THE EFFECT OF POLYETHYLENE GLYCOL 6000 AND OTHER CHEMICAL PRETREATMENT ON ASPARAGUS SEED GERMINATION

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
WAYNE BOYD COUSINS

1975

ABSTRACT

THE EFFECT OF POLYETHYLENE GLYCOL 6000 AND OTHER CHEMICAL PRETREATMENT ON ASPARAGUS SEED GERMINATION

By

Wayne B. Cousins

Asparagus seeds (*Asparagus officinalis* L.) were pretreated in different chemical solutions to improve germination in cool sandy soils. Soaking seeds for sixteen days in an aerated polyethylene glycol (PEG) 6000 solution (40g/100 ml distilled water) enhanced speed of seedling emergence. Total emergence and total germination were also improved by soaking seeds in PEG 6000 for sixteen days. Pretreatment in $CaCl_2$ (10^{-2}M) solution for 24 hours improved speed of germination. When a $CaCl_2$ (10^{-2}M) solution soak followed sixteen days of PEG 6000 pretreatment, the greatest improvement in speed of emergence was obtained. When seeds were soaked in PEG 6000 for sixteen days followed by $CaCl_2$ for one day, 44% emergence was obtained seventeen days after sowing compared to 1% emergence for untreated seeds.

Moisture content of asparagus seeds soaked in PEG 6000 was inversely related to PEG 6000 concentration. It appears that PEG 6000 has an affinity for water. When the concentration of PEG 6000 increased, hydrogen bonding between water and the ether oxygens of PEG increased; in turn the amount of water imbibed by the seed is reduced. It appears that the percent water within the seed is critical for radicle emergence. If the concentration of PEG 6000 is 40g/100 ml distilled water, the critical percent moisture needed for radicle emergence is not obtained. This

allows the seed to be presoaked for long periods of time (16 days) without germination or apparent detrimental effects.

THE EFFECT OF POLYETHYLENE GLYCOL 6000 AND OTHER CHEMICAL PRETREATMENT ON ASPARAGUS SEED GERMINATION

by

Wayne Boyd Cousins

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Horticulture

1975

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Professor Hugh Price for his help as advisor and Chairman of the advisory committee, and to Professors Jim Motes and Clarence Hansen for their guidance as this work was being conducted and for their review and suggestions in the preparation of this manuscript.

The author also wishes to thank those members of the Department of Horticulture who made their laboratory facilities available during this period.

TABLE OF CONTENTS

Title	Page
List of Tables	iv
List of Figures	vi
Introduction	1
Literature Review	1
Materials and Methods	7
Section I	
Chemical Pretreatment of Asparagus Seeds	7
Sections II and III	
Polyethylene Glycol Pretreatment of Asparagus Seeds	10
Results and Discussion	8
Section I	
Chemical Pretreatment of Asparagus Seeds	8
Section II	
Polyethylene Glycol Pretreatment of Asparagus Seeds	13
Section III	
PEG 6000 and $CaCl_2$ Pretreatment of Asparagus Seeds	25
Summary and Conclusion	31
List of References	35
Appendix	

LIST OF TABLES

Title of Table		Page
Table 1.	Effect of chemical pretreatment of asparagus seed for 24 hours on subsequent development after 36 days in a petri dish (50 seeds total per replication)	9
Table 2.	Effect of polyethylene glycol (PEG) 6000 pretreatment of asparagus seeds on total percent seedling emergence after 50 days (25 seeds total per replication)	16
Table 3.	Effect of polyethylene glycol (PEG) 6000 pretreatment of asparagus seeds on time to 50% of total emergence (25 seeds total per replication)	17
Table 4.	Effect of polyethylene glycol (PEG) 6000 pretreatment of asparagus seeds on time to 50% of total germination (25 seeds total per replication)	18
Table 5.	Effect of polyethylene glycol (PEG) 6000 and calcium chloride pretreatment of asparagus seeds on the number seedlings emerged after seventeen days from sowing (100 seeds total per replication)	26
Table 6.	Effect of polyethylene glycol (PEG) 6000 and calcium chloride pretreatment of asparagus seeds on total percent emergence after 45 days from sowing (100 seeds total per replication)	27
Table 7.	Effect of polyethylene glycol (PEG) 6000 and calcium chloride pretreatment of asparagus seeds on total percent germination after 21 days in a petri dish (100 seeds total per replication)	28
Table 8.	Effect of cold treatment of asparagus seeds on subsequent seedling development after 36 days in a petri dish (50 seeds total per replication)	39

Title of Table | Page

Table 9. Effect of cold storage on asparagus seeds later soaked in gibberellic acid 4/7 on subsequent seedling development after 40 days in a petri dish (50 seeds total per replication) 40

Table 10. Effect of Ethephon pretreatment of asparagus seeds on subsequent seedling development after 40 days in a petri dish (50 seed total per replication) 41

Table 11. Effect of concentrated H_2SO_4 scarification of asparagus seeds on germination (33 seeds per replication) 42

LIST OF FIGURES

Title of Figure		Page
Figure 1.	Effect of pretreating asparagus seeds in polyethylene glycol (PEG) 6000 on percent moisture within the seed (10 seeds total per replication)	20
Figure 2.	Effect of polyethylene glycol (PEG) 6000 pretreatment for sixteen days on asparagus seed germination (25 seeds total per replication)	22
Figure 3.	Effect of polyethylene glycol (PEG) 6000 pretreatment for sixteen days on asparagus seedling emergence (25 seeds total per replication)	24
Figure 4.	Effect of pretreating asparagus seeds in 40g of polyethylene glycol (PEG) 6000 per 100 ml of water for sixteen days and/or one day of soaking in calcium chloride on the emergence rate (100 seeds total per replication)	30

INTRODUCTION

In today's world of mechanization, producing crops efficiently is one of the main goals of a grower. Direct seeding has become a very important practice to increase efficiency for the grower. Compared to transplanting, direct seeding provides more disease free seedlings, more flexibility in planting schedules and plant population and greater speed in planting as well as lower establishment cost. (38).

In Michigan 17,000 acres of asparagus (*Asparagus officinalis* L.) were harvested in 1974 producing 12,750 tons. This is an increase of 2,500 acres harvested over 1972. The market value of asparagus harvested in Michigan in 1974 was $8.6 million or greater than $500 an acre.

The conventional method of establishing a field of asparagus is with one or two year old crowns planted in the bottom of a seven to eight inch trench. However, planting crowns is a very expensive operation costing approximately $258 an acre (2). Establishing an asparagus field by direct seeding has presented one major problem. It takes from three to six weeks for the seedlings to emerge from cool, sandy soils (7). When asparagus seeds are planted in the bottom of a six inch v-shaped trench, erosion of the sides of the trench often occurs prior to seedling emergence and covers the seed with four to six inches of soil. The seeds may never emerge from this depth resulting in a poor stand which is uneconomical to maintain.

Asparagus spear diameter is affected by crown depth. If asparagus is direct seeded at .75 to 1.0 inch depth and not lowered, the average weight per spear is 40 to 50 percent less than the spear weight from asparagus established by crowns at the same time (29). This reduction is not desirable because small spear size reduces the value and quality of the crop. To solve this problem, Michigan State University has developed a Lister-transplanter (1) to lower direct seeded asparagus crowns to a desired depth. This machine lifts a ribbon of soil containing the crowns onto a conveyor belt. While the crowns are on the conveyor, a middle buster forms a trench nine to ten inches deep into which the ribbon of soil is lowered. Using the Lister-transplanter method for lowering crowns, the total cost of establishing an acre of asparagus is $174 (2). However, obtaining an adequate stand of field seedlings is still an important factor.

Early work by Borthwick (7) on asparagus germination showed that if seeds were soaked in water for 110 hours at 30°C, then germinated at room temperature, 64% of the seeds had germinated after two days while none of the non-soaked seeds had germinated. Asparagus seeds showed no signs of dormancy and would germinate readily after harvesting. Borthwick also showed that the optimum germination temperature for asparagus seeds was 86°F.

Komoti (17) showed that placing asparagus seeds in a 0 to 5°C cold storage for two months resulted in better germination than was obtained from seeds that were not cold treated. Germination was also improved by scarifing the seeds in concentrated H_2SO_4 for ten minutes. After four days the acid treated seeds obtained 84% germination while only 5% of the untreated seeds germinated. In contrast to Borthwick, Komoti reports that there was dormancy present in asparagus seeds just after harvest and that the dormancy was intensified by drying.

Growth regulators have been added to seeds to improve germination. Bradbeer et al. (8) showed that treating hazel nut seeds with gibberellic acid increased germination by substituting for stratification which is normally required for germination. In the case of hazel seeds abscisic acid was the chemical retarding germination. Treatment with gibberellic acid and/or stratification reduced the concentration of abscisic acid or gibberellic acid synthesis was increased. Ketring et al. (16) found that gibberellic acid treatment of peanut seeds increased the production of ethylene gas and increased germination. Toole et al. (37) reported that an ethylene treatment for peanut seeds increased germination; however, when ethylene was combined with carbon dioxide the best germination rate was obtained. Work done on tomato seeds (32) also showed an improvement in germination rate with the addition of ethylene and carbon dioxide. Lettuce (3) and rape (35) were some of the other crops which ethylene has increased germination.

'Hardening' has improved germination. It is a process of soaking the seeds for 24 hours then drying the seeds for 24 hours. This cycle may be repeated three or four times. Austin et al. (4) and Hegarty (9) reported that carrot seeds which were hardened had a higher rate of germination than did the untreated seeds. Hegarty also found that two of five cultivars of corn seed tested showed a significant improvement for hardened seeds compared to untreated seeds. Hegarty also reported that carrot seeds hardened in a solution of K_2HPO_4 instead of water improved in germination rate.

Inorganic salts have been used to improve seed germination. Oyer et al. (28) found that soaking tomato seeds in solutions of KNO_3 and

K_3PO_4 improved germination at suboptimal temperatures. Other work done with KNO_3(10) showed an increase in germination for pigweed, yellow foxtail, timothy and barnyardgrass. Work done on pollen (6,19) showed that pretreating the spores with calcium increased germination.

Water and polyethylene glycol 6000 have been used to increase germination. Bleak et al. (5) showed that emergence of several forage grasses was greatly improved by pretreatment for 30 to 60 hours in 16°C water. Heydecker et al. (11) reported that presoaking onion, carrot, beet and celery seeds with polyethylene glycol (PEG) 6000 greatly improved the rate of germination. In the case of onion, seeds pretreatment for 23 days in 29g of PEG 6000 per 100 ml of distilled water, the treated seeds germinated in 20 hours at 10°C compared to 9.3 days for untreated seeds. These results were obtained by allowing seeds to air dry for one hour after their removal from the PEG 6000 solution. However, if the same onion seeds were allowed to air dry fully then the pretreated seeds required three days to germinate which was 6.3 days sooner than non-soaked seeds. Heydecker et al. (11) feels that PEG 6000 places an osmotic suction on the seed which counteracts the suction of the imbibing seed. In other words, if the osmotic potential of the solution was too weak then the seeds would germinate in the solution; however, if the osmotic potential was too high then insufficient water is absorbed and the seed's metabolism is not stimulated.

One advantage for soaking seeds in PEG 6000 is that the molecule is very large and cannot pass into the plant cells (11,13,15,21,24,25,36). This permits prolonged pretreatment of seeds without chemical harm.

However, the larger PEG molecules such as 6000 and 20,000 contain ions of aluminum and magnesium which may be toxic to the plants (13,20,21). Janes (13) reported that pepper roots placed in a solution from one particular lot of PEG 4000 were brown and appeared flaccid after 24 hours. If the same solution were passed through a column of standard Bantam demineralizing resin, the roots grown in the solution showed no signs of injury after seven days. Studies have been conducted where no toxic effects develop when the plants are grown in unpurified solutions of PEG 6000 and PEG 20,000 (15,25,26). Mexal _et al._ (24) reported that osmotic potentials of -12 bars for PEG 4000 and -7 bars for PEG 6000 decreased the oxygen availability severely. This may be the source of some toxic effects when seeds are soaked in PEG 6000 and not the aluminum and magnesium ions (12).

Another problem is that the osmotic potential for polyethylene glycol changes inversely with temperature. Taking this into account Michel _et al._ (27) has developed an equation to calculate the osmotic potential. The equation is as follows:

$$\text{Osmotic potential} = -(1.18\times10^{-2})C-(1.18\times10^{-4})C^2+(2.67\times10^{-4})CT+(8.39\times10^{-7})C^2T$$

where,

C= concentration of PEG in g/kg water

T= temperature in degrees C

The changes in the osmotic potential as a function of temperature are speculated to be caused by hydrogen bonding between PEG and water, and as the temperature increases the hydrogen bonding is reduced producing a lower osmotic concentration.

Work done on pea seeds (22), grown in a -506 joules/kg solution of PEG 6000 showed a decrease in germination rate. Similar results (14) were obtained for lettuce, sunflower and citrus seeds grown in -1.1, -2.3 and -4.1 bars of PEG 6000 solution.

Even though very little work has been published dealing with pretreating asparagus seeds to improve germination rate, the work done with other seeds is enormous. However, most of the work has been in the area of dormancy breaking and light substitution. Still there are many ideas and treatments which have been tried on other crops which can be performed on asparagus to determine if the seeds will germinate faster. This thesis reports results of research conducted to achieve faster germination of asparagus seeds in cool soils.

MATERIALS AND METHODS

Section I

Chemical pretreatment of asparagus seeds

Asparagus officinalis L. (cv. Mary Washington) seeds were soaked in different chemical solutions for 24 hours. The four treatments were potassium nitrate (KNO_3), calcium chloride ($CaCl_2$), gibberellic acid (GA 4/7) and Alar. Seeds that were not soaked before germination were the control. After 24 hours of soaking, the seeds were removed and allowed to air dry for 24 hours. Fifty seeds from each treatment were placed in a 10-cm petri dish which contained one 9-cm Whatman #1 filter paper. Approximately five milliliters of distilled water was added to the seeds in each petri dish. The petri dishes were placed in a 68°F growth chamber and kept in total darkness. To assure an adequate water supply for the seeds, the dishes were checked daily. Each treatment was replicated five times. After 36 days the seeds were removed and fresh weight, shoot length and germination count were determined.

RESULTS AND DISCUSSION

Section I

Chemical pretreatment of asparagus seeds

Seedlings from asparagus seeds presoaked in $CaCl_2$ at the highest concentration (10^{-2}M) and seeds soaked in an intermediate concentration Alar (10^{-3}M) were greater in both fresh weight and shoot length than seedlings from seeds that were not pretreated (Table 1). Seedlings from seeds soaked in the highest concentration of Alar (10^{-2}M) showed a marked improvement over untreated seeds in average shoot length; however, there was no significant difference in fresh weight compared to seedlings from untreated seeds. The rest of the treatments were not significantly different from untreated seeds except seeds soaked in the lowest concentration of Alar (10^{-4}M) which showed a significant decline in fresh weight. Total germination was not significantly different even though it varied from a low of 22.4 for seeds soaked in 10^{-3}M of $CaCl_2$ to a high of 32.4 for seeds soaked in 10^{-2}M of $CaCl_2$.

Table 1. Effect of chemical pretreatment of asparagus seed for 24 hours on subsequent seedling development after 36 days in a petri dish (50 seed total per replication)

Chemical	Conc. (M)	Fresh Weight (gm)	Average Shoot Length (mm)	Number of Seeds Germinated
KNO_3	10^{-2}	0.38 ab*	12.1 a	25.8 a
KNO_3	10^{-3}	0.33 ab	10.8 a	22.6 a
$CaCl_2$	10^{-2}	0.92 d	31.2 c	32.4 a
$CaCl_2$	10^{-3}	0.29 ab	12.2 a	22.4 a
GA 4/7	10^{-3}	0.55 bc	14.5 a	27.8 a
GA 4/7	10^{-4}	0.35 ab	15.5 a	24.0 a
Alar	10^{-2}	0.77 cd	24.1 b	29.0 a
Alar	10^{-3}	0.93 d	26.0 bc	29.0 a
Alar	10^{-4}	0.21 a	8.4 a	28.0 a
Alar and GA 4/7	10^{-3} 10^{-4}	0.35 ab	11.4 a	26.2 a
Not Soaked (Control)		0.55 bc	14.3 a	30.6 a

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

MATERIALS AND METHODS

Section II and III

Polyethylene glycol pretreatment of asparagus seeds

Asparagus officinalis L. (cv. Mary Washington) seeds were presoaked in nine concentrations of polyethylene glycol (PEG) 6000. Flasks containing 150 ml of the PEG solution and 1000 seeds were aerated using compressed air passed through an aeration stone. The flow of air to each flask was kept constant by a clamp at the head of the line running to each flask. The air flow was adjusted so that small air bubbles could be seen saturating the solution. Water was added to flasks each day to replace evaporation loss. Solutions were thereby maintained at the desired concentration. In section II samples were taken periodically during the 30 days of pretreatment. At each sampling 60 seeds were removed from each of four replicated flasks. Of the 60 seeds removed 25 seeds were used in a germination test, 25 seeds were used in an emergence test and the final 10 seeds were used to determine percent moisture. Seeds remaining in the flasks were placed in a fresh solution of PEG 6000. The seeds used for the germination and emergence studies were rinsed in distilled water to remove the excess PEG 6000 and allowed to air dry for 24 hours before being planted. The seeds for the percent moisture determinations were also rinsed with water, blotted dry and placed in crucibles and weighed.

The seeds used for the emergence study were planted 1/2 inch deep in 2 parts loam and a 1 part sand soil mix. The soil was sterilized before being placed in a 20x14x3 inch flat in a 65°F greenhouse. No fertilizer was added to the soil. The flats were watered twice a week and counts were made on the emerged seedlings when the shoots were 1/2 inch tall. Counts were made twice weekly for three weeks. Fifty days after sowing the shoots were counted and cut at the soil surface for fresh and dry weight determinations. The shoots were placed in a 150°F oven for 48 hours, removed, cooled for 30 minutes and weighed to determine dry weight.

For the germination test petri dishes were covered with Parafilm with a 1 cm^2 hole cut in the Parafilm on one side of the dish (18). Two pieces of 7-cm diameter Whatman #2 filter paper were placed on top of the Parafilm with a small portion of the filter paper cut and placed through the hole to act as a wick. Twenty five ml of distilled water was placed in the petri dish and 25 seeds were placed on top of the filter paper and covered with the petri dish lid. This method allowed the seeds to be in contact with water but not submerged. The dishes were then placed in a 18°C incubator. Counts of the germinated seeds started seven days after the seeds were placed in the petri dish and continued two to three times a week until 17 of the 25 seeds had germinated or 33 days had passed whichever came first. Seeds were counted as germinated if the radicle was visible.

To determine percent moisture content (30), ten seeds per replication were placed in a crucible and weighed following their removal from the different PEG 6000 pretreatments. They were then placed in a 130°C oven for 60 minutes after which they were removed and allowed to air cool for 30 minutes before they were weighed.

In section III the PEG 6000 concentration giving the greatest increase in speed of seedling emergence was combined with the $CaCl_2$ presoaking described in section I. The asparagus seeds were presoaked in a 40 gram/100 ml solution of PEG 6000 utilizing the aeration system described previously. Ten days after initiation of the presoaking treatments, the PEG 6000 solution in one-half of the aerated flasks was replaced with fresh solution; the remaining flasks retained the original PEG 6000 solution. At 16, 19, and 22 days after the presoaking treatments were initiated, samples of 600 seeds from each flask were removed. Of the 600 seeds, 100 seeds were used for a germination test and an additional 100 seeds were used for an emergence test. The remaining 400 seeds were split into two lots and soaked for an additional day in two concentrations of $CaCl_2$ before being prepared for a germination and emergence test. Four hundred additional seeds (not presoaked in PEG 6000) were also split in two lots and soaked in the different concentrations of $CaCl_2$. Six replications were used per treatment. The experiment in section III was terminated after 45 days for the emergence test and after 21 days for the germination test.

RESULTS AND DISCUSSION

Section II

<u>Polyethylene glycol pretreatment of asparagus seeds</u>

There was no significant difference in total seedling emergence (Table 2) for any treatment through the 13th day. After sixteen days of soaking the seeds soaked in 20 and 40 grams of PEG 6000, solutions had a 67 and 73% seedling stand respectively, while the untreated seeds had a seedling stand of only 44%. Seeds soaked in a 40 gram solution of PEG 6000 had significantly greater percent seedling emergence than untreated seeds after 20, 23, 27 and 30 days of presoaking. Seeds soaked in a 35 gram solution of PEG 6000 for 20, 23 and 30 days also had significantly greater percent seedling emergence than the untreated seeds.

All seeds pretreated for 27 days required significantly less time for 50% of total seedling emergence (Table 3) than untreated seeds except seeds soaked in a 20 and 25 gram solution of PEG 6000. Seeds presoaked for sixteen days in a 40 gram solution of PEG 6000 were not significantly different than untreated seeds. However, it took only 23.4 days for 9.1 seedlings (50% of seedlings emerged) to emerge while the untreated seeds took 25.5 days for only 5.5 seedlings (50% of seedlings emerged) to emerge. Although the results were not significantly different between seeds soaked in a 40 gram solution of PEG 6000 for sixteen days and untreated seeds, it did take less time for more seedlings from the treated seeds to emerge. Seeds presoaked for sixteen days in a 40 gram solution of PEG 6000 solution required significantly less time for 50% of total petri dish germination than untreated seeds (Table 4).

Percent moisture in seeds soaked in concentrations of 25 grams to 40 grams of PEG 6000/100 ml water showed that the higher the concentration of PEG the lower the amount of water imbibed by the seeds over a period of time (Figure 1). Seeds soaked in a 5 gram/100 ml water solution of PEG 6000 imbibed water faster than seeds soaked in distilled water. It is possible that PEG 6000 at a low concentration has very limited hydrogen bonding of water to ether oxygens of PEG 6000. Since the osmotic pressure in the PEG 6000 solution is less than distilled water, the seed will imbibe water faster in a 5 gram/100 ml water solution of PEG 6000 because of the greater diffusion pressure deficit. However, as the concentration of PEG 6000 increases the disruption within the PEG molecule increases, in turn, increasing the amount of hydrogen bonding of water to the ether oxygens of PEG 6000 (27). Even though the diffusion pressure deficit is also increasing, less water is available to be imbibed by the seed.

The different pretreatment concentrations appeared to have a toxic effect develop (Table 2). Treatments underlined in Table 2 gave the best total emergence before a steady decline in total emergence occurred. If seeds presoaked in a 10 gram/100 ml water solution of PEG 6000 were used as the example, the best total emergence occurred after six days of pretreatment in PEG 6000 and steadily declined as the seeds remained in solution longer. It also appeared that as the solution concentration increased more days of presoaking were required before the toxic effect was induced. In the case of seeds presoaked for six days in a 10 gram solution of PEG 6000, the seeds internally probably reached a critical point in their pregermination status. If the seeds were soaked longer than six days, damage to the seed probably resulted when the seed was air dried. Seeds soaked in a 5 gram/100 ml water solution of PEG 6000 attained a 70% total emergence after three days of presoaking. Soaking the seeds in the 5 gram/100 ml water solution of PEG 6000 longer than three days showed a steady decline in total emergence whereas seeds soaked in distilled water required eight days of soaking before they reached maximum total emergence.

Asparagus seeds presoaked in 40 gram/100 ml water solution of PEG 6000 germinated in the petri dish much sooner than untreated seeds (Figure 2) and were significantly better than untreated seeds through the 12th day. For shoot emergence (Figure 3) the asparagus seeds presoaked in 40 gram/100 ml water solution of PEG 6000 emerged significantly better than untreated seeds for each day counted except the 20th day.

Seeds presoaked in distilled water, 5 gram, 10 gram and 15 gram/100 ml water solution of PEG 6000 germinated while presoaking. This was why there was missing data for these pretreatments in Tables 2, 3 and 4.

Table 2. Effect of polyethylene glycol (PEG) 6000 pretreatment of asparagus seeds on total percent seedling emergence after 50 days (25 seeds total per replication)

PEG Conc.	Percent Emerged							
	Days Soaked							
g/100ml H_2O	3	6	8	13	16	20	23	27
0	52 a*	51 bc	<u>69 a</u>	45 a	33 d			
5	<u>70 a</u>	63 abc	56 a					
10	65 a	<u>78 a</u>	59 a	55 a	49 bcd			
15	66 a	55 bc	59 a	<u>65 a</u>	64 abc	51 ab	42 b	48 b
20	66 a	75 ab	59 a	57 a	<u>67 ab</u>	50 ab	57 ab	43 b
25	66 a	54 bc	53 a	61 a	<u>64 abc</u>	57 ab	55 ab	51 b
30	65 a	69 abc	60 a	50 a	60 abc	42 b	<u>63 ab</u>	59 ab
35	71 a	50 c	67 a	57 a	62 abc	<u>67 a</u>	65 a	62 ab
40	63 a	68 abc	67 a	64 a	73 a	66 a	66 a	<u>74 a</u>
Not Soaked	57 a	59 abc	65 a	65 a	44 cd	40 b	48 b	44 b

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test)

Table 3. Effect of polyethylene glycol (PEG) 6000 pretreatment of asparagus seeds on time to 50% of total emergence (25 seeds total per replication)

PEG Conc.	Days to 50% of Total Emergence							
	Days Soaked							
g/100ml H_2O	3	6	8	13	16	20	23	27
0	22.4 a*	23.1 a	21.9 ab	24.4 a	29.6 a			
5	22.4 a	22.2 a	22.9 ab					
10	22.4 a	21.8 a	23.0 a	22.1 ab	26.0 bcd			
15	20.8 a	22.3 a	21 3 ab	22.8 abc	27.4 abc	24.8 abc	25.4 bc	22.5 b
20	22.5 a	22.8 a	22.7 ab	22.5 abc	27.4 abc	25.9 ab	28.0 ab	24.8 ab
25	22.4 a	21.8 a	23.5 a	24.3 a	28.5 ab	27.3 a	29.8 a	23.2 ab
30	22.5 a	22.5 a	23.3 a	21.5 bc	27.0 abc	24.9 abc	27.9 ab	22.3 b
35	21.6 a	21.8 a	22.3 ab	23.8 ab	26.0 bcd	25.0 abc	25.7 bc	22.4 b
40	21.9 a	20.8 a	20.4 b	20.7 c	23.4 d	21.8 c	24.4 c	21.4 b
Not Soaked	21.6 a	21.8 a	20.9 ab	20.8 c	25.5 cd	23.5 bc	25.8 bc	25.9 a

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 4. Effect of polyethylene glycol (PEG) 6000 pretreatment of asparagus seeds on time to 50% of total germination (25 seeds total per replication)

PEG Conc.	Days to 50% of Total Germination					
	Days Soaked					
g/100ml H_2O	13	16	20	23	27	30
0	13.3 a*	13.6 abc				
10	12.1 a	15.6 ab				
15	11.3 ab	15.8 a	14.2 ab	12.6 a	10.7 bc	
20	13.3 a	12.8 c	11.7 bc	12.3 a	13.2 ab	10.7 a
25	11.2 ab	12.5 c	14.9 a	13.2 a	13.6 a	10.0 a
30	11.2 ab	13.1 bc	14.3 ab	11.0 a	12.5 abc	10.5 a
35	11.7 ab	14.9 abc	12.6 abc	12.2 a	11.7 abc	10.7 a
40	10.0 b	9.6 d	10.3 c	11.1 a	10.1 c	9.7 a
Not Soaked	11.1 ab	13.0 bc	11.6 bc	10.3 a	11.3 abc	9.9 a

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

Figure 1. Effect of pretreating asparagus seeds in polyethylene glycol (PEG) 6000 on percent moisture within the seed (10 seeds total per replication)

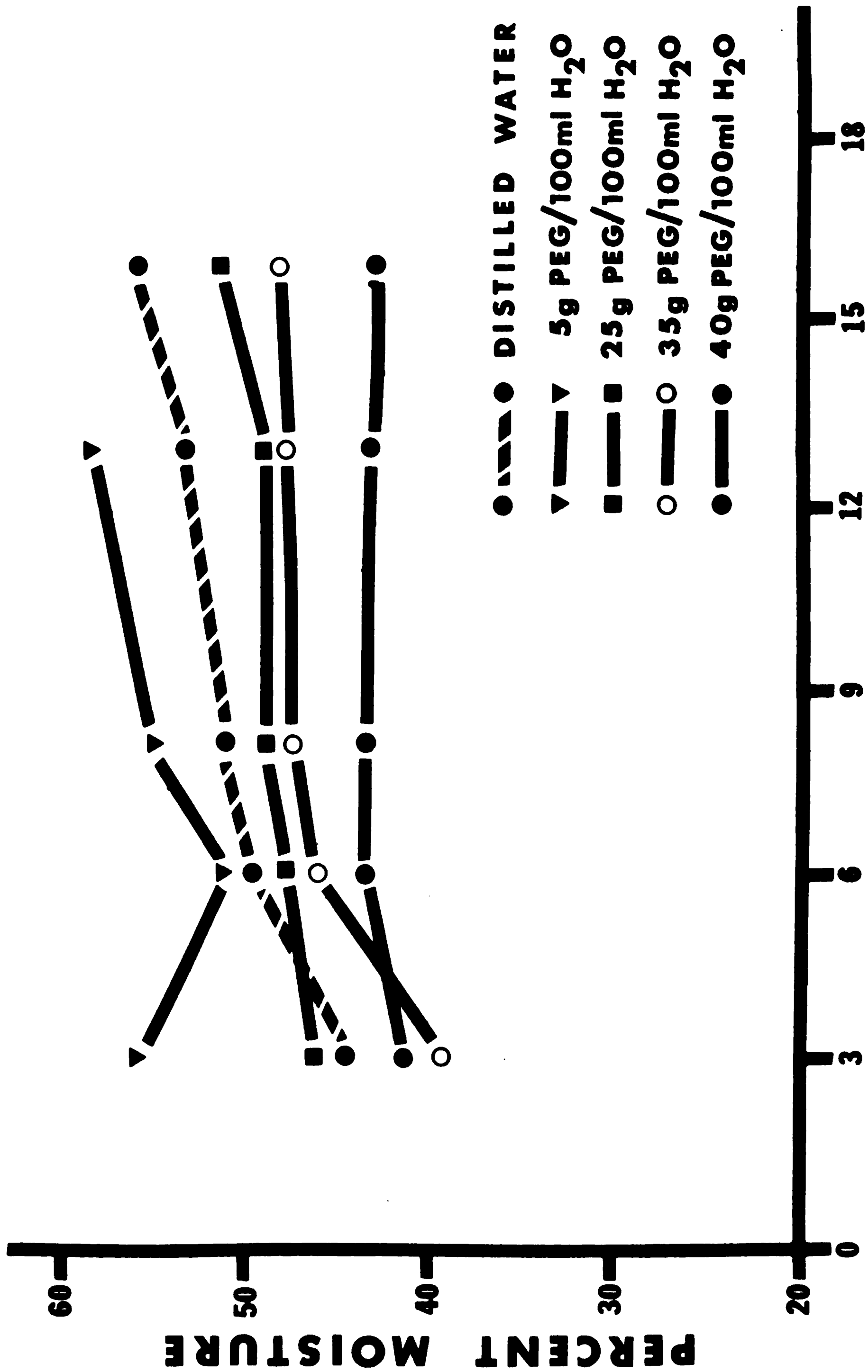
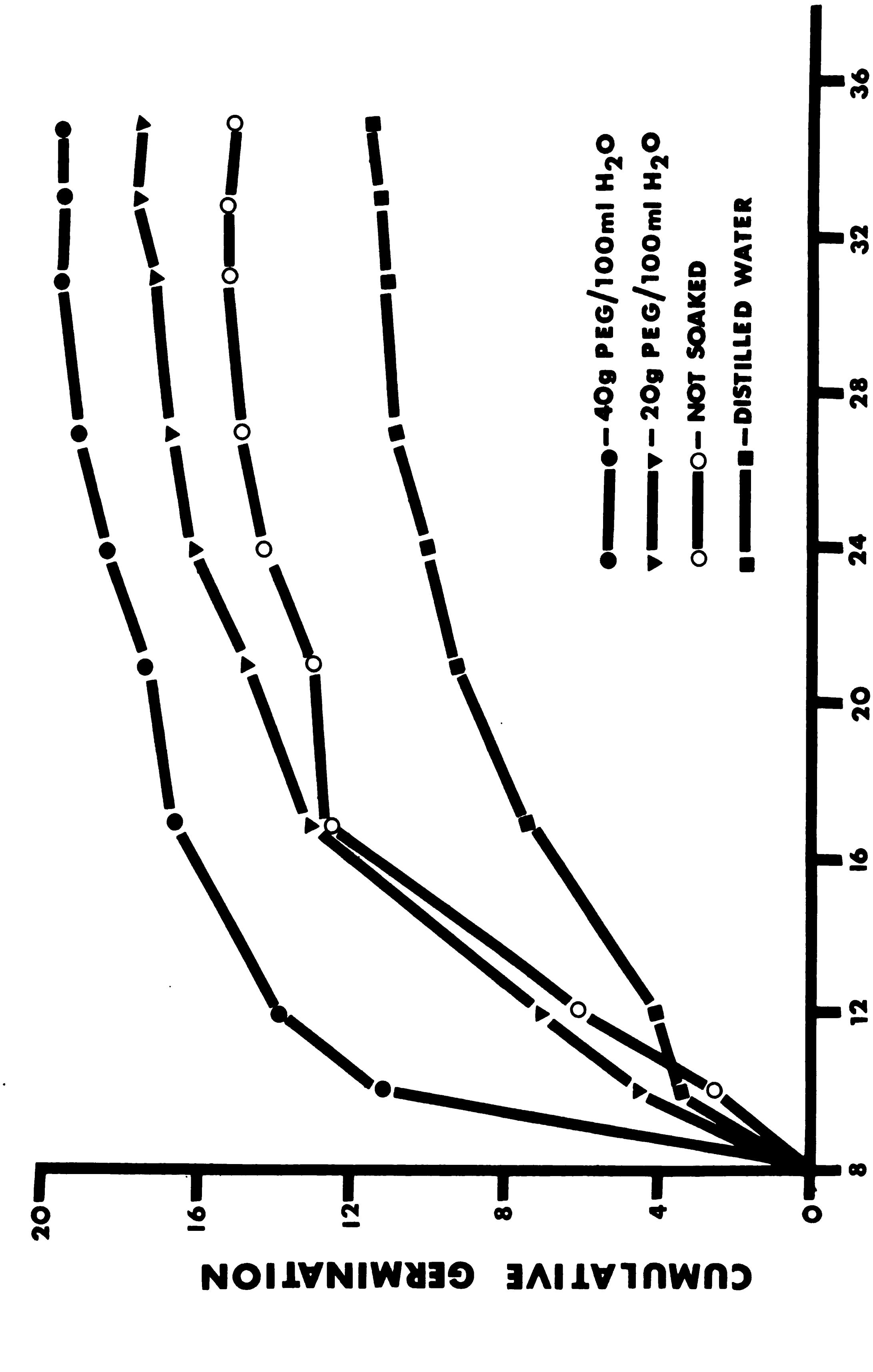
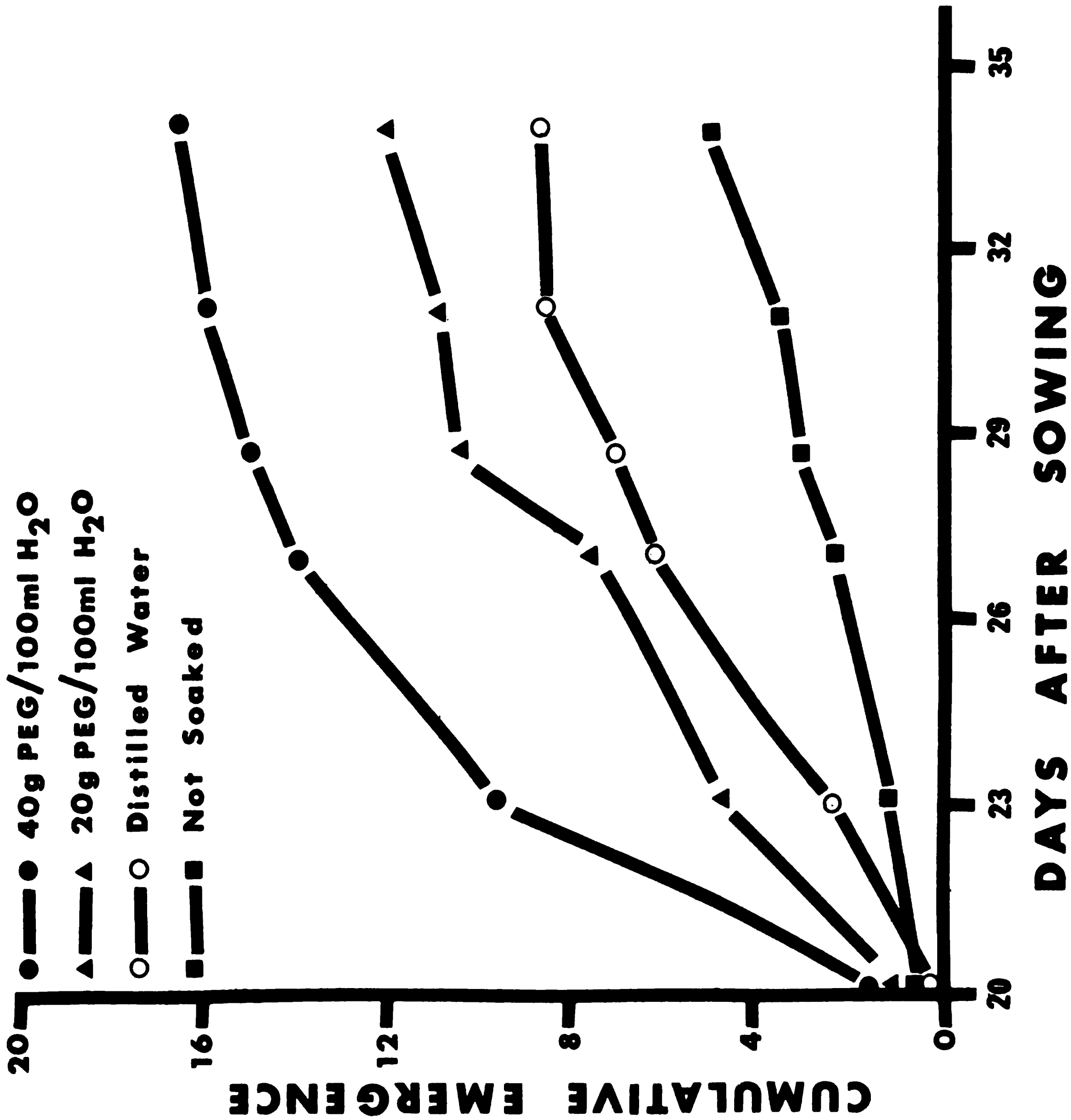

DISTILLED WATER
5g PEG/100ml H_2O
25g PEG/100ml H_2O
35g PEG/100ml H_2O
40g PEG/100ml H_2O
DAYS SOAKED
0
3
6
9
12
15
18
PERCENT MOISTURE
20
30
40
50
60

Figure 2. Effect of polyethylene glycol (PEG) 6000 pretreatment for sixteen days on asparagus seed germination (25 seeds total per replication)



CUMULATIVE GERMINATION
0
4
8
12
16
20
DAYS AFTER PREGERMINATION TREATMENT
8
12
16
20
24
28
32
36
40g PEG/100ml H_2O
20g PEG/100ml H_2O
NOT SOAKED
DISTILLED WATER

Figure 3. Effect of polyethylene glycol (PEG) 6000 pretreatment for sixteen days on asparagus seedling emergence (25 seeds total per replication)


40g PEG/100ml H_2O
20g PEG/100ml H_2O
Distilled Water
Not Soaked
CUMULATIVE EMERGENCE
0
4
8
12
16
20
20
23
26
29
32
35
DAYS AFTER SOWING

RESULTS AND DISCUSSION

Section III

PEG 6000 and $CaCl_2$ pretreatment of asparagus seeds

There was no interaction between seeds soaked in the same solution and seeds soaked in the changed solution. Seeds treated in a 40 gram/ 100 ml water solution of PEG 6000 for sixteen days significantly improved germination and emergence (Tables 5, 6, 7 and Figure 4) compared to untreated seeds. However, when seeds pretreated in PEG 6000 for sixteen days had an additional day of pretreatment in a $CaCl_2$ solution, seedling emergence seventeen days after sowing was enhanced significantly compared to seeds treated in PEG. However, there were no significant differences for total germination and total emergence for seeds presoaked in PEG 6000 for sixteen days compared to seeds soaked in PEG 6000 for sixteen days followed by one day soaking in $CaCl_2$ (Tables 6 and 7).

Seeds soaked in PEG 6000 for 19 and 22 days and $CaCl_2$ showed a slight decline in beneficial results when compared to sixteen days of presoaking (Tables 5 and 6). There was no doubt that soaking in PEG 6000 for sixteen days plus one day in a $CaCl_2$ solution is the best pretreatment.

Table 5. Effect of polyethylene glycol (PEG) 6000 and calcium chloride pretreatment of asparagus seeds on the number seedlings emerged after seventeen days from sowing (100 seeds total per replication)

PEG 6000	$CaCl_2$	Number Emerged		
		Days Soaked		
40g/100ml H_2O	(M)	16	19	22
PEG	$1x10^{-2}$	44.0 c*	31.6 c	39.8 c
PEG	$5x10^{-2}$	36.7 c	29.1 c	29.7 b
PEG	-----	27.9 b	18.3 b	29.7 b
---	$1x10^{-2}$	3.5 a	3.8 a	11.2 a
---	$5x10^{-2}$	4.0 a	6.2 a	15.8 a
Not Soaked		1.0 a	1.8 a	8.7 a

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 6. Effect of polyethylene glycol (PEG) 6000 and calcium chloride pretreatment of asparagus seeds on total percent emergence after 45 days from sowing (100 seeds total per replication)

PEG 6000	$CaCl_2$	Percent Emerged		
		Days Soaked		
40g/100ml H_2O	(M)	16	19	22
PEG	$1x10^{-2}$	79.9 c*	68.7 cd	62.5 b
PEG	$5x10^{-2}$	72.5 c	72.6 d	53.1 ab
PEG	-----	73.6 c	58.2 bc	52.3 ab
---	$1x10^{-2}$	43.5 b	52.8 ab	54.7 b
---	$5x10^{-2}$	45.7 b	60.0 bc	56.3 b
Not Soaked		27.3 a	46.5 a	42.5 a

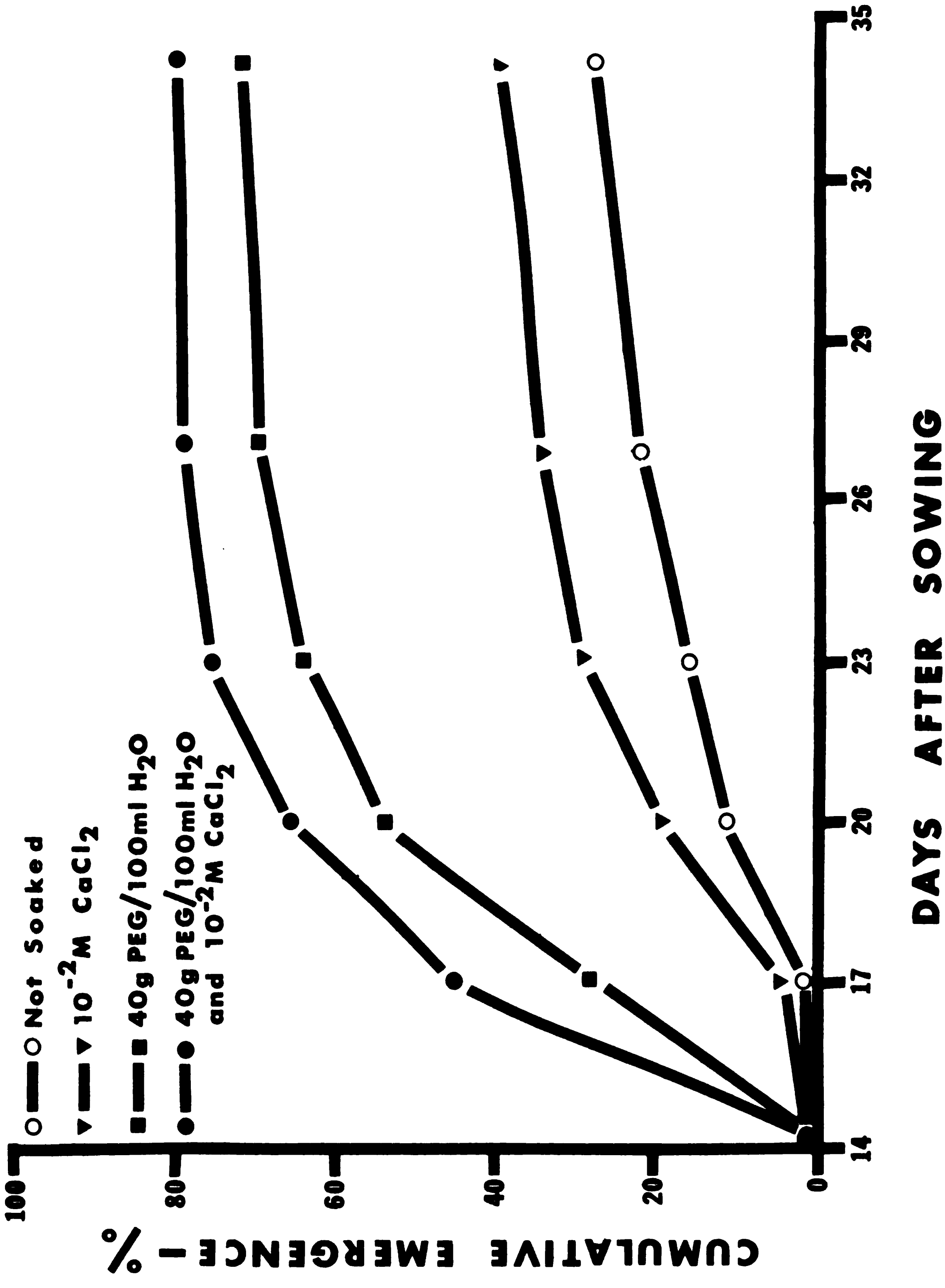
*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 7. Effect of polyethylene glycol (PEG) 6000 and calcium chloride pretreatment of asparagus seeds on the total percent germination after 21 days in a petri dish (100 seeds total per replication)

PEG 6000	$CaCl_2$	Percent Germinated		
		Days Soaked		
40g/100ml H_2O	(M)	16	19	22
PEG	$1x10^{-2}$	84.3 b*	79.3 a	83.0 a
PEG	$5x10^{-2}$	79.7 b	79.2 a	81.6 a
PEG	-----	80.5 b	70.5 a	75.0 a
---	$1x10^{-2}$	66.7 a	75.3 a	73.3 a
---	$5x10^{-2}$	66.3 a	75.2 a	74.2 a
Not Soaked		61.0 a	78.7 a	70.8 a

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

Figure 4. Effect of pretreating asparagus seeds in 40g of polyethylene glycol (PEG) 6000 per 100ml of water for sixteen days and/or one day of soaking in calcium chloride on the emergence rate (100 seeds total per replication)


Not Soaked
10^{-2}M $CaCl_2$
40g PEG/100ml H_2O
40g PEG/100ml H_2O and 10^{-2}M $CaCl_2$
CUMULATIVE EMERGENCE – %
100
80
60
40
20
0
14
17
20
23
26
29
32
35
DAYS AFTER SOWING

SUMMARY AND CONCLUSION

Total seedling emergence was greatly enhanced by presoaking in a 40 gram/100 ml water solution of PEG 6000. Heydecker et al. (11) theorized that pretreatment in PEG 6000 allows the 'slow' and 'fast' seeds to reach the same stage of preparedness for germination. With asparagus seeds it is possible that there is this difference within the seed population. Some groups of seeds could contain more of a population of 'slow' seeds than 'fast' seeds causing poor emergence. Experiments in sections II and III showed a large variability in percentage of total emergence for untreated seeds. Poor uniformity for direct seeded asparagus would also cause a problem in the field. Seeds treated (Tables 5 and 6) with PEG 6000 and $CaCl_2$ improve chances for a suitable stand of seedlings by allowing the seedlings to emerge faster in cool soils, reducing the time the seed stays dormant in the soil.

PEG 6000 has an interesting effect on water uptake of the seed. Shull (33) found cocklebur seeds had an osmotic potential greater than 900 atmospheres. If water is imbibed by the seed as Meyer and Anderson (23) postulate, then asparagus seeds soaked in higher concentrations of PEG 6000 should absorb more water than seeds soaked in distilled water. However, this is not the case with PEG 6000. As the concentration increases, PEG 6000 increases its affinity for water (27). If a concentration of PEG 6000 is selected as the 40 gram/100 ml water solution which was used on asparagus seeds, then less water is imbibed by the seed compared to imbibition in distilled water (Figure 1). This decrease in water imbibition allows the seed to advance its pregermination metabolism part way to germination but because of the reduced water uptake the seed will not germinate in the solution.

Apparently the seed does not have to completely germinate for it to be damaged by either presoaking or drying (Table 2). In this case drying the seed is the damaging effect. The underlined numbers show the best total emergence in the particular solutions. After this soaking time the total percent emergence drops off steadily. It is possible that presoaking the seed longer advances the seed beyond a desirable stage of pregermination and air drying the seed for 24 hours damages it. High concentration of PEG 6000 solution increases the soaking duration necessary to induce seed damage by air drying.

With asparagus seeds it is possible that Ca^{+2} is bound by pectins in the middle lamella (6). It also could trigger the metabolism of carbohydrates such as cellulose, callose and pectin as it does in pollen germination, in turn possibly benefiting asparagus seed germination.

The results obtained by working with PEG 6000 are very exciting. By pretreating asparagus seed for sixteen days in a 40 gram/100 ml water solution of PEG 6000 and one day in a $1x10^{-2}M$ solution of $CaCl_2$ more direct seeding of asparagus beds could be used at a savings to the grower who now plants crowns. This is just the beginning of the experiments that could be performed using PEG 6000. One area could be pretreating seeds with a PEG 6000 solution and a nutrient mixture instead of water. Another area would be PEG 6000 treating of asparagus seeds with a higher concentration of the chemical, and finally PEG 6000 treating of seeds followed by soaking in different chemical solutions. This work shows much promise and should definitely be continued.

LIST OF REFERENCES

LIST OF REFERENCES

1. Anonymous. 1973. Progress report of asparagus research. Research Report 217. Michigan State Univ. Agric. Expr. Station.

2. Anonymous. 1974. Report of asparagus research. Agric. Engr. Dept. Michigan State University.

3. Abeles, F. B. and J. Lonski. 1969. Stimulation of lettuce seed germination by ethylene. Plant Physiol. 44:277-280.

4. Austin, R. B., P. C. Longden, and J. Hutchinson. 1969. Some effects of 'hardening' carrot seeds. Ann. Bot. 33:883-895.

5. Bleak, A. T. and W. Keller. 1972. Germination and emergence of selected forage species following preplanting seed treatments. Crop Sci. 21:9-13.

6. Brewbaker, J. L. and B. H. Kwack. 1963. The essential role of calcium ions in pollen germination and pollen tube growth. Am. J. Bot. 50:859-865.

7. Borthwick, H. A. 1925. Factors influencing the rate of germination of the seed of Asparagus officinalis. Calif. Tech. Paper 18.

8. Bradbeer, J. W. and N. F. Pinfield. 1967. Studies in seed dormancy. III. The effects of gibberellin on dormant seeds of Corylus avellana L. New Phytol. 66:515-523.

9. Hegarty, T. W. 1970. The possibility of increasing field establishment by seed hardening. Hort. Res. 10:59-64.

10. Hendricks, S. B. and R. B. Taylorson. 1974. Promotion of seed germination by nitraten nitrite, hydroxylamine, and ammonium salts. Plant Physiol. 54:304-309.

11. Heydecker, W., J. Higgins, and R. Gulliver. 1973. Bringing seeds to the brink of germination. The Grower. 81:1134-1135.

12. Janes, B. E. 1966. Adjustment mechanisms of plants subjected to varied osmotic pressures of nutrient solution. Soil Science. 101:180-188.

13. Janes, B. E. 1974. The effect of molecular size, concentration in nutrient solution, and exposure time on the amount and distribution of polyethylene glycol in pepper plants. Plant Physiol. 54:226-230.

14. Kaufmann, M. R. 1969. Effects of water potential on germination of lettuce, sunflower, and citrus seeds. Can. J. Bot. 47:1761-1764.

15. Kaul, R. 1966. Relative growth rates of spring wheat, oats, and barley under polyethylene glycol-induced water stress. Can. J. Plant Science. 47:611-617.

16. Ketring, D. L. and P.W. morgan. 1971. Physiology of oil seeds. II. Dormancy release in virginia-type peanut seeds by growth regulators. Plant Physiol. 47:488-492.

17. Komoti, S. 1958. studies on temperature treatments of seeds. II. Dormancy and germinating temperature in garden asparagus seeds. Hakkaido Nogyo Shikenjo Res. Bul. of the Nat. Agric. Expr. Station. 73:9-19.

18. Knudson, L. L. and T. W. Tibbitts. 1973. Wick-action method for germination of seeds. Hort. Sci. 8:472.

19. Kwack, B. H. 1964. The effect of calcium on pollen germination. Proc. Amer. Soc. Hort. Sci. 86:818-823.

20. Lagerwerff, G. V., G. Ogata, and H. E. Eagle. 1961. Control of osmotic pressure of culture solutions with polyethylene glycol. Science. 133:1486-1487.

21. Lawlor, D. W. 1970. Absorption of polyethylene glycols by plants and their effect on plant growth. New Phytol. 69:501-513.

22. Manohar, M. S. 1966. Effect of "osmotic" systems on germination of peas (Pisum sativum L.). Planta. 71:81-86.

23. Meyer B. S. and D. B. Anderson. 1952. Plant Physiology. D. Van Nostrand Company, Inc. New York.

24. Mexal, J., J. T. Fisher, J. Osteryoung, and C. P. P. Reid. 1975. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. Plant Physiol. 55:20-24.

25. Mexal, J. and C. P. P. Reid. 1973. The growth of selected mycorrhizal fungi in response to induced water stress. Can. J. Bot. 51:1579-1588.

26. Michel, B. E. 1970. Carbowax 6000 compared with mannitol as a suppressant of cucumber hypocotyl elongation. Plant Physiol. 45:507-509.

27. Michel, B. E. and M. R. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51:914-916.

28. Oyer, E.B. and D. E. Koehler. 1966. A method of treating tomato seeds to hasten germination and emergence at sub-optimal temperatures. Proc XVII. Intern. Hort. Congr. I. p. 626.

29. Putnam, A. R. 1972. Efficany of a zero-tillage cultural system for asparagus produced from seeds and crowns. J. Amer. Soc. Hort. Sci. 97:621-624.

30. Roberts, E. H. and D. L. Roberts. 1972. Moisture content of seeds. In: Viability of Seeds. (E. H. Roberts). Syracuse Univ. Press. p. 424-429.

31. Robbins, W. W. and H. A. Borthwick. 1925. Development of the seed of *Asparagus officinalis*. Bot. Gaz. 80:426-438.

32. Sanok, W. J. 1972. The influence of carbon dioxide and ethylene preplant seed treatment on respiration and germination of tomato at low temperatures. M. S. Thesis. Michigan State University.

33. Shull, C. A. 1916. Measurement of the surface forces in soils. Bot. Gaz. 62:1-31.

34. Takatori, F. H., J. I. Stillman, and B. Power. 1968. Direct seeding of asparagus. Calif. Agric. 22:8-10.

35. Takayanagi, K. and J. F. Harrington. 1971. Enhancement of germination rate of aged seeds by ethylene. Plant Physiol. 47:521-524.

36. Tarkow, H., W. C. Feist, and C. F. Southerland. 1966. Interaction of wood with polymeric materials. Penetration versus molecular size. Forest Prod. J. 16:61-65.

37. Toole, V. K., W. K. Bailey, and E. H. Toole. 1964. Factors influencing dormancy of peanut seeds. Plant Physiol. 39:822-832.

38. Wilcox, G. E. and P. E. Johnson. 1970. Direct seeding tomatoes for mechanical harvest. Research Progress Report 366. Purdue Univ. Agric. Expr. Station.

APPENDIX

Table 8. Effect of cold treatment of asparagus seeds on subsequent seedling development after 36 days in a petri dish (50 seeds total per replication)

Cold Treatment (8°F) (hours)	Fresh Weight (grams)	Average Shoot Length (mm)	Number of Seeds Germinated
0	0.110	5.1	22.8
1	0.134	5.2	25.6
2	0.089	4.8	26.8
3	0.224	5.1	23.0
4	0.057	3.7	19.2

There are no significant differences

Table 9. Effect of cold storage on asparagus seeds later soaked in gibberellic acid 4/7 on subsequent seedling development after 40 days in a petri dish (50 seeds total per replication).

GA 4/7 Concentration (M)	Fresh Weight (grams)		Average Shoot Length (mm)		Number of Seeds germinated	
	Control	Cold Storage	Control	Cold Storage	Control	Cold Storage
10^{-3}	0.51 ab*	0.71 a	8.0 ab	15.9 d	32.4 a	32.0 a
10^{-4}	0.42 b	0.47 b	7.5 ab	12.1 bc	30.2 a	29.6 a
10^{-5}	0.52 ab	0.40 b	9.3 a	9.1 abc	33.5 a	29.6 a
Not Soaked	0.59 ab	0.39 b	8.2 ab	5.7 ab	38.4 a	36.0 a

*Means within columns followed by common letters are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 10. Effect of Ethephon pretreatment of asparagus seeds on subsequent seedling development after 40 days in a petri dish (50 seed total per replication)

Ethephon (ppm)	Fresh Weight (gm)	Average Shoot Length (mm)	Number of Seeds Germinated
1000	0.016	2.6	15.2
750	0.010	2.1	13.4
500	0.036	1.0	14.8
250	0.110	5.1	13.4
Not Soaked	0.110	5.1	22.8

Table 11. Effect of concentrated H_2SO_4 scarification of asparagus seeds on germination (33 seeds per replication)

Time in H_2SO_4	Percent Germination	
	Days in Petri Dishes	
(min)	11	28
5	0.1	0.3
10	0.0	0.0
15	0.0	0.3
Not Soaked	12.7	20.0