

STORAGE OF PREGERMINATED
SNAPDRAGON (ANTIRRHINUM MAJUS) SEED
IN THREE HYDROGELS

by

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INTRODUCTION

As a greenhouse cut-flower and bedding plant, Antirrhinum Majus (snapdragon) possesses one increasingly important and desirable characteristic: a low temperature requirement (15.5 C) for optimal growth and development (40). This fuel-efficient crop could be improved if the germination temperature requirement of 22 C for five to twelve days (13) were eliminated.

British scientists have developed a technique of field seedling establishment that has potential for the greenhouse snapdragon grower. The technique consists of pregerminating the seed and sowing it via a viscous liquid carrier to prevent damaging the emerged radicle. A greenhouse germination temperature is thus eliminated and the grower could benefit from other advantages associated with "fluid drilling."

A fundamental objective of seed treatment studies has been to improve seedling performance. Such an improvement can be measured through a number of responses: earlier time to emergence, greater uniformity of emergence, a reduction in total time to harvest, increased percent emergence and an increase in total yield (33).

As a seed treatment, fluid drilling has been shown to favor the above-mentioned responses. However, the problem of storage of pregerminated seed has not been addressed. Once the seed has germinated, it is committed to growing and efforts at suspending that growth through cold storage have not been made. Currently, once the seed is germinated, it is blended into the gel and sown. Storage is not recommended. However, delays in sowing the prepared seed could occur, thus the reason for this study.

The objectives of this study were:

1. To determine the feasibility of storing pregerminated seed in the fluid sowing medium.
2. To evaluate the effect of three storage gels, two storage temperatures and two storage durations on seedling vigor.
3. To determine if oxygen was a limiting factor in holding pregerminated seed in gel.

REVIEW OF LITERATURE

The Technique

Fluid drilling is a new technique of seed sowing that has potential for application to a large number of crops, including greenhouse floral crops. Growers who want to keep abreast of this new technique of seedling establishment may find storage of pregerminated seed an added benefit to a method which has proven to be commercially useful in England.

Many techniques aimed at improving seedling establishment have been documented. Carlson (6) stated that direct seeding is a highly desirable means of seedling establishment but precise standards for maximum germination are lacking. Zink (65) attempted to reduce one of the major limiting factors in seedling production (labor involved in thinning and transplanting) by using pelleted lettuce seed. He found that pelleted seed lowered germination percent and emergence but average spacing between plants after thinning was similar for pelleted and non-pelleted seed. Pelleting therefore, resulted in a reduction of time needed for thinning. Furthermore, precision planting was noted as being possible through the use of pelleted seed. In addition, it was noted that nutrients and seed protectants could be incorporated into the pellet, thus further improving seedling performance (33). However, improved rate of germination did not result from this method of seed treatment.

Punch planting as an alternate means of sowing lettuce seed under adverse conditions showed increased germination percentage when compared to the conventional method of seed sowing (7). Chancellor (8) explored a

seed taping system on small seed crops as a possible way of achieving precision planting, increasing crop population and reducing labor through reduction in thinning. For the system to be economically useful, he established a goal of 80% emergence but was unable to obtain greater than 58% while using seed of 92% germination. Further, it was found that the tape planting technique gave less reliable seedling establishment than the conventional dry sowing method (15).

Smith et al. (51) looked for a relationship of seed size to seedling growth and vigor through the technique of seed grading. It was found that fractioning a seed lot by weight resulted in greater crop population uniformity. However, such a means of achieving crop uniformity was discounted as economically unfeasible.

A seed sheet technique has been used in England with some success in precision planting and crop uniformity (6). This method was much like Chancellor's tape system (8) with the exception that the sheets were prepared in advance to sowing and stored; while the taping method involved placing the seed on the tape as it was being sown. This practice was found to be unacceptable to the American grower because of the high cost of preparing and inventorying the seed sheets.

A mechanical vacuum seeder which automatically plants individual dry seeds in greenhouse flats is being used by some of the bedding plant growers in the industry (1). Problems with this method are related to seed size variability within seed lots and between species. In addition, the grower must have a high enough volume of production to justify the large capital expenditure required.

Another seed sowing technique which has been investigated is plug-mix (24, 28). The method involves planting ungerminated seed in moist plugs

of Cornell Peat-Lite mix. Although results were generally favorable, some plantings suffered from lack of uniformity in emergence due to fluctuating temperatures and moisture content in the seed zone (60). Weed competition was also considered a negative aspect of this method of seedling establishment. However, some of the problems associated with plug-mixing were overcome by incorporating pregerminated seed in the plug (5, 46, 60).

The concept of sowing pregerminated seed originated in England in 1966 (18), while the Weed Research Station was making efforts to revive killed pasture. Originally, the pregerminated seed was sown via water, but later it was found that a solution of higher viscosity was a better carrier and protector of the fragile radicle. Both in England and in the United States, fluid drilling (as the technique became known) experiments have focused exclusively on vegetable crops.

Celery (2, 47, 48), lettuce (15, 21, 23, 25), parsnip (22), carrots (49), onion (38) tomatoes (26, 44, 46) green pepper (39, 56) and beets (39) are among the crops that have been investigated. All of these crops have shown some positive responses to the fluid drilling technique. Such improvement has been shown through comparative studies of this method to some of those methods of seedling establishment mentioned earlier.

Celery yield increased as a result of earlier and higher percent emergence of pregerminated seed compared to dry sown seed (2, 16). Earlier emergence and maturity, as well as higher final percent emergence of lettuce seed was found when comparing pregerminated fluid sown seed to graded and ungraded dry seed (22). Variability in emergence of pregerminated seed was also found to be reduced in comparison to seed pelleting (23).

Studies on sugar beets, a crop known for its lack of uniformity in emergence, yielded conflicting results (39). While Longden was able to see an improvement in uniformity of emergence in both field and laboratory studies, he was unable to obtain consistently higher and earlier yields for the entire 230 days growing season in the field (39). Another crop which was used to compare fluid drilling to dry sowing was onion. A 21 day difference in emergence was observed between the two methods (83). This earlier emergence of fluid-drilled seed resulted in an ultimate 37.5 percent increase in bulb diameter at harvest.

A comparison of the fluid drilling technique to osmotica conditioning, a pre-sowing seed treatment known to speed up germination (33), showed that the former was an improvement over the latter in terms of emergence rate (22). A reduction in spread of emergence was also reported.

In comparing fluid drilling to various other techniques of seedling establishment, the most consistent effects of this new concept have been earlier emergence, higher percent emergence and greater uniformity of crop population (3, 5, 21, 46, 48). In England, a National Vegetable Research Station review of investigations in that country, confirmed these general advantages for six crops (49). In the United States, Taylor (56) reported similar results for six vegetable crops.

The Crop

Antirrhinum majus (snapdragon) is one of the main crops grown from seed by the greenhouse grower (18). High labor and fuel costs, special facilities needed for seed germination and lack of uniformity in seedling yield are adverse factors of this method of propagation (20).

In order for the grower to obtain the expected 40% transplant population from a trade packet of 2000 seeds, good germinating facilities

are necessary (1). This consists of an environment with an average temperature of 25 C, lights, and high humidity (13). The latter may be provided by either an intermittent misting system (27) or by sealing pre-sown flats in plastic sleeves (1). Either method is costly and seeds typically take one to two weeks to germinate (13).

Once the seed germinates and the seedling has a set of true leaves, it is transplanted by hand directly to the bench or into "Jiffy-pots". Quality of the end product is affected by the method chosen, but cost is a consideration (35). Direct benching produces higher quality plants, but is not practiced as widely as it takes an additional month of production space. By using the "Jiffy-pot" intermediate technique, many year-round growers are able to get in an additional crop (63).

Another quality-related factor a grower of snapdragons must consider is single spiked vs. multiple spiked plants (20). The former practice yields higher quality flowers, but requires more seedlings for a given number of spikes, thus increasing the cost of production. Regardless of the method of production chosen, cost is high and will continue to increase as fuel and labor costs increase. Seedling establishment techniques which will increase rate of germination, percent emergence and reduce fuel and labor costs, are much needed by the floriculture industry (6).

Storage

The aspect of pregerminated seed storage has not been documented as most of the research to date has concentrated on planting the seed immediately following germination. References to storage of seed in the pregerminated condition are few, vague and inconclusive.

The storage of pregerminated seed in humid air at 1 C and 5 C for periods of up to seven days has been reported (49). This report also indicated that a number of species can be held at 1 C in a tank of aerated water with negligible root growth or loss of viability. Fluid Drilling Ltd. sales literature described a system for storing pregerminated seed under cold storage at 0 C to 1 C in water under continual aeration (19).

Pregerminated tomato seed held at 5 C and 10 C for up to eight days was not injured, although holding the seed at 10 C for longer than eight days resulted in hypocotyl growth (44). (The method of storage was not mentioned.) It was further found that exposure of pregerminated seed to four, eight, and twelve days at 0 C was progressively more injurious. Irwin and Price (30) determined that pregerminated pepper seed held at 5 C for up to 12 days were uninjured, but 0 C caused injury after a short exposure. Pepper seed held at 0 C in polyethylene bags for four days were found to emerge more slowly than freshly germinated seed. Furthermore a slight amount of low temperature injury resulted from low temperature storage, but when the chilled seed was removed to more favorable conditions the injury appeared to be reversible. The effects of cold temperature on live tissue, specifically root tissue, is well documented.

Christiansen (10) reported a reduction of root growth of cotton seedlings immediately following chilling, but indicated that long term or cumulative effects of chilling on cotton seedlings had not been proven. Chilling during early seedling growth after initiation of germination in cotton resulted in root cortex damage as evidenced by necrotic zones (11).

Creencia and Bramlage (14) noted that visible chilling injury on corn seedlings was preceded by a rise in oxygen uptake, but this response was reversible on returning the damaged seedling to a more favorable temperature

within 36 hours. However, they found that some seedlings were unable to recover from the chilling injury after being exposed for 48-60 hours. Their findings of a reduction in initial rate of growth were later confirmed (30).

A study of the influence of chilling on electrolyte permeability, oxygen uptake and 2,4 dinitrophenol in leaf discs revealed that the longer period of chilling resulted in strongly increased electrolyte permeability, decreased oxygen uptake and a disappearance of the uncoupling effect of 2,4 dinitrophenol. In general, these effects were reversible within four days of chilling upon returning to 25 C (55).

Christiansen et al. (12) found that the loss of organic substances from cotton radicles was increased by chilling, anaerobiosis or acidic conditions. This loss was found to decrease upon correction of the injurious conditions and by the introduction of divalent cations (Mg^{++} , Ca^{++}). The site of injury was found to be near the root tip based on the location of greatest leakage of ninhydrin-reactive compounds onto filter paper on which injured seedlings had been kept for 24 hours.

The effect of anaerobic conditions on root growth of cuttings standing in water was studied by Zimmerman (64). He found that aerating the water with oxygen increased the rate of root growth. The use of hydrogen peroxide and potassium permanganate as oxygen-generating agents was found effective. Tinga (58) determined that decreasing the level of oxygen below that of normal air progressively decreased root initiation and growth of carnations. Similar results were found in aeration studies on roses. A linear relationship between oxygen availability ranging from 21% to 1% and shoot growth was found (50). A 1% oxygen level in the root environment caused death, while 5% and higher quantities of oxygen resulted in increases in root dry weight.

MATERIALS AND METHODS

General

Seeds of Antirrhinum majus F₁ hybrid Yellow 'W. Virginia' of response groups 1, 2 and 3 were obtained from the A. H. Hummert Seed Company and germinated in glass columns of distilled water under continual aeration. Incoming air was filtered and humidified by sending the air through an aquarium air stone placed in 250 mls of water in a 500 ml Erlenmeyer flask. The air was then delivered to the columns of water through a hose which was fitted with another air stone and placed in a plastic funnel which served as the base for the glass columns. This arrangement resulted in the delivery of small humidified and filtered air bubbles to the germinating medium: distilled water. The glass columns were surrounded by a water bath fitted with a small aquarium heater which maintained a constant 26 C in the columns. This germinating system was adapted from a similar one described in detail by Salter (47). Under these conditions, the snapdragon seed germinated and developed a 1.5 mm radicle within forty-eight hours.

Germinated seeds were separated from the non-germinated ones by using the specific gravity technique developed by Taylor et al. (57). Separation of snapdragon seed was achieved by placing the seed in a .771 M solution of sucrose having a specific gravity of 1.10. In this solution, the germinated seeds floated, whilst the non-germinated ones settled out due to their difference in density, thus making separation quick and easy via a drain-off valve at the base of the separatory funnel. Seeds were then flushed with fresh water to avoid damage due to the osmotic effect of sucrose. The germinated seed was then transferred to Petri dishes where

it was randomly separated into groups of twenty-two seeds for further transfer to the storage vials.

There were three gel treatments plus a control which consisted of seed storage in water. The gels were 0.4% (w/w) Permasorb-29, 1.17% (w/w) Laponite and 1.5% (w/w) Natrosol powder and water. A homogenous mixture of relatively low viscosity was obtained by mixing the powder and water at a slow speed in a Waring Blender for twenty seconds. Vials having a 15 ml capacity were filled with 5 mls of gel. The control consisted of the same number of seeds in 5 mls of distilled water. Two storage temperatures, 0 C and 5 C were used. Two storage durations, four and eight days were used. All the treatments were replicated three times.

At the end of the storage period, the seeds were removed from the gel and planted in four Petri dishes (for each replicate) filled with 20 gms dry "Jiffy-mix" wetted with 30 mls of distilled water. After several unsuccessful plantings, it was determined that the optimal level of substratum moisture had to be found. More than 30 ml resulted in rotting of the seedlings and less than 30 ml resulted in desiccation. Seeds thus planted were grown in a Mangelsdorf Germinator at 22 C and 95% humidity.

After seven days, the seedlings were removed from the growing medium and roots and shoots were measured. Percent emergence was calculated. All data thus collected was subjected to an analysis of variance to test treatment effects and interactions.

Respiration Rate

A Warburg respirometer was used to determine the respiration rate of freshly germinated seed with radicle lengths of 1.5 and 3 mm. This

measure of vigor was also determined for seed that had been stored in Natrosol for four and eight days at 5 C. The manometric techniques described by Umbreit et al. (59) and the equation given by Wharton and McCarty (62) were used in determining the respiration rate of the germinated treated and untreated seed.

Oxygen Diffusion Rate

Using the Jensen Model B Oxygen Diffusion Ratemeter, the diffusion rate of oxygen in the three gels and water at 0 C, 5 C and 22 C was determined. Fifty mls gel or water was placed in a 125 ml beaker. A platinum microelectrode, reference electrode and anode were inserted in the media and allowed to equilibrate four minutes before readings were taken as described in the instruction booklet (31). The average of three readings was recorded. The microampere reading was converted to oxygen diffusion rates in $\text{g/cm}^2 \text{ min}$ to facilitate comparison with the literature. The constant, .060 multiplied by the reading yielded micrograms/ $\text{cm}^2 \text{ min}$.

Germination in Gel

Thirty ungerminated seeds were placed in groups of ten in three positions of Laponite gel in 15 ml capacity vials with and without bottoms. Ten dry seeds were randomly picked from the seed packet and distributed throughout the bottom of the vial. They were then covered with 5 mls of gel. A second group of ten seeds were distributed on the surface thus formed by the gel. Another 5 mls of gel were poured onto these seeds, thus positioning them between two 5 ml layers of gel. A final ten seeds were then scattered on the surface of the top layer. The

base of the bottomless vials was a #80 mesh brass strainer which served as a tray for all the vials. The seeds in the bottomless vials were positioned in the same manner as those in the bottomed vials. The tray holding all the vials was placed in a Mangelsdorf Germinator at a temperature of 26 C and 95% humidity. Humidity was monitored with a Bacharach Relative Humidity Meter Model Serdex B. After four days, percent germination and radicle length were measured.

RESULTS AND DISCUSSION

Post-storage growth of pregerminated seed appeared to fall into three categories: Fully emerged, half emerged (showing only root damage), and non-emerged (showing root and shoot damage). Therefore, results were separated by those criteria.

Water storage resulted in the highest percent emergence, with an overall mean of 94% for four and eight days of storage at 0 C and 5 C (Table 1). Natrosol also resulted in a high survival rate regardless of storage length and temperature. On the other hand, Permasorb and Laponite had a fatal effect on a large number of seedlings. The eight day storage for both Laponite and Permasorb was worse than the four day storage. The 5 C storage temperature was worse for Permasorb than for Laponite, while the 0 C storage temperature had a more damaging effect on seedlings stored in Laponite than in Permasorb. Overall, Laponite and Permasorb had more damaging effects on percent emergence than did Natrosol and water.

Values listed in Table 2 represent the percent of the total which failed to emerge fully, i.e., there was shoot emergence but no obvious root development. Laponite and Permasorb resulted in a higher percent of seedlings with only root damage. Within these two gels, seedlings stored at 0 C suffered more root damage than those stored at 5 C. The least root damage in the absence of shoot damage was seen in the Natrosol and water treatments, especially at the 0 C storage temperature.

Overall non-emergence (Table 3) was the highest for Permasorb and Laponite. Permasorb had higher emergence for seedlings held four days at 0 C than eight days at 5 C, while Laponite had the highest non-emergence value for seedlings stored eight days at 0 C (Table 1). The same conclusions can be drawn from Table 3. Natrosol storage resulted in an overall

TABLE 1. Effect of storage temperature, time in storage and gel type on mean percent of seedlings fully emerged. Emergence was determined seven days post-storage at 22 C, 95% humidity.

Gel:	<u>Permasorb</u>		<u>Laponite</u>		<u>Natrosol</u>		<u>Water</u>	
Days Stored:	4	8	4	8	4	8	4	8
(%) ^z								
Temperature (C)								
0	63	40	48	25	92	84	96	90
5	58	29	58	55	90	91	95	95

^z100% = 20 seeds. Values represent the mean of 3 replications. Percentages were subjected to angular transformation prior to calculation of the means.

TABLE 2. Effect of storage temperature, time in storage and gel type on mean percent of emerged seedlings showing only root damage. Measurements on growth were made seven days post-storage at 22 C, 95% humidity.

Gel:	<u>Permasorb</u>		<u>Laponite</u>		<u>Natrosol</u>		<u>Water</u>	
Days Stored:	4	8	4	8	4	8	4	8
(%) ^z								
Temperature (C)								
0	8	24	24	11	1	1	1	5
5	8	8	9	8	3	2	5	5

^z100% = 20 seeds. Values represent the mean of 3 replications. Percentages were subjected to angular transformation prior to calculation of the means.

TABLE 3. Effect of storage temperature, time in storage and gel type on mean percent of non-emerged seedlings. Emergence was determined seven days post-storage at 22 C, 95% humidity.

Gel:	<u>Permasorb</u>		<u>Laponite</u>		<u>Natrosol</u>		<u>Water</u>	
Days Stored:	4	8	4	8	4	8	4	8
$(\%)^Z$								
Temperature (C)								
0	29	36	28	64	7	15	3	5
5	34	63	33	37	7	7	0	0

^Z100% = 20 seeds. Values represent the mean of 3 replications. Percentages were subjected to angular transformation prior to calculation of the means.

non-emergence mean of 9%, with lowest percent emergence at 0 C storage for eight days. Similarly, water storage, which had the lowest overall non-emergence for eight days at 0 C resulted in some seedling damage. No seedlings suffered shoot damage at 5 C storage. Since some shoots were damaged by the lower storage temperature for both the four day and the eight day storage, it would seem that the 0 C storage temperature had the most damaging effect on total emergence.

Likewise, root and shoot growth resulted in slower growth for seedlings stored for eight days at 0 C (Table 4). Storage in Natrosol yielded post-storage growth rates comparable to seedlings stored in water. Root and shoot post-storage growth was least for seedlings stored in Permasorb or Laponite; tissue growth was comparable within these two gels. Within these two gels the best shoot and root growth occurred after four days of storage at 5 C.

Table 5 represents a summary of data more fully detailed in Tables 1-4. Increased storage time resulted in reduced shoot and root growth, while increased storage temperature resulted in increased growth. Post-storage growth from the Natrosol gel treatment was much better than growth after storage in Laponite or Permasorb. Mean shoot and root growth after storage in Laponite or Permasorb were not significantly different, but both root and shoot growth after storage in those two gels was significantly lower than growth after storage in Natrosol. Storage time had no effect on overall specific root damage, but specific root damage appeared to be influenced by storage temperature with storage at 0 C causing more damage than storage at 5 C. Seedlings stored in Permasorb and Laponite experienced greater specific root damage than

TABLE 4. Effect of storage temperature, time in storage and gel type on post-storage root and shoot growth of seedlings. Growth measurements were made seven days post-storage at 22 C, 95% humidity in light.

Gel:	<u>Permasorb</u>		<u>Laponite</u>		<u>Natrosol</u>		<u>Water</u>	
Days Stored:	<u>4</u>	<u>8</u>	<u>4</u>	<u>8</u>	<u>4</u>	<u>8</u>	<u>4</u>	<u>8</u>
	(%) ^z					(mm of growth)		
Tissue:								
<u>SHOOT</u>								
0 C	80	44	56	44	117	77	31.8	39.7
5 C	82	53	82	71	107	95	33.4	35.5
<u>ROOT</u>								
0 C	79	51	66	53	105	87	10.2	10.2
5 C	95	54	93	69	124	87	9.7	10.9

^zValues represent a percent of seedlings stored in water. Shoot measurements were made on "fully emerged" and "half emerged" seedlings. Root measurements were taken only from "fully emerged" seedlings. See Table 5.

TABLE 5. Effect of different gels, time and temperature of storage on pregerminated snapdragon seed mean percent emergence and tissue growth.

	Fully Emerged	Half Emerged ^z	Root	Shoot
	(%) ^y	(%)	(Growth,mm)	(Growth,mm)
<u>Time</u>				
4 days	85a ^x	7a	9.14a ^w	28.32a ^w
8 days	65b	8a	7.29b	24.03b
<u>Temp C</u>				
0	80a	9a	7.56b	24.39b
5	80a	4b	8.82a	28.03a
<u>Gel</u>				
Permasorb	53b	10b	7.04b	22.35b
Laponite	53b	10b	7.34b	21.82b
Natrosol	99a	1a	10.13a	34.30a

^zHalf emerged = shoot emergence but no root growth.

^yPercentages underwent angular transformation prior to statistical analysis.

^xMean separation within columns by Duncan's multiple range test, 5%.

^wShoot measurements were made on "fully emerged" and "half emerged" seedlings. Root measurements were taken only from "fully emerged" seedlings. See Table 5.

those stored in Natrosol. Furthermore, increasing storage time had a detrimental effect on full emergence, while storage temperature had no influence on full emergence. Overall, storage in Natrosol regardless of time or temperature, was less injurious than Laponite or Permasorb storage.

Roots appeared to be more susceptible to the adverse storage conditions than were shoots. However, it is possible that shoot injury caused the root injury. Root development would not be supported by an injured shoot, therefore both organs would perish. This would be evidenced as no emergence. Hence, Table 3 may represent the extent of storage-induced shoot damage.

Roots appear to be particularly sensitive to the oxygen content of their environment (34, 36, 37). Conditions favoring water logging damage (34) may be likened to the conditions created by gel storage. Flooding reduces the oxygen levels of the root environment, a condition which if persistent, will result in root death (36). Low oxygen concentration or low oxygen diffusion rate is particularly damaging in the early stages of growth (37).

While it was the intent of this study to attempt to suspend radicle growth through cold storage in gel, it was hoped that growth would resume once the pregerminated seed was sown in a favorable growing environment. If the minimal respiration rate of the tissue could be maintained, no growth would occur, but life processes would be sustained. Resumption of normal growth would be expected after removal from cold storage (14). However, extensive damage was sustained by the radicle in many cases, indicating that the storage medium, duration and/or temperature exceeded the critical levels for retaining seed viability.

The literature indicated that the most likely injurious factors resulting from low temperature storage under low oxygen tension could be 1) lack of oxygen or oxygen diffusion (37, 52, 54) and 2) chilling injury (11, 13). How these two conditions affect viable tissue and how viable tissue responds to these factors is also discussed in the literature (5, 12, 42). For example, Stolzy et al. (54) found that root initiation was reduced or stopped at oxygen diffusion rates of less than $18 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$ and Kaack and Kristensen (32) noted that mustard seed failed to emerge at oxygen concentrations below 2% and that emergence percentage increased with increased oxygen up to atmospheric levels. Christiansen (9) noted root tip abortion in cotton seedlings due to chilling injury at 10 C.

Another gel variable, which could partially account for the damaging effects of the storage medium on radicle vigor, was pH. Laponite had the highest pH (8.9) and resulted in very much damage to roots (Table 6). However, Permasorb had a close-to-neutral pH of 6.5 and was equally damaging. Water and Natrosol, the least damaging gel treatments, had pH's of 8.2 and 7.2 respectively. These differences in pH of gels probably had no effect on radicle vigor. Experiments with roots grown in a series of solutions ranging from pH 3.8 to 10.5 showed no significant differences in intercellular air space formation (42). Increased air space formation was attributed to low oxygen and not to differences in pH.

Although the oxygen concentration of the storage media was not determined the rate of oxygen diffusion through the gel at various temperatures was determined (Table 6). Storage media which caused the fewest ill-effects on overall seedling performance, had the highest oxygen diffusion rates (ODR). However, those very rates were lower than the ODRs found to be critical

TABLE 6. pH and oxygen diffusion rate of storage gels.^z

<u>Gel:</u>	<u>pH</u>	Temperature C		
		0	5	22
(Oxygen diffusion in rate, $\text{O}_2\text{cm}^{-2}\text{min}^{-1} \times 10^8$)				
Permasorb	6.5	9	12	15
Laponite	8.9	10	14	16
Natrosol	7.2	13	17	19
Water	8.2	13	19	24

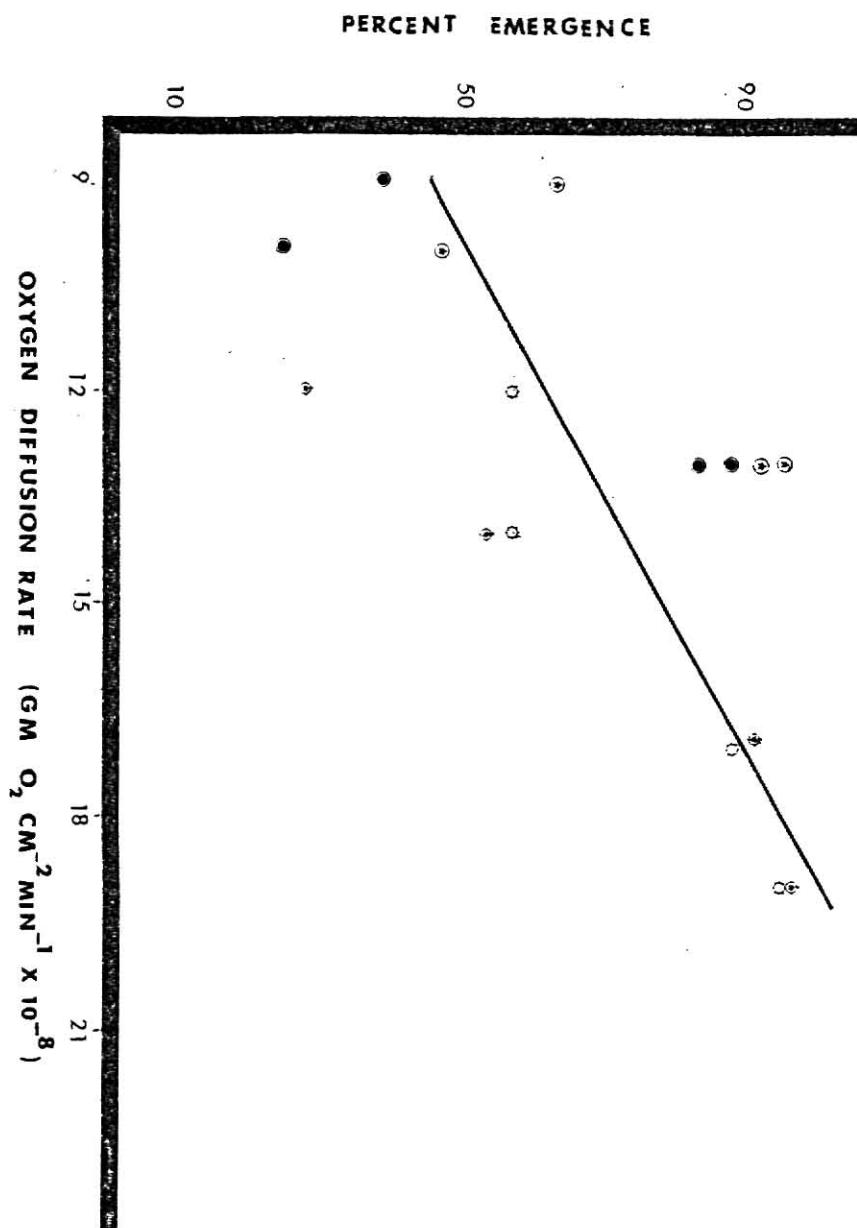
^zReadings were taken with a Jensen oxygen diffusion ratemeter.

and $10 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$ for Permasorb and Laponite respectively was lower than that of Natrosol and water. A correlation between ODR and post-storage seedling emergence seemed to exist. A correlation coefficient of .68 was seen for percent emergence at both storage temperatures and durations as a function of ODR (Figure 1). No such correlation was found for shoot and root growth and ODR. Increased storage temperature resulted in increased ODR (Table 6) and also resulted in increased percent emergence (Table 1). Natrosol and water, which had the least damaging effect on seedling vigor (Table 5), had higher ODRs than did Laponite and Permasorb - both of which were very injurious to root tissue. Furthermore, ODR was lowest at 0 C for all gels, thus partially explaining the greater damage attributed to low temperature in all storage gels and storage durations.

Stolzy et al. (54) determined that $18 \text{ to } 23 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$ was the critical level necessary for snapdragon growth. In the present study, growth during storage was not desired. Instead, minimal or suspended growth without permanent damage was the goal. Since snapdragon radicle tissue maintained viability at an ODR of $13 \text{ to } 19 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$ (Natrosol and water), but suffered much damage in media with an ODR of $9 \text{ to } 14 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$ (Laponite and Permasorb), the former ODR is apparently sufficient for maintenance respiration, while an ODR less than $14 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$ is insufficient for survival.

ODR may not be the only factor affecting seedling response to storage. The question of how seedlings survived the storage period, as evidenced by post-storage growth, in a medium with less than the critical ODR may have partial explanation in the observations made by Kramer (34). He noted that roots produced and grown in water were different anatomically

Figure 1. Percent emergence of seedlings stored in Permasorb, Laponite, Natrosol and water at 0 C and 5 C for four and eight days as a function of the oxygen diffusion rate of those gels. () represents four day storage at 5 C, () represents eight day storage at 5 C, () represents four day storage at 0 C, and () represent eight day storage at 0 C.



and physiologically from those produced in soil. Unaerated culture solutions cause roots to develop large intercellular air spaces due to cellular collapse following death from lack of oxygen (42). The loss of cells resulted in less need for oxygen. Life maintenance at lower levels of oxygen would thus be possible. In addition, it was found that roots developed in water respired normally in lower oxygen concentrations than roots developed in well aerated media (52).

Because Natrosol and water had the higher ODRs and post-storage seedling performance was at acceptable levels, a respiration rate study was performed on seedlings stored in those two media in order to determine what effect, if any, storage had on that measurement of seedling vigor. Storage at 5 C in water and Natrosol for four days resulted in a decreased respiration rate (Table 7). This may be attributed to a reduction of cell number due to 1) cellular collapse, 2) other forms of chilling injury, 3) pH of the gel, or 4) a combination of these factors. Additionally, the respiration rate was lower for seeds stored in Natrosol than those stored in water. The fact that seedlings stored in Natrosol had greater growth after four days of storage in spite of the lower respiration rate than the water storage may be explained as follows. Although one would expect the treatment showing a higher respiration rate to result in greater seedling growth, the opposite appeared to be true for the four day storage period. Some cells may have been killed during storage, so the respiration rate per cell after storage in Natrosol may be the same as that in water. The eight day storage results were consistent with expectations; root and shoot growth was greater for seedlings with the higher respiration rate.

TABLE 7. Respiration rate of pregerminated snapdragon seed stored at 5 C. 100 seedlings per vial were used. After removal from the gel, seedlings were washed with distilled water to remove as much of the gel as possible, without damaging the radicle. Seedlings were then placed in 1 mm of water in a Warburg respirometer flask. Respiration was measured at 25 C using a Warburg respirometer.

Storage Time	Radicle Length	Storage Medium	Respiration
(days)	(mm)		(gm O_2 min ⁻¹ seedling ⁻¹ X 10 ⁹)
0	3.0	-	19.8
	1.5	-	5.1
4	1.5	water	3.9
	1.5	Natrosol	2.0
8	1.5	water	3.2
	1.5	Natrosol	2.3

A correlation between respiration rate and ODR seemed to exist. Seedlings stored in Natrosol ($ODR = 17 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$) had respiration rates of $2 \times 10^{-9} \text{ gm min}^{-1} \text{ seedling}^{-1}$ after four days and $2.3 \times 10^{-9} \text{ gm min}^{-1} \text{ seedling}^{-1}$ after eight days storage. Seedlings stored in water (which had the slightly higher ODR of $19 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$) had respiration rates of $3.9 \times 10^{-9} \text{ gm min}^{-1} \text{ seedling}^{-1}$ for four days and $3.2 \times 10^{-9} \text{ gm min}^{-1} \text{ seedling}^{-1}$ for eight day storage. Thus, the respiration of seedlings after storage in different media appeared to be related to the ODR of the media. An inverse relationship between respiration rate and storage time was observed. Increased respiration rate resulted from decreased storage time. Respiration rate of seedlings after storage in Laponite or Permasorb was not determined since these two gel treatments had proven to be significantly inferior to Natrosol and completely unacceptable as storage media.

In addition to low percentage emergence of seeds stored in Permasorb or Laponite, the morphological effects of these two gels on root growth was cause for eliminating them. The root growth pattern of the various treatments showed distinct differences, ranging from a fairly normal downward growing tap root - to right angled initial growth - to complete curling of the initial growth into a "pigtail" pattern prior to normal, geotropic growth. Root growth of seedlings stored in water most approximated the typical snapdragon tap root. Root growth of Natrosol-stored seedlings was fairly similar to that of water stored seedlings, though some tendency toward curling was evident. Natrosol storage also resulted in necrosis of root tips, inducing some lateral roots to develop. These adventitious roots appeared thicker and growth length was comparable to non-adventitious roots. A large percentage of the roots from seedlings

stored in Laponite exhibited growth radial to the axis of the shoot for the initial growth before growing tangential to the shoot axis. The greatest distortion in root growth pattern was seen in the Permasorb treated roots. These roots exhibited initial upward growth, sometimes making a complete circle prior to normal downward growth. In addition to these distortions, root growth of seedlings stored in all media was somewhat wiry and in some cases almost hair-like. Post-storage growth of none of the roots, even those stored in water, exhibited thick and apparently healthy growth similar to freshly germinated seedlings. However, seedlings stored in water exhibited root growth somewhat similar to that observed in non-stored seeds and showed some beginnings of root hairs.

All gels have been tested for phytotoxicity for fluid sowing and found to be acceptable fluid sowing gels. However, the curling of the root in the initial stages of growth could be a form of epinasty resulting from the flood-like storage environment under the most acute oxygen deficiencies. The greatest distortion was seen in gels with the lowest ODR. Additionally, root tip distortion due to chilling injury has been reported (10, 11). However, were this the case, the distortion would have been evidenced in all gel treatments. A combination of low temperature 0 C and 5 C, coupled with a specific critical ODR may be the answer.

Shoot morphology appeared very similar for all treatments, though mean length varied with treatments (Table 4). Overall, those seedlings stored in water for eight days exhibited greater growth than those stored for four days suggesting that perhaps growth during storage in water was never arrested, but rather was a continuous process gaining speed after sowing. On the other hand, an inverse correlation of storage time and growth was seen in the gel treatments: increased storage time resulted in less growth. This would suggest that gel storage did indeed arrest

growth during storage and only those that were not severely damaged were able to recuperate following a lag period. In addition, temperature had an effect on growth. Seedlings stored at the lower temperature possessed slower post-storage growth than those stored at the higher storage temperature. It should be noted that growth measurements were made just one week after sowing and a longer period of observation might have resulted in full recuperation of seedlings stored at 0 C and ultimate yield would have been comparable (14).

As noted in the Review of Literature, the sowing of pregerminated seed has many advantages. Uniformity of shoot growth, one of those advantages, was noted particularly in the Natrosol and water treatments. However, this primary advantage of sowing pregerminated seed was lost in the Laponite and Permasorb gel treatments as evidenced by the large number of half emerged seedlings (Table 2). Those seedlings which were only half emerged would have probably died or at best, been extremely stunted.

Another well documented advantage of sowing pregerminated seed is that of faster and more uniform emergence. However, uniformity of growth of seedlings stored in all gels was not observed, apparently due to the inconsistent root development. Generally, root growth was more uniform than shoot growth. Root growth may be a better indicator of the ill effects of storage treatments, since in the absence of roots, normal growth could not occur. Roots were probably most obviously sensitive to the storage medium since shoots were still protected by the seed coat from direct exposure to the gel.

In the preliminary studies, difficulties were encountered in getting roots to develop in spite of the apparently vigorous seedlings. The first methods used in fluid sowing the post-storage pregerminated seeds

met with failure. Direct sowing into 10cm X 10cm greenhouse plastic trays of "Jiffy-mix" in a growth chamber proved unsuccessful as the surface of the medium dried out faster than that at the bottom of the tray causing the gel to crust on the surface. A second unsuccessful method used was one described by Parker (43). Seeds were planted on the surface of growing media containing the optimal water level, sealed in a Petri dish and placed at a 45° angle in the growing chamber. This technique enables the observer to take root measurements nondestructively through the glass. The results of these efforts were even more catastrophic, perhaps due to the exacerbated low oxygen diffusion conditions created by compacting the medium around the gel imbued radicle and sealing against atmospheric oxygen. The conditions which finally yielded results were obtained by simulating greenhouse conditions in a shallow container. "Jiffy-mix" was placed loosely in a Petri dish and moistened to 60% water (w/w). The pregerminated seedlings were then sown about 3 mm deep. The medium was never moistened again, but the humidity in the growing chamber, a Mangelsdorf germinator, was maintained at 95%.

An attempt was also made to increase the oxygen level of the gels by the use of chemicals. That oxygen was the limiting factor in the storage of pregerminated seed in gel was confirmed early in the study through an experiment with germinating dry seed in gel. The experiment that implicated oxygen deficiency as the limiting factor in gel consisted of positioning ungerminated seed between layers of Laponite gel. It was believed that seeds would not germinate in the absence of oxygen.

Percent germination results and subsequent growth of the radicle at three levels in Laponite gel and gel mixed with hydrogen peroxide and catalase can be seen in Table 8. Those seeds situated at the bottom and

TABLE 8. Percent germination of dry snapdragon seed in Laponite and mean radicle length three days after germination.

Gel level:	Vial with bottom		Vial without bottom		H ₂ O ₂ + Catalase ^x	
	germination % ^z	radicle length mm	germination %	radicle length mm	germination	radicle length mm
Top	90-100 ^y	1.8 - 1.5	90	1.6 - 1	-	-
Middle	50-60	1 - .9	90 - 70	.9	-	-
Bottom	40-50	1 - .6	90	1.9 - 1.8	-	-
Mixed	50	1	90 - 80	1.9 - .9	90	1.6 - 1.1

^zBased on n = 10 for each layer.

^yValues represent the range of observations (r = 2). Both replicates were identical when only one value is given.

^xFinal concentrations were 3.6 mM H₂O₂ and 1.3×10^{-1} gm catalase (21000 units/gm)/ml.

middle levels had a much lower percent germination than those on the surface layer. Mixing the seeds throughout the gel resulted in higher germination of those closer to the surface-atmosphere interface than those closer to the bottom. Attempts were made to measure depth for each seed before removal for measurement in order to see if there was a correlation between radicle length and depth of gel. This was not feasible with the seed mixed randomly throughout the gel. However, in the discretely arranged seed trials, there was a decrease in percent germination with increased depth. Table 8 shows an average germination of 45% for the bottom and 95% for the surface. Other preliminary trials showed zero germination at the bottom of the vial. Radicle length three days after germination also decreased with increased depth of gel, though radicle growth during this period was almost negligible.

A second aspect of this experiment consisted of layering ungerminated seed between 5 mls of gel in a bottomless vial (Table 8). The base of the vial consisted of a fine mesh copper strainer which allowed atmospheric air entry. Hence, seeds at the bottom of these vials should have been exposed to the same oxygen concentrations as seeds at the top of the vials. This resulted in identical percent emergence for seeds situated at both the top surface and the bottom surface. Additionally, percent germination of seeds situated in the middle layer was considerably higher when the bottom of the vial was removed. Mean radicle growth did not show the same linear decrease with gel depth. Instead, the bottom layer appeared to be favored by gel pressure and air availability as evidence by a slightly higher mean radicle length. However, middle layer radicle growth for the bottomless vial was shorter than for those seeds germinated in the vial with a glass base. This can only be explained as follows. Since there was a higher number of seeds which germinated, seeds that

seeds which germinated, seeds that must have been respiring and utilizing oxygen, there may have been less oxygen available for interior seeds. Thus respiration and radicle growth of seeds in the interior would have been reduced.

A third aspect of this experiment consisted of arranging the dry seed between layers of gel treated with hydrogen peroxide and catalase. However, the oxidizing reaction caused the gel to apparently lose some of its viscosity and the seeds became randomly mixed throughout the gel. Notwithstanding, this mixing of the seeds throughout the gel did not result in similar germination to mixed seeds in vials with glass bases. Instead, results were comparable to the surface germination percentage of both bottomed and non-bottomed vials. Furthermore, the mean radicle growth of the mixed hydrogen peroxide and catalase treatment was also comparable to results of seed situated on the surface. This was viewed as evidence that oxygen was the limiting factor in germination as well as growth of seedlings in gel. That some seeds were able to germinate in gel under low oxygen conditions is supported by Kaack and Kristensen (32) who found that germinability of cucumber and celosia seeds in an anaerobic environment was possible. However, they reported a decrease in the rate of germination under these adverse conditions.

Knowing that oxygen was the limiting factor in the storage of pregerminated seed, the amount of oxygen required per pregerminated seed immediately following germination at room temperature was determined. Knowledge of the per seed oxygen requirement (Table 7) and the oxygen diffusion rates in gels (Table 6) was to be used in altering the storage medium to meet the seedlings' oxygen needs. That ODR values of the gels were in a critical range can be illustrated by the following analysis. This point of consideration is based on a snapdragon seedling with a

1.5 mm long radicle having a 0.5 mm diameter. If the hypocotyl and cotyledons were roughly spherical, 1 mm in diameter, the seedling would have a surface area of $0.055 \text{ cm}^2 \text{ seed}^{-1}$. A seed with a respiration rate (Table 7) of $5.1 \times 10^{-9} \text{ gm min}^{-1} \text{ seed}^{-1}$ would require a medium with an ODR of at least $9.3 \times 10^{-8} \text{ gm cm}^{-2} \text{ min}^{-1}$, a value quite close (given errors in approximating seedling surface area) to the oxygen diffusion rates observed in the gels (Table 6).

The attempt to make a given amount of oxygen available to the seeds through the use of catalase to release oxygen from hydrogen peroxide, a chemical which had been effectively used as an oxygenator (64) had to be abandoned because it apparently lowered the viscosity of the gel. Other chemicals with potential for oxygenating the gels, including potassium permanganate (44), calcium sulfate and magnesium sulfate (12), were tried but proved to be ineffective as they caused gel desolidification. Thus, oxygenation of the gel by chemical means was not feasible. Use of chemicals for altering the pH of the gels was equally unsuccessful. However, pH alteration could have been done by lowering the pH of water prior to blending in the gel. (P. O'Brian, Laporte (United States) Inc., personal comm.).

Since the gels examined were deficient in oxygen and proved to have an adverse effect on radicle vigor and, since the oxygen level of the gels could not be altered through chemical means, cold storage of pregerminated seed in gels was not found to be completely successful.

A second injurious factor on post-storage seedling vigor was temperature. Decreasing the temperature from 5 C to 0 C resulted in decreased percent emergence, decreased shoot growth and decreased ODRs which in turn had a negative effect on post-storage seedling vigor. A third

injurious factor was length of storage time. Increasing the storage period generally resulted in a decrease in percent emergence and shoot growth. Increasing the storage period also resulted in decreasing respiration rates. Overall, water storage resulted in the least damaging effects, but Natrosol was found to be comparable. Laponite and Permasorb were not included in the respiration study because of the extreme ill effects they produced on seedlings as evidenced by low percent emergence, erratic and reduced shoot growth, high incidence of root damage, and distorting tendencies on root morphology.

SUMMARY AND CONCLUSIONS

This study was designed to determine the feasibility of storing pregerminated seed in the fluid sowing medium by evaluating three gels at two storage temperatures for two storage durations. Whether oxygen was the limiting factor in holding pregerminated seed in gel was also to be ascertained.

After storing pregerminated seed in three gels, Laponite, Permasorb, and Natrosol and comparing those results with storage in water using percent emergence, percent root damaged seedlings, and shoot growth; it was determined that gel storage was not a perfectly satisfactory option to fluid sowing. The effects of the various gels were different, but generally they could be categorized into two groups: reasonably acceptable and definitely unacceptable. Those in the former category were Natrosol and water, while Laponite and Permasorb fell into the latter category.

For Laponite and Permasorb an overall percent emergence mean across temperature and storage duration was 47%, while for Natrosol and water, it was 92%. Greater root damage was found in the seedlings stored in Laponite or Permasorb than in those stored in Natrosol or water. In addition, overall shoot growth was greatest for Natrosol or water and least for Laponite or Permasorb at both storage temperatures and durations.

Temperature was found to significantly influence emergence, root damage, shoot growth, respiration rate and oxygen diffusion rates. The 0 C temperature had more negative ill effects on the aforementioned variables than did the 5 C storage temperature. These results were attributed to chilling injury and lower oxygen availability at the lower temperature.

A low oxygen diffusion rate (lowest being $9 \times 10^{-8} \text{ cm}^{-2} \text{ min}^{-1}$ for Permasorb) in combination with low temperature seemed to result in the greatest damage to seedlings as evidenced by post-storage vigor measurements. In addition to low percentage emergence, high percent half emergence and reduced shoot growth; distortion in initial post-storage root growth was observed in all gels. At higher ODRs and higher storage temperature these effects were not as prominent.

Oxygen appeared to be a major limiting factor in pregerminated seed storage in gels. Germination of dry seed in gel was affected. Seeds on the surface of the gel had a mean 95% germination, while germination decreased to 45% with increased depth within the gel. Removing the bottom of the germination vessel resulted in comparable germination percentage at the bottom and the top surfaces. Furthermore, this caused an increase in germination in the middle layer of seeds within the gel. Similarly, radicle length of germinated seed in the gel showed greater growth when closer to the oxygen source. Further evidence that oxygen was deficient in the gel was obtained by increasing the oxygen supply of the gel with hydrogen peroxide and catalase. This resulted in higher percent germination throughout the gel and radicle growth comparable to that of seeds germinated on the surface.

In conclusion, Natrosol and water were found to be acceptable as storage media but with reservations. Because of the ill effects that were evidenced from the treatments, storage of pregerminated seed would not be recommended. However, if seeds had been germinated and a delay in sowing were necessary, it would be best that the pregerminated seeds be stored in either Natrosol or water at 5 C for the shortest time necessary. If the pregerminated seeds were already in Laponite or Permasorb ready for fluid sowing, removal from the gel would be suggested.

The fragile condition of the seedlings after storage as evidenced by difficulties encountered in determining the proper growing conditions could be an indication of a possible unfavorable final product. Without further studies to determine the effect on final yield, storing snapdragon seed in gel for longer than absolutely necessary would not be recommended since seedling quality was inversely related to storage time. Studies on the effect of storage of pregerminated seed in Natrosol or water on flower size, color, texture and time to harvest would be a good follow-up to this initial study.

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STORAGE OF PREGERMINATED
SNAPDRAGON (ANTIRRHINUM MAJUS) SEED
IN THREE HYDROGELS

by

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Three hydrogels were evaluated to determine the feasibility of cold storage of pregerminated snapdragon seed in gel. The criteria used in evaluating the effects of the gel on seedling vigor were: percent full emergence, percent half emergence and root and shoot growth. Storage at two temperatures, 0 C and 5 C for four and eight days was evaluated.

The overall effect of the gels fell into two categories: reasonably acceptable for Natrosol and water and definitely unacceptable for Permasorb and Laponite. Significant differences were found between these two categories with Laponite and Permasorb resulting in very damaging effects on post-storage seedling performance. An average of 47% of the seedlings stored in Laponite or Permasorb emerged, while 92% of the seedlings stored in Natrosol or water emerged fully. Laponite and Permasorb resulted in significantly higher root damage in the absence of apparent shoot damage than Natrosol or water. Root and shoot growth was also significantly lower than root and shoot growth following storage in Natrosol or water.

Within these two subcategories of gels, the effects of storage temperature and length of storage time were evaluated. Storage temperature did not significantly influence full emergence of stored seedlings. However, storage temperature did influence root damage in the absence of shoot injury, with storage at 0 C being most damaging. Root and shoot growth was also significantly higher after 5 C storage than 0 C storage. Length of storage time had a significant effect on full emergence; a shorter storage time resulted in greater emergence. Root and shoot growth was significantly greater for seedlings stored for four days than for those stored for eight days. Post-storage respiration rates for

seedlings stored in water and Natrosol for four and eight days was found to be highest for the four day water stored seedlings. Natrosol stored seedlings for eight days had the lowest respiration rate. The respiration rate of seedlings stored in Laponite and Permasorb was not determined since they resulted below the acceptable level in the other measurements of vigor.

A low oxygen diffusion rate (ODR) in the gels, particularly Laponite and Permasorb was determined to be a major factor contributing to the injurious effect of storage on seedling vigor. Since decreased temperature resulted in decreased ODR, temperature was determined to be a second major limiting factor of cold gel storage. Percent emergence as a function of ODR was found to have a positive coefficient of correlation.

Oxygen diffusion was determined to be the limiting factor in germination of seeds in gel. A higher percent germination was evidenced on the surface of the gel and increased depth of seeds in gel decreased germination percentage from 95% to 45%. Removing the bottom of the germination vessel also increased the percent of seed germinating at the bottom of the gel. This was seen as evidence of oxygen deficiency in the gel as the main limiting factor for cold gel storage.