-D were used in the germination media. Two experiments using nonstratified scotch thistle achenes with GA and IAA were conducted. Three studies each using GA and IAA, and two with 2,4-D on stratified achenes were conducted.

Achene Coat

Scarification with coarse sandpaper, removal of approximately 1 mm. of the radicle end of the achene tip, or exposure to concentrated sulfuric acid for 0, 0.5, 1, 2, 3, 4, or 5 minutes constituted achene coat treatments. After exposure to the acid, the achenes were placed in cheesecloth and immediately washed for 3 to 5 minutes with running tap water. This experiment was repeated three times on stratified as well as nonstratified achenes.

pH

Solutions with pH's of 2.2, 3, 4, 5, 6, 7, or 8 were prepared using McIlvain's buffer.[4] The solutions were combinations of 0.1 M citric acid and 0.2 M disodium phosphate in various ratios. Confirmation of pH was accomplished by using a glass electrode pH meter. However, there was evidently an inhibiting salt effect on the achenes since no germination occurred at pH 7. Therefore, distilled water was brought to various pH's using HCl or NaOH. A solution of pH 10 was added to the study. The change in pH of these nonbuffered solutions was about 0.4 pH units in 10 days towards neutrality as read with a glass electrode pH meter. Two experiments were conducted to evaluate the effect of pH.

Salinity

The effect of sodium chloride on germination was evaluated using media containing 0, 250, 500, 1,000, 5,000, 10,000, 15,000, or 20,000 p.p.m.w. NaCl on a mg./l basis in five different studies. Reaction of seedlings to 0, 50, 100, 150, 300, 450, 600, 900, 1,500, or 2,000 p.p.m. NaCl was evaluated by incorporating the desired amount of salt in soil on a μg/g basis. The experiment was conducted twice.

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[4] Hodgman, Charles D., Robert C. Weast, and Samuel M. Selby. 1958. Handbook of Physics and Chemistry. Chem. Rubber Pub. Co., Cleveland, Ohio 3456 pp.

Air-dry, Sharpsburg silty clay loam soil with about 23 m.e. Na/100 g., mixed with sand at a 3:1 ratio was thoroughly mixed with the salt for 3 hours on a rotating mixer. Twenty achenes were planted 1-cm. deep in 3-inch diameter plastic pots containing the desired salt in soil concentrations. Seedling emergence and survival were recorded periodically. Seedlings were harvested after 21 days, oven-dried, and weighed to estimate the effect of salt on production.

Moisture Stress

Moisture stress was simulated in 5 experiments by using solutions of d-mannite (mannitol). Osmotic pressures of 0, and at 2 atm. from 4 to 20 atm., were formulated using the formula given by Powell and Pfeifer (13).

Osmotic pressure (OP) in atmospheres was calculated as OP=gRT/mV where "g" is grams of mannitol, "R" is 0.825 liters atm. per degree per mole, "T" is absolute temperature, "m" is molecular weight of mannitol, and "V" is the volume in liters. Thus, the amount of mannitol required for an osmotic pressure of "P" was calculated from g=PVm/RT.

Inhibitors in the Achene Coat

Two studies were conducted to evaluate the effect of extract from scotch thistle achenes on bioassay crops. In the first study, 400 achenes were soaked in 100 ml. distilled water, ethyl alcohol or acetone for 5 hours.

The extract was diluted to 0, 25, and 50% in distilled water for germination media. Bioassay was accomplished by applying 7 ml. of the various concentrations of extract to petri dishes containing 25 seeds of cucumber (*Cucumis sativa* L.), corn (*Zea mays* L.), soybean (*Glycine max* L.), or wheat (*Triticum aestivum* L.). Response to scotch thistle extract was measured as percentage germination and extent of root and shoot elongation.

In the second study, one set of achenes was soaked in distilled water at room temperature. Another set was brought to 100 C. in distilled water, covered with "Saran"[5] and cooled to room temperature. A third treatment tested the effect of scotch thistle achenes on bioassay crops by placing 100 achenes in the germination dish with the crops. Cucumber and corn were used for the bioassay species.

Germination of soaked scotch thistle achenes was compared to that of unsoaked achenes in three studies to further test for an inhibiting substance.

RESULTS AND DISCUSSION

Prechilling

Although imbibition is thought of as the first step toward germination, certain partial processes must occur long before rehydration of the embryo (8). Seeds of many species require exposure to low temperature before they will give maximum germination at a favorable temperature.

Scotch thistle achenes, freshly harvested and graded, germinated about 28± 2% in an alternating 20 to 30 C. germinator. Storage under wet or dry conditions at 0 or 5 C. for seven to sixteen days greatly increased germination (Figure 3).

This response differs somewhat from Mayer and Paljakoff-Mayber (9) in that they indicated that full imbibition is required before seeds will respond to temperature treatment. However, it is known that after-ripening of some seeds can take place in dry storage (4).

Regardless of moisture level, activity was greater from scotch thistle achenes stored at 0 C. for at least 16 days. However, after 3 weeks of storage, germination decreased to 50 to 75% of untreated achenes except at 5 C. and wet storage. After the initial increase in germination percentage, germination decreased steadily under cold temperatures with time, regardless of moisture level.

There was no significant difference between germination of non-stratified scotch thistle achenes and those stored at 0 C. for all

[5] Trade name, cited for identification of product used, is not necessarily endorsed by the USDA over similar products.

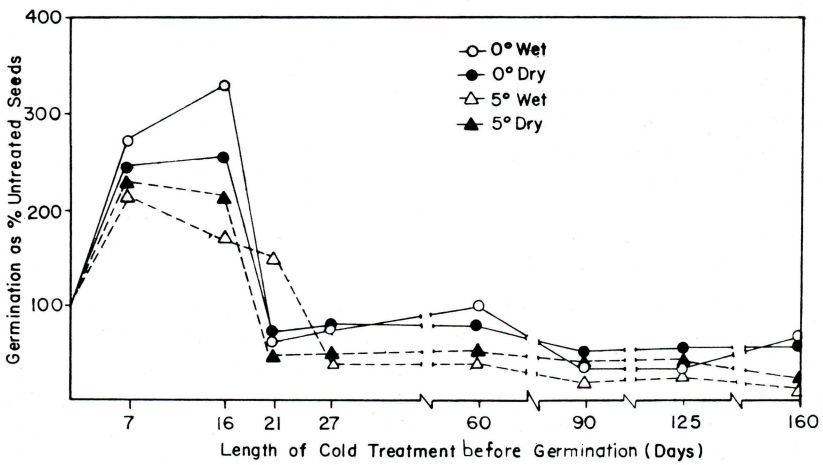

Figure 3. The effect of storage at 0 or 5 C. on the germination percentage of imbibed or dry scotch thistle achenes. The germinating chamber alternated from 20 to 30 C.

sampling dates from the 21st to 160th day of storage. After 160 days, germination was 20 and 14% of untreated achenes after storage in conditions of 5 C. dry and 5 C. wet, respectively.

The effect of temperature is presumably manifested through its relation to metabolism and stimulation of growth. For instance, the embryonic axis of cherry (*Prunus axium* L.) seeds increased in dry weight, total length and cell number when stored under moist conditions at 5 C. (9). Therefore, it is possible that in some seeds the effect on metabolism at a temperature that would allow some physiological activity could be more detrimental than at a lower temperature where the processes are slowed to the point that activity is negligible.

These data indicate that immaturity of the embryo is probably not the cause of low germination in scotch thistle. It is pertinent to mention that nonstratified scotch thistle embryos were capable of reducing tetrazolium dye. This might indicate that an achene coat factor could be important in regulating germination and that stratification probably had a neutralizing effect on this factor.

Ambient Temperature

Stratification involves temperature exposure prior to placing the seeds in an environment suitable for germination. In the following discussion, "stratified" achenes refers to achenes stored in dry beakers at 0 C. for 15 days.

Temperature at the time of seed germination is among the most important of specific conditions that must be met (4). Different seeds have different temperatures at which they germinate best, and extremes in temperature inhibit germination of many seeds (9).

Germination of nonstratified scotch thistle achenes was higher at 25 and 28 C. than at other temperatures (Figure 4). The trend shown in Figure 4 was the same under light or dark conditions. Constant temperatures usually permit only a small percentage of the germination obtained from alternating temperatures (4). However, the use of constant temperatures may help establish tolerance limits for germination.

There was little increase in average percentage germination after 10 days with scotch thistle achenes, and percentage germination was approximately 20% under the most favorable temperatures (Figure 4). Storage on moist filter paper for 10 days at 0 and 5 C. resulted in 30 and 20% germination, respectively, ten days after transfer to 28 C. The average percentage germination of nonstratified scotch thistle achenes in a 20 to 30 C. alternating temperature was about 28%.

Seedling growth under constant temperature was favored by the same temperatures giving highest germination percentage (Table 1). Length and fresh weight of seedlings was greater under 25 and 28 C.

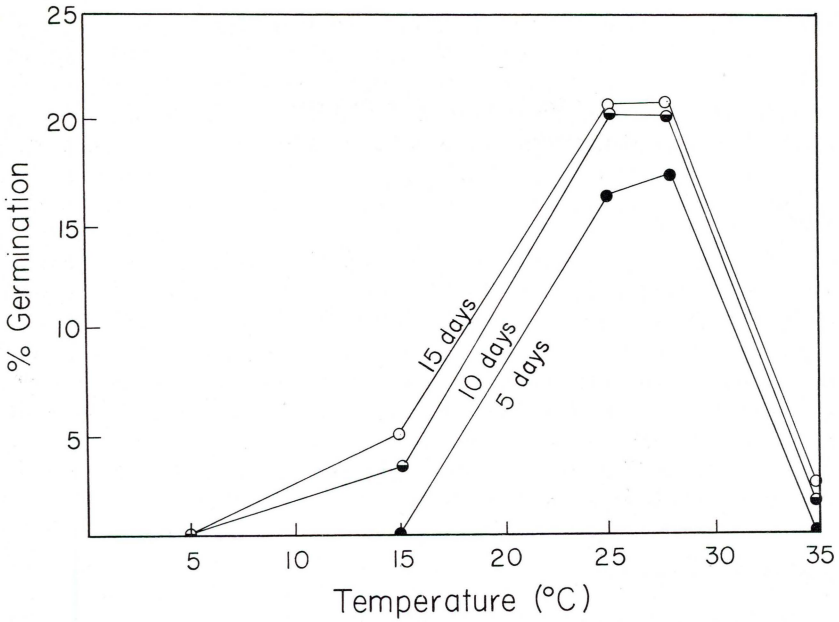

Figure 4. Average percentage germination of nonstratified scotch thistle achenes at various constant temperatures in the germinator after 5, 10, and 15 days.

than under 15 or 35 C. Maximum growth, as previously noted for percentage germination, occurred from achenes stored at 0 C. for 10 days, and then transferred to 28 C. Cold treatment improved germination as noted in Figure 3. The effect on seedling growth was probably not an increase in vigor; but the result of a longer growing period due to decreased time required for germination after cold treatment.

The optimum temperature for germination is that one which allows maximum germination in the shortest time (9). Germination, as well as seedling growth, was highest at 25 and 28 C. constant temperatures. Since a 20 and 30 C. sequence alternating on 16 and 8 hours, respectively, gave about 85% germination of stratified achenes,

Table 1. Average lengths and fresh weights of scotch thistle seedlings 10 days after germination at various constant temperatures.[a]

Temperature C.	Seedling length mm.	Fresh wt/seedling mg.
0 to 28[b]	28.5 a	4.0 a
5 to 28[b]	25.0 a	1.4 c
15	4.8 b	1.7 c
25	22.6 a	2.5 ab
28	25.0 a	2.2 b
35	2.3 b	0.3 d

[a] Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

[b] Stored at 0 or 5 C. for 10 days, then at 28 C. for 10 days.

this sequence was selected for use in all other experiments. This does not necessarily exclude other temperature regimes that might give similar results. It is the range of temperature that is important (9). The optimum range of temperature varies with age, physiological state, and kind of seed (15).

Light Requirement

Length of day, or actually, length of dark period affects growth processes in plant and animal life (7). It is known that the requirement for light often may be substituted for by other factors such as temperature.

As previously noted, nonstratified scotch thistle achenes were not sensitive to light. Germination ranged from 20 to 30% under constant temperature in light or dark, or under an alternating temperature and light regime. However, after stratification, scotch thistle achenes were light sensitive (Table 2).

In alternating light at 20 to 30 C., germination averaged 80%. However, in covered petri dishes, germination was only 26%. The use of 30 p.p.m.w. GA in the germination media in the light gave a slight increase in germination.

However, of more importance is the increase in germination of achenes stored in the dark with GA used as the germination media. Germination was not significantly different from that of achenes receiving intermittent light periods during germination. Increased germination in the dark from GA also excludes the possibility of the aluminum foil wrap lowering gas exchange to a detrimental level.

Total fresh weight of seedlings was lowered in petri dishes receiving no light, but this was a function of reduced germination, not reduced growth rate (Table 2). Seedlings grown in the dark for this short period were etiolated, but weights and lengths of individuals were not significantly different from seedlings under light. GA

Table 2. Average percentage germination and seedling development of stratified scotch thistle achenes as affected by 10 days in light or dark in the germinator and in media containing 30 p.p.m.w. GA or IAA.[a]

Treatment		% germination	Fresh weights of seedlings		Seedling length mm.
Chemical	Light		total g.	Average mg.	
None	+	80 a	1.2 a	31 ab	38 a
None	—	26 c	0.5 c	37 a	44 a
GA	+	88 a	1.2 a	27 bc	44 a
GA	—	81 a	1.2 a	30 ab	30 ab
IAA	+	87 a	0.9 b	20 c	20 b
IAA	—	46 b	0.7 c	28 b	27 ab

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

did not affect total fresh-weight production under light, but the seedlings tended to be longer. Seedlings grown in the dark in GA did not have the etiolated characteristics of those receiving no GA and grown in the dark.

IAA did not affect germination of achenes under intermittent light, but did reduce fresh-weights and seedling lengths (Table 2). IAA increased germination of achenes in the dark, but not as much as GA. The reaction of seedling growth was similar to dark conditions without IAA except that seedling lengths were not increased.

These data indicate that there might be an inhibitory substance in the achene coats of scotch thistle. Once the inhibitor is broken down, the achene coat is sensitive to light. GA then is able to substitute for this requirement.

Depth of Planting

The depth from which a shoot must emerge may be decisive as to whether or not a plant becomes established. Depth of planting undoubtedly directly influences emergence through its effects on light, moisture, gas exchange and by physical limits on the distance an

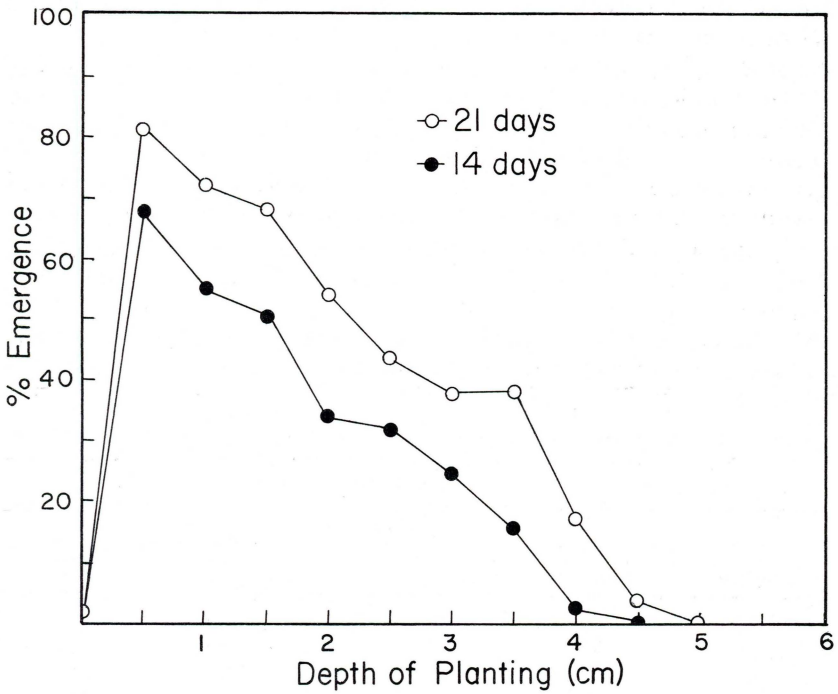

Figure 5. The effect of depth of planting on the emergence of stratified scotch thistle achenes in the greenhouse after 14 and 21 days from a Sharpsburg silty clay loam soil with a bulk density of 1.1.

embryonic plant can develop on the stored food supply in the seed. Burnside (2) showed that emergence of wild cane (*Sorghum bicolor* (L.) Moench) was affected by depth of planting and temperature whereas soil type and other factors were of lesser importance.

Before stratified achenes of scotch thistle would germinate, they required coverage by soil (Figure 5), or at least maximum contact with the soil. Surface planted achenes that germinated had been washed into the soil due to overhead watering and were partially buried. Any achenes left completely exposed on the surface did not germinate. This was probably a function of moisture availability.

Scotch thistle achenes given proper treatment germinated well without maximum substrate contact in petri dishes and they showed a definite light requirement. However, the petri dishes were covered and the filter paper saturated with water. Condensation within the dishes indicated saturation of the air. Thus, water was available for imbibition from the ambient atmosphere.

Under greenhouse conditions (Figure 5), high evaporation rates probably decreased the available water on the soil surface such that quantities sufficient for imbibition were not available. Emergence was greatest from seeds covered with 0.5 cm. soil. Overhead watering by sprinkler irrigation exposed a portion of these achenes to light. Also, the depth of light penetration into the soil is not known. Emergence decreased with increasing depth. After 21 days, only 5% of the achenes buried 4.5 cm. deep had emerged. Emergence at 28 days was no different than at 21 days.

At depths of 1.5 to 4 cm., some achenes germinated but failed to emerge during the experiment (Table 3). When all achenes were replanted at the 1-cm. depth, there was an inverse relationship between germination and emergence from the original depths. Analysis of

Table 3. Effect of depth of planting on the percentage germination and emergence of stratified scotch thistle achenes.[a]

Planting depth (cm.)	% emergence after 21 days	% germinated but not emerged	% germination after replanting at 1 cm. deep	Total
0	5 a	0 a	77 a	82 a
0.5	81 e	0 a	5 cd	86 a
1.0	78 e	0 a	0 d	78 a
1.5	71 e	10 b	6 cd	87 a
2.0	60 d	12 b	10 c	82 a
2.5	50 cd	10 b	23 bc	83 a
3.0	48 c	21 b	10 c	79 a
3.5	48 c	0 a	39 b	87 a
4.0	20 b	12 b	46 b	78 a
4.5	6 a	0 a	78 a	84 a
5.0	0 a	0 a	81 a	81 a
5.5	0 a	0 a	86 a	86 a
6.0	0 a	0 a	84 a	84 a

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 4. Effect of various concentrations of GA and IAA on the average germination of stratified scotch achenes.[a]

Concentration p.p.m.w.	% germination					
	Days in GA			Days in Iaa		
	5	7	10	5	7	10
0	51 a	79 a	88 a	47 a	76 b	90 a
8	65 ab	91 ab	91 ab	37 b	89 a	89 a
15	55 a	92 ab	92 ab	35 b	84 ab	87 a
31	55 a	97 b	97 b	16 c	84 ab	88 a
63	67 ab	99 b	99 b	9 cd	76 b	91 a
125	80 b	95 b	95 b	9 cd	66 bc	76 b
250	77 b	96 b	96 b	0 d	44 c	67 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

variance on the total number of seedlings recovered during the experiment showed no significant differences among depths. Thus, the failure of achenes to germinate at deeper levels was not due to secondary dormancy.

Growth Regulators

Growth regulators affect many growth processes. The metabolic blocks to sugar accumulation and utilization naturally inhibit germination of wild oats (*Avena fatua* L.) (12). Exogenous GA overcomes these blocks. Simpson (14) later demonstrated the requirement of an exogenous source of sugar, amino acids, and GA before germination and subsequent growth of naked, dormant, wild oat embryos would take place.

Neither GA nor IAA stimulated germination of nonstratified scotch thistle achenes. However, after stratification, the achenes were sensitive to both chemicals (Table 4).

GA increased germination after 5 days at 125 and 250 p.p.m.w. (Table 4). After 7 days, all concentrations had increased germination; but after 10 days, the untreated achenes nearly equalled the germina-

Table 5. Average fresh weights and lengths of scotch thistle seedlings germinated and grown for 10 days in various concentrations of gibberellic acid.[a]

GA concentration p.p.m.w.	Fresh weight of seedlings		Seedling lengths mm.
	Total g.	Average mg.	
0	1.2 a	28 a	47 ab
8	1.1 a	27 a	37 abc
15	1.3 a	30 a	49 ab
31	1.2 a	27 a	55 a
63	1.2 a	29 a	47 ab
125	1.2 a	27 a	27 bc
250	1.2 a	27 a	20 c

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

Table 6. Average fresh weights and lengths of scotch thistle seedlings germinated and grown for 10 days in various concentrations of indoleacetic acid.[a]

IAA concentration p.p.m.w.	Fresh weight of seedlings		Seedling lengths mm.
	Total g.	Average mg.	
0	1.2 a	29 a	48 a
8	1.0 a	25 a	28 c
15	1.0 a	22 b	36 b
31	1.3 a	23 ab	22 cd
63	0.9 a	23 ab	23 cd
125	0.7 ab	19 b	17 de
250	0.6 b	4 c	10 e

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

tion of those in two lower concentrations of GA. GA had no effect on fresh-weight of seedlings but reduced seedling length, as measured from the cotyledons to the tip of the primary radicles (Table 5). The decrease in length appeared to be a result of decreased growth of the primary radicle. There also appeared to be a stimulation in production of secondary roots. In general, seedlings growing in solutions of GA had a deeper green color and healthier overall appearance than those growing in distilled water.

IAA retarded germination so that at 5 days all concentrations had significantly fewer germinated achenes (Table 4). However, after 7 or 10 days, only the highest concentrations depressed germination. The higher concentrations of IAA significantly reduced total fresh-weight production of scotch thistle seedlings after 10 days (Table 6). All concentrations of IAA decreased seedling length.

There are reports of low 2,4-D concentrations stimulating germination of some species, but no such reaction occurred with scotch thistle (Table 7). All concentrations inhibited germination at 5 days but by 10 days, only concentrations of 25 p.p.m.w. or more significantly reduced germination.

Total fresh weights were reduced by all concentrations, and there was a trend in reduced weights of individual seedlings although only 100 p.p.m.w. 2,4-D differed significantly from the controls. Radicle lengths were greatly reduced and shoot lengths of scotch thistle achenes were significantly shorter than the controls regardless of 2,4-D concentration. However, the hypocotyl and radicles of treated seedlings were greater in circumference and extremely turgid. This accounts for the drastic reduction in lengths although the decrease in fresh weight was much more gradual with increasing concentrations.

At the time of evaluation, seedlings growing in concentrations up to 50 p.p.m.w. 2,4-D appeared capable of further growth although there is no way to predict how long they might have survived. Assuming equal distribution of an application of 2,4-D at 2 lb/A through

Table 7. Effect of various concentrations of 2,4-D on the average percentage germination and early seedling growth of scotch thistle.[a]

2,4-D concentration	% germination		Fresh weight of seedlings after 10 days		Seedling length (% of 0 p.p.m.) after 10 days	
p.p.m.w.	5 days	10 days	Total g.	Average mg.	Root	Shoot
0	68 a	97 ab	1.8 a	38 a	100 a	100 a
2	42 b	92 ab	1.5 b	31 a	17 b	63 b
3	38 b	96 ab	1.5 b	31 a	14 b	54 b
6	28 bc	98 a	1.1 c	25 a	10 b	50 b
12.5	22 c	91 b	1.0 cd	23 a	10 b	46 b
25	12 d	82 c	0.9 d	20 ab	10 b	46 b
50	8 de	40 d	0.4 e	19 ab	10 b	42 b
75	1 e	6 e	0.1 f	20 ab	10 b	42 b
100	0 e	1 e	0.1 f	10 b	10 b	42 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 2 experiments.

the top 6-inch soil layer, the resulting concentration would be 1 p.p.m. If all the 2,4-D were concentrated in the upper 1-inch layer of soil and none was absorbed by soil colloids, degraded by soil microbes, volatilized, leached out by soil water, or otherwise dissipated, the resulting concentration available would be about 6 p.p.m.

We realize no direct correlation between field and laboratory conditions is possible, but these data indicate resistance of scotch thistle achenes to normal field rates of 2,4-D. The herbicidal effect of 2,4-D, then, must be exerted on the seedling.

Achene Coat

The outer coverings of an embryo may restrict germination by preventing entrance of water and gases, by germination inhibitors, or by simple mechanical constraint of the embryo. Achene coat treatments did not affect germination of nonstratified achenes. The response of stratified scotch thistle achenes to various coat treatments was consistent among experiments.

Treatment with sulfuric acid increased germination after 3 days over untreated achenes and those receiving mechanical treatments (Table 8). Acid-treated achenes germinated the same as untreated achenes after 7 days, but there was a trend for increased germination in up to 4 minutes of sulfuric acid after 10 and 15 days. Although there was only 12% difference in achenes treated with acid for 4 minutes and untreated achenes, this difference was significant.

Scarification with coarse sandpaper did not affect germination (Table 8). Removal of 1 mm. of the radicular end of the achene coat reduced germination at the 7-, 10- and 15-day evaluations. This was due to damage incurred by the embryo during treatment. The radicles emerged from the achene coat but were severely damaged and had large brown spots on the damaged portions. There was less total fresh-

Table 8. Effect of various achene coat treatments on the average percentage germination after stratification of scotch thistle achenes.[a]

Treatment	% germination			
	3 days	7 days	10 days	15 days
None	36 a	88 a	88 b	88 b
1 mm. of achene tip removed	42 a	49 b	68 c	69 c
sandpaper	37 a	82 a	85 b	89 b
Concentrated H_2SO_4:				
30 seconds	67 b	87 a	88 b	89 b
1 minute	82 c	87 a	88 b	90 b
2 minutes	75 bc	93 a	93 ab	93 ab
3 minutes	83 c	84 a	92 ab	95 ab
4 minutes	84 c	91 a	100 a	100 a
5 minutes	68 b	84 a	84 b	86 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

weight production from this treatment due to lower germination and smaller seedlings (Table 9). The radicles were shorter than untreated achenes which also reduced the ratio of shoot to radicle.

Sulfuric acid treatment damaged some seedlings and induced abnormal germination patterns. Most seedlings from acid-treated achenes were not as well developed as those from untreated achenes (Table 9). Both roots and shoots were affected, but shoots more than roots as shown by comparing ratios. As acid concentration increased, there was a tendency for the cotyledons to emerge first rather than the radicles. Many seedlings with radicles of normal length were devoid of secondary roots.

Although acid-treated achenes were washed in running water before being placed in the germinator, there is no way of measuring pH changes that might have occurred in the achene coat and consequent effects on the embryo and seedling.

Table 9. Effect of various achene coat treatments on development of seedlings from stratified scotch thistle 10 days after germination.[a]

Treatment	Fresh weight of seedlings		Seedling length in mm.			Root/shoot ratio
	Total g.	Average mg.	Root	Shoot	Total	
None	2.4 a	51 ab	71 a	20 a	91 a	3.6 ab
Scarification	2.6 a	52 a	66 a	20 a	86 a	3.3 ab
Tip removed	1.9 b	44 abc	43 b	16 ab	59 bc	2.7 b
Concentrated H_2SO_4:						
0.5 minute	1.6 b	36 c	63 ab	12 b	75 ab	5.3 a
1 minute	1.7 b	39 c	53 ab	13 b	66 b	4.1 ab
2 minutes	1.9 b	42 bc	62 ab	11 b	73 ab	5.6 a
3 minutes	1.8 b	39 c	54 ab	17 ab	71 b	3.2 ab
4 minutes	1.8 b	42 bc	53 ab	11 b	64 b	4.8 ab
5 minutes	1.7 b	38 c	43 b	12 b	55 c	3.6 ab

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

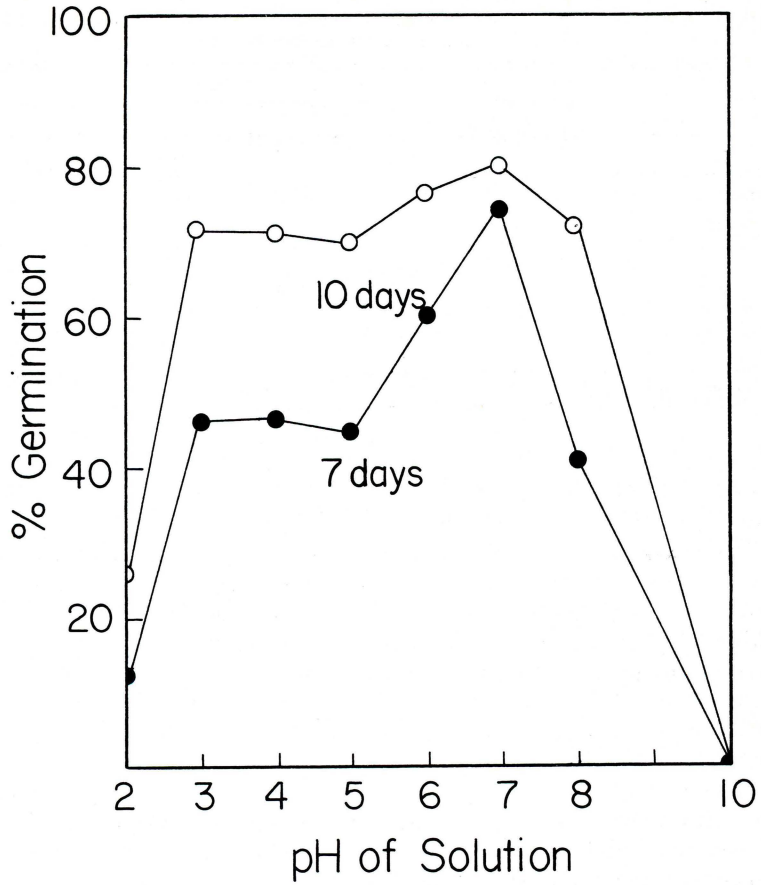

Figure 6. Effect of pH of the germination media on the average percentage germination of stratified scotch thistle achenes 7 and 10 days after initiating the experiments.

Other studies indicate the presence of an inhibitor in the achene coat, and that the stimulating effect of stratification might be due to inactivation of the inhibitory substance. Soaking in acid could have leached or broken down any inhibitor remaining in the seed coat after stratification and thus improved rate and amount of germination. This possibility is more likely than restriction of germination due to constraint by the achene coat since mechanical treatment did not improve rate or total percentage germination.

pH

Stratified scotch thistle achenes had highest and most rapid germination in media of pH 7 (Figure 6). After 10 days, percentage

Table 10. Effect of pH of the germination media on early lengths of seedlings from stratified scotch thistle achenes.[a]

pH	Seedling length mm.					
	Shoot		Root		Total	
	7 days	14 days	7 days	14 days	7 days	14 days
2	1 a	9 a	3 a	3 a	4 a	12 a
3	8 b	20 b	11 b	27 b	19 b	47 b
4	9 b	16 b	16 b	25 b	25 b	41 b
5	9 b	16 b	14 b	30 b	23 b	39 b
6	8 b	17 b	17 b	32 b	25 b	49 b
7	7 b	13 b	14 b	20 b	21 b	33 b
8	7 b	16 b	14 b	25 b	21 b	41 b
10	.....	4 a	.....	2 a	.....	6 a

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 2 experiments.

germination in pH range 3 to 8 ranged from 70 to 80%. These data indicate a wide latitude in pH for germination, but with a tendency to be favored by a neutral to slightly acid substrate. There was no difference in seedling lengths from pH 3 to 8 at either 7 or 14 days (Table 10). There were no morphological abnormalities or growth pattern differences in seedlings growing in this range. These data would also lessen consideration of the probability that pH changes in the achene coat due to acid treatment would detrimentally affect germination.

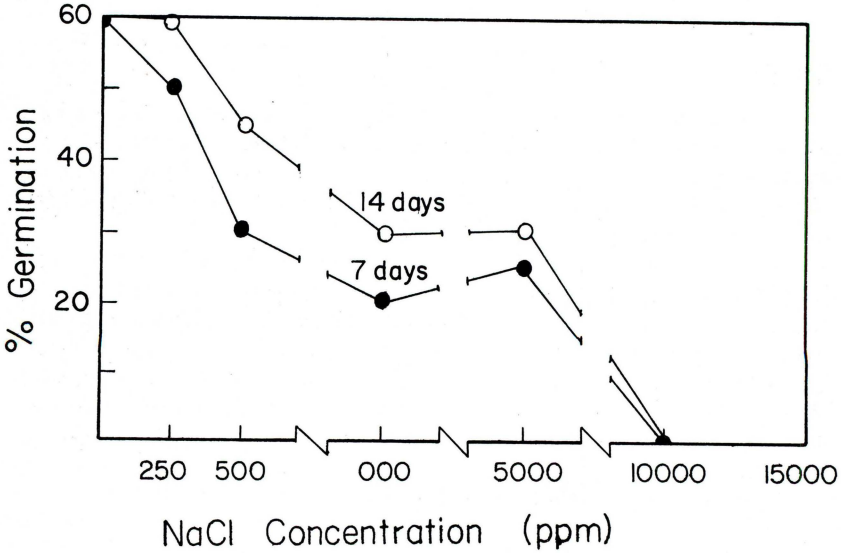

Figure 7. Average percentage germination of nonstratified scotch thistle achenes after 7 and 14 days in media containing various concentrations of sodium chloride.

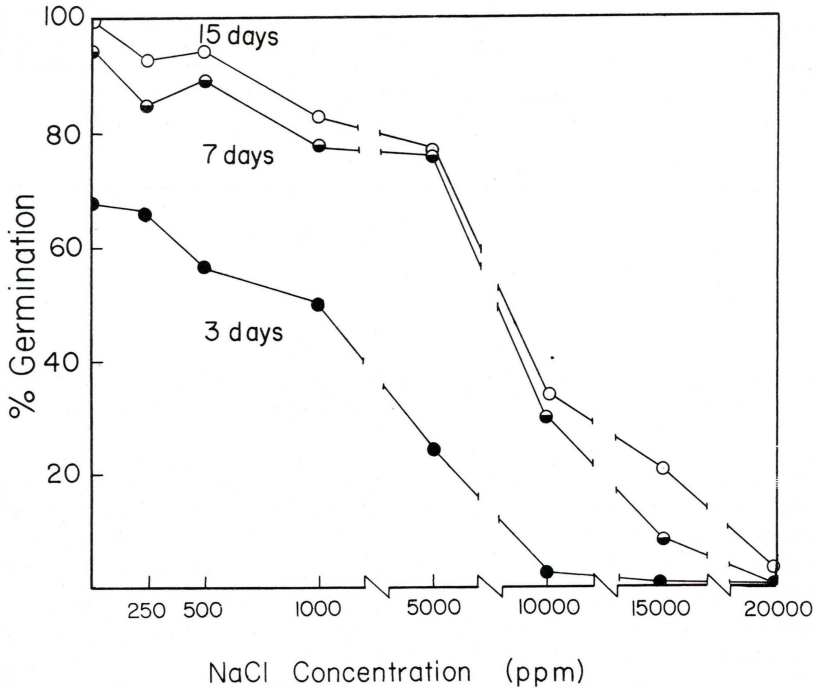

Figure 8. Average percentage germination of stratified scotch thistle achenes in media containing various concentrations of sodium chloride.

Salinity

Soil conditions have a strong effect on germination and seedling growth. Sodium chloride level is an important soil factor in determining the species that occupy a given area. Soil salinity decreases the ease with which seeds may take up water and facilitates the entry of ions in sufficient amounts to be toxic (1).

Nonstratified scotch thistle achenes germinated in media containing up to 5,000 p.p.m.w. NaCl (Figure 7); and for some undetermined reason, nonstratified achenes germinated at a higher rate than expected. Germination percentage was greater after 14 days than at 7 days, but no germination occurred in concentrations higher in NaCl than 5,000 p.p.m.w. There was no increase in germination at 21 days as compared to 14 days regardless of salt concentration (data not shown).

Stratification increased the rate of germination in all salt concentrations and also the capability of scotch thistle achenes to tolerate higher salt levels than nonstratified achenes (Figure 8). There was about 10% germination of stratified achenes in 15,000 p.p.m.w. NaCl after 7 days. After 15 days, the difference in germination between 0

Table 11. Average fresh weights and lengths of seedlings from stratified scotch thistle achenes 10 days after germination in various sodium chloride concentrations.[a]

NaCl concentration p.p.m.w.	Fresh weights of seedlings		Seedling length mm.
	Total g.	Average mg.	
0	1.4 a	32 a	70 a
250	1.1 a	25 a	30 b
500	1.2 a	27 a	29 b
1,000	1.2 a	31 a	33 b
5,000	0.7 b	26 a	15 c
10,000	0.2 c	19 b	8 cd
15,000	0.1 c	13 b	5 d
20,000	0 c	0 b	0 d

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 5 experiments.

and 5,000 p.p.m.w. was only 19% and about 2% of the achenes germinated in 20,000 p.p.m.w. NaCl. The effect of increasing salt concentrations on early seedling growth after 10 days showed the same trend as germination (Table 11).

The amount of stress that an achene will stand from salt solutions before germination may or may not be related to tolerances of the seedling and its requirements for establishment. After 21 days seedlings from stratified scotch thistle achenes in soil containing up to 450 p.p.m. NaCl emerged at about the same rate as in soil containing no additional salt (Figure 9).

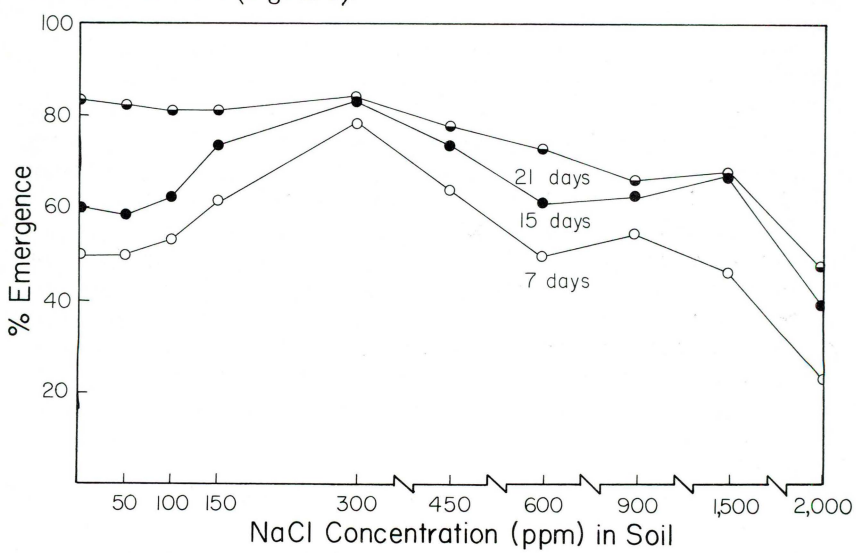

Figure 9. Average percentage emergenc of stratifid scotch thistle achenes from soils after additions of various concentrations of sodium chloride in the greenhouse.

Table 12. Average oven-dry weights of 18-day old scotch thistle seedlings grown in soil after addition of various concentrations of sodium chloride.[a]

NaCl concentrations p.p.m.w.	Average oven-dry topgrowth weights (mg.) after 18 days	
	Total	Average
0	120 a	8.2 a
50	95 a	7.3 a
100	106 a	8.7 a
150	109 a	6.9 ab
300	114 a	7.3 a
450	86 ab	5.6 ab
600	96 a	6.3 ab
900	64 b	4.6 b
1,500	74 b	5.5 ab
2,000	45 b	3.7 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 2 experiments.

After 7 and 15 days there appeared to be an increased emergence rate in 100 to 450 p.p.m. additional salt. Seedlings grown in petri dishes were retarded in growth when the salt concentration was between 1,000 and 5,000 p.p.m.w. (Table 11). Seedlings grown in soil for 18 days under greenhouse conditions were retarded in growth when the additional salt level was raised to 900 p.p.m. (Table 12). Therefore, it is concluded that scotch thistle seedlings can become established in salt concentrations of approximately 1,000 p.p.m.

Moisture Stress

Seeds have unique optimum moisture levels for germination as plants do for other growth processes. Doneen and MacGillivary (6) demonstrated the variation among several vegetables as to their moisture requirements for germination. Dale and Harrison (5) showed that wild carrot (*Daucas carota* L.) germination was not confined to any particular season, but was highly correlated with soil moisture.

Sodium chloride has been used to simulate moisture stress through increased osmotic pressure in the germination media. However, the direct relation of a NaCl solution to moisture tension is complicated by the effect of the sodium and chlorine ions on plant tissue. For this reason, mannitol was used instead of NaCl to simulate moisture stress on scotch thistle achenes.

Mannitol has been used by workers to simulate drought stress on different species and then correlated with soil moisture content (13). Stratified scotch thistle achenes germinated in osmotic pressures of 16 atmospheres after a week, and in up to 20 atm. after 13 days (Figure 10). The tendency was a fairly steady decrease in germination as osmotic pressure in the germination media was increased, but differences were not significant at either date until pressures of 10 to 12 atmospheres were reached. After 10 days in the last three experiments

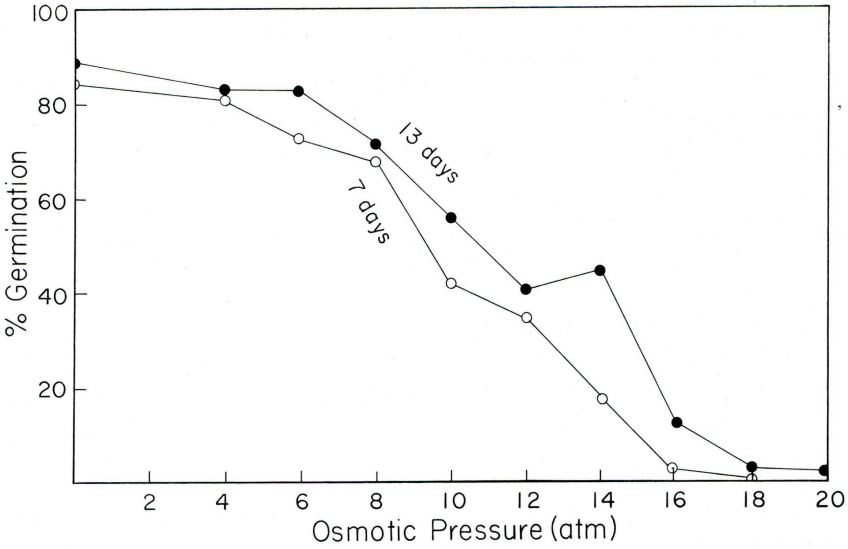

Figure 10. Effect of various moisture tensions on the average percentage germination of stratified scotch thistle achenes. Data represent average of two experiments.

(Table 13), there was no germination in osmotic pressures above 14 atmospheres.

Seedling growth followed the same trend as germination. Seedling weights and lengths, however, were significantly reduced at 6 to 8 atmospheres. These data point out that although the achene may germinate in an environment of low available water, the seedling requires a less extreme situation for normal development.

Table 13. Effect of various osmotic pressures in the germination media on the average percentage germination and seedling growth of stratified scotch thistle achenes after 10 days.[a]

Osmotic pressure (atm.)	% germination	Fresh weight of seedlings		Average seedling length mm.
		Total g.	Average mg.	
0	88 a	1.5 a	34 a	58 a
4	80 a	1.3 a	31 ab	40 b
6	65 a	0.3 b	21 b	14 c
8	60 ab	0.1 c	14 c	7 cd
10	42 b	0.1 c	14 c	6 cd
12	40 b	0.1 c	10 cd	4 cd
14	22 bc	0.1 c	6 d	3 cd
16	0 c	0 c	0 d	0 d
18	0 c	0 c	0 d	0 d
20	0 c	0 c	0 d	0 d

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 3 experiments.

Table 14. Average percentage germination of nonstratified scotch thistle achenes after soaking for 5 hours in various solvents.[a]

Solvent	% germination
Water	44 a
Acetone	5 c
Ethanol	12 bc
Distilled water control	24 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 2 experiments.

Inhibitors in the Achene Coat

Endogenous plant chemicals may either stimulate or inhibit germination. These chemicals usually reside in the seed coat. Loss of dormancy during the weeks after maturity of wheat seeds has been correlated with natural inactivation of an inhibitor in the seed coat (11). Stratification of silverberry (*Elaeagnus commutata* Bernh.) seeds increased germination, but removal of the endocarps of unstratified seeds resulted in 85 to 100% germination within 10 days (3). A water extract of the endocarps retarded growth of wheat seedlings and of seedlings from naked silverberry seeds.

Soaking of nonstratified scotch thistle achenes for 5 hours in distilled water at room temperature and decanting the liquid significantly increased percentage germination (Table 14). Acetone and ethyl alcohol lowered germination, but a dark brown color developed in all solvents after two hours soaking.

Light increased the germination of stratified achenes that had been soaked as compared to unsoaked achenes (Table 15). GA gave the same effect as the soaking treatment. The combination of soaking, treatment with GA and intermittent light during germination, gave 100% germination. The addition of GA, or soaking achenes, or a combination of the two, resulted in germination equal to or greater than that in light.

These reactions could conceivably be related to the previously

Table 15. Interrelationships of the light requirement for germination of stratified scotch thistle achenes after 6 days with soaking and gibberellic acid.[a]

Treatment	% germination
Light	80 a
Light + soaking in distilled water (5 hrs)	99 b
Light + gibberellic acid (30 p.p.m.w.)	91 b
Light + soaking + gibberellic acid	99 b
Dark	43 c
Dark + soaking	90 ab
Dark + gibberellic acid	79 a
Dark + soaking + gibberellic acid	99 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test. Data represent average of 2 experiments.

Table 16. Effect of extracts from soaking scotch thistle achenes on the average percentage germination of 4 bioassay crops after 7 days.[a]

Solvent	% media as extract	Germination as % solvent control			
		Corn	Soybeans	Cucumber	Wheat
Water	50	96	96	97	104
Water	25	100	110	103	111
Acetone	50	77*	88	ng[b]	ng
Acetone	25	120*	132*	86	ng

[a] Means followed by an asterisk show a significant difference in germination at the 5% level as compared to germination in the same solvent without extract.

[b] The symbol "ng" indicates no germination.

hypothesized germination inhibitor in the achene coat, which, in view of light requirements for germination, mediates light sensitivity in the achenes and is broken down by stratification, or is removed by soaking. GA in some way inactivates the inhibitor.

The extract, diluted in water to 25%, increased germination slightly in all bioassay crops except corn (Table 16). A 50% concentration had little effect on germination in soybeans, corn and cucumber. A similar trend occurred in corn and soybeans when acetone was used as the solvent and the differences were significant at the 5% level. Cucumber and wheat were susceptible to the acetone. Increasing concentrations of extracts in distilled water at room temperature or brought to 100 C. had no effect on cucumber and corn germination in the second study (Table 17).

Germination and root lengths of either bioassay crop were not affected by the presence of scotch thistle achenes in the petri dish although large brown spots formed under each thistle achene as the substance diffused from them. Cucumber shoot lengths were significantly shorter than those in solvent controls in some concentrations

Table 17. Average percentage germination of corn and cucumber seeds and growth of seedlings in extracts from soaking scotch thistle in cold or hot water.[a]

	% media as extract	% germination		Length as % solvent controls			
				Roots		Shoots	
		cucumber	corn	cucumber	corn	cucumber	corn
	0	79	96	100	100	100	100
Cold:	100	83	93	97	77	72*	84
	80	69	95	76	63*	82	68
	60	73	91	91	77	39*	92
	40	75	94	147*	57*	83	136*
	20	76	91	108	93	59*	104
Hot:	100	76	91	76	87	59*	90
	80	86	93	82	120	83	68
	60	81	92	70*	80	56*	88
	40	79	94	58*	63*	52*	76
0 + 100 Scotch thistle achenes		74	93	94	103	52*	320*

[a] Means followed by an asterisk are significantly different from 0% media with LSD = .05.

Table 18. Effect of extracts from soaking scotch thistle achenes on the average shoot and root growth of seedlings of 4 bioassay crops.[a]

Solvent	% media as extract	% change in root lengths as compared to solvent controls			
		Corn	Soybeans	Cucumber	Wheat
Water	50	−20*	+ 1	− 11	+ 96
Water	25	+27*	+186	− 5	+ 88
Acetone	50	+11	+162*	ng[b]	ng
Acetone	25	+76*	+489	− 80	ng

Solvent	% media as extract	% change in shoot lengths as compared to solvent controls			
		Corn	Soybeans	Cucumber	Wheat
Water	50	− 3	.....	−14*	+ 33*
Water	25	+17	.....	+ 2	+ 67*
Acetone	50	+17*	.....	ng	ng
Acetone	25	+84*	.....	− 7	ng

[a] Means followed by an asterisk show a significant difference in length at the 5% level as compared to the solvent controls.

of water extracts (Tables 17 and 18). The presence of scotch thistle achenes also lowered cucumber shoot lengths while corn shoot growth was stimulated (Table 17). Corn roots and shoots were longer than solvent controls when a 25% dilution of water extract was used (Table 18).

SUMMARY AND CONCLUSIONS

Chilling for 7 to 16 days at 0 or 5 C. before placing them in the germinator increased the germination of scotch thistle achenes. Embryos from unstratified achenes were capable of reducing tetrazolium. This would indicate that the germination regulating mechanism is in some tissue region other than the embryo.

Chilling also sensitized the achene to light. GA would substitute for the light requirement. Maximum germination occurred from stratified achenes in intermittent light and 20 to 30 C. alternating temperatures.

Soaking the achenes in distilled water for 5 hours improved germination significantly, but not to the degree that stratification did. A dark colored substance can be extracted from the achene coat by soaking; this could be a light regulating pigment in view of the reaction of scotch thistle achenes to light. Water extracts from scotch thistle achenes did not affect germination of corn or cucumber, but affected their root and shoot growth.

Scarification with sandpaper or removal of 1 mm. of the radicular tip of the achene did not improve germination. This eliminates mechanical constraint as a germination regulator. These data sug-

gest that the germination regulating factor in scotch thistle is located in the achene coat. Stratification either deactivates the inhibiting factor or in some way speeds its diffusion from the achene coat.

Scotch thistle achenes had highest emergence from 0.5 cm. deep in a greenhouse soil with a bulk density of 1.1. Emergence occurred up to depths of 4.5 cm. Optimum germination occurred under temperatures of 25 to 28 C. Scotch thistle achenes germinated best in media of pH 7, but tolerated a range from 3 to 8. Achenes and seedlings tolerated high concentrations of salt, and seedlings were established in the greenhouse in 1,000 p.p.m. NaCl in soil. Although germination decreased with increasing osmotic pressure in the germination media, it was not significantly lowered until a pressure of 10 to 12 atm. was reached.

Scotch thistle achenes tolerated up to 6 p.p.m.w. 2,4-D with no reduction in germination after 10 days. Root length was decreased 90% and shoots 50%.

These studies demonstrate the wide range of environmental factors tolerated by scotch thistle achenes and seedlings. The potential of this weed to become established in a diverse ecological range is indicated by its ability to withstand extremes in moisture, temperature, and salt concentration.

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