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Lauren E. Carlson

University of Minnesota, St. Paul

T. H. King

University of Minnesota, St. Paul

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The Effect of Environmental Factors on the Quality of Stored Pea Seed¹

LAUREN E. CARLSON and T. H. KING

University of Minnesota, St. Paul

INTRODUCTION: The factors that determine whether stored pea seed will remain as quality seed or be invaded and deteriorated by storage molds, (*Aspergillus* species), are moisture content, temperature, and duration of storage period. Fields (1961) demonstrated that pea seed placed in common storage at a moisture content of 12% increased to 15% moisture content in 3 months in the fall of the year in Minnesota and remained at this moisture content the rest of the winter. According to Christensen (1955) many of the storage fungi will invade seed and cause deterioration if the moisture content is above 13.2% and the temperature is optimum. According to the United States Weather Bureau the relative humidity in the North Central States during the fall and winter is on the average 75% and above. Fields (1961) demonstrated that pea seed in equilibrium with a 75% relative humidity has a moisture content of 15% and in equilibrium with an 85% relative humidity, a moisture content of 17%. It is obvious that when seed is placed in common storage where the relative humidity averages above 75%, deterioration could occur if temperatures were optimum. Therefore, an attempt was made to determine if high moisture pea seed would decrease in moisture when stored in different environments and also if the type of container in which it was stored would influence the fluctuation of the moisture content of the seed. In addition an attempt was made to determine if treatment of the pea seed prior to storage would control mold infestation that may occur during handling and to determine any effect of the treatment on germination and vigor.

MATERIALS AND METHOD: Preparation and handling of seed before and after storage. Seed for the studies was grown on the Libby, McNeill and Libby Agricultural Research Plots at Spring Valley, Minnesota in the summer of 1960. When the seed had a moisture content of 25% it was harvested and brought to the University of Minnesota for artificial drying. The seed was dried at 28°C., having been determined in previous studies as a favorable drying temperature to use in order to obtain quality seed. The seed was dried to two different moisture contents and then each lot was treated in four separate ways as demonstrated in Table 2. Each of the four separate seed lots was placed in cloth bags and 3 mil plastic bags. The seed was stored in 2 different geographical areas, Boise, Idaho, and St. Paul, Minnesota. At the Boise site,

peas were stored in unheated but protected buildings. At the St. Paul site, peas were stored in a heated building and also in an unheated but protected building.

Sub-samples were removed from the seed lots 4 and 8 months after the beginning of the storage period. The seed removed from the cloth bags was placed in plastic lined envelopes and sent to the University of Minnesota, Department of Plant Pathology and Botany, for processing. The seed stored in the plastic bags was originally packaged with one sub-sample per bag. This method of storage was devised to prevent any change in the moisture content of the seed remaining in storage. The seed was stored from September to May and checks during the storage period were made to determine the per cent of germination, vigor, mold invasion, and moisture content.

The germination and vigor of the seed was determined by a procedure devised and tested by the Raw Products Research Bureau, National Canners Association.² The test evaluates the quality of a seed lot by determining the number of normal seedlings obtained in a germination test. The test is called a "vigor test." Each lot is given a vigor rating according to its reaction in the following:

1. Fill a 2½-inch deep plastic container one-third full of "Vermiculite" which has water added at the rate of 5 parts Vermiculite to 2 parts water.
2. Place seeds in the container, space evenly, and press seeds down firmly.
3. Fill the remainder of the plastic container with the same mixture of Vermiculite as used before and press firmly.
4. Spray the top of the Vermiculite with a fungicide.
5. Place plastic containers in an incubation room for 6 days at 24°C.
6. After 6 days record the results in the following manner:
 - a) Count number of seeds germinated and calculated the percentage germinated.
 - b) The pea seedlings with tops 1½ inches shorter than the average plant height are ranked as plants with short tops.
 - c) All the plants with roots that are shorter than half the length of the top are ranked as plants with short roots.
 - d) The number of plants with short tops and short roots are added together and subtracted from the number of seeds that germinated. This figure is

¹Paper No. 4859, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

²Raw Products Research Bureau, National Canner's Association, Washington, D.C.

then used to calculate the vigor rating on a percentage basis.

The moisture content of seed was determined by oven drying 10 grams of seed for 5 days at 105° C. After 5 days the seed was weighed again and the percentage of moisture in the seed calculated on a wet weight basis.

The percentage of seed which yielded storage molds (*Aspergillus* species) was determined by surface disinfecting the seed for 1½-2 minutes with a 1% solution of sodium hypochlorite to which 1 drop of the surfactant, "Tween 20," had been added. The seed was then cultured on a medium containing 7.5% NaCl, 2.0% malt extract and 1.7% agar and incubated for 6-10 days at room temperature (25° C). The fungi that grew out of the seed were identified and the percentage of seeds yielding fungi was calculated.

The various species of *Aspergillus* used to artificially inoculate pea seed were isolated from pea seed harvested from by-passed (exceeded canning maturity) pea fields in Southern Minnesota.

RESULTS: The influence of environment and treatment prior to storage on mold invasion. Table 1 shows the per cent mold invasion of the seed subjected to the four treatments and stored during the fall and winter of 1960 and 1961 in three different environments. Storage fungi were isolated from three of the four pre-storage treatments during storage in cloth and plastic bags at the three different environments. Seed which was treated with a fungicide (Captan) and not inoculated with storage fungi did not yield any storage fungi from seeds stored in either cloth or plastic bags during the 8 month storage period.

Seed with an original moisture content of 11% that was not treated but inoculated and stored in cloth and plastic bags yielded approximately the same percentage of invaded seeds when stored at all three sites during the two periodic checks for mold invasion. Seed with an

original moisture content of 18% which was not treated but inoculated and stored in cloth bags yielded an average of 15% invaded seeds, when stored at the three areas after 8 months. The same seed which was stored in plastic bags and stored at St. Paul, in an unheated building, a heated building and at Boise in an unheated building yielded 70%, 94%, and 52% invaded seeds, respectively after 8 months of storage.

The application of a fungicide to inoculated seed did not appear to prevent invasion of the seed by storage molds. Inoculated, but treated seed yielded up to 9% invaded seed when stored in cloth and plastic bags with an original moisture content of 11 and 18%.

The check treatment (non-inoculated and non-treated) yielded invaded seeds in serious amounts only when it was stored with an original moisture content of 18%. After 8 months the check treatment yielded no invaded seeds from seed stored in cloth bags while seed stored in plastic bags yielded 73% invaded seeds in seed stored at St. Paul in an unheated building, 30% in a heated building, and 21% at Boise, Idaho, in an unheated building.

The invasion of seed in the control treatment indicated that seed can become contaminated with storage fungus spores before being placed in the plastic bags and that if the moisture content of the seed is optimum for growth, invasion will take place. The relative humidity surrounding seed with a high moisture content in a plastic bag in a heated building is very high and would be optimum for high invasion of the seed whether it was artificially inoculated or not. Seed that was only treated prior to storage and stored in cloth and plastic bags yielded no storage fungi. The results indicate that mold free seed should be chemically treated before storage to prevent invasion from storage fungi. However, if the seed is above 14 or 15% moisture content it should be dried before treatment because of possible chemical toxicity. The high

TABLE 1. A comparison of the percentage of seed yielding storage fungi or molds after 8 months of storage at three locations and the influence of types of storage containers and treatments.

Area	Type of storage container	Original moisture content	Non-treated ^a non-inoc.	Treated non-inoc.	Treated inoc.	Non-treated inoc.
St. Paul unheated	Cloth	11%	1 ^b	0	1	12
	Plastic		0	0	0	12
St. Paul heated	Cloth	11%	0	0	1	11
	Plastic		0	0	0	14
Boise unheated	Cloth	11%	0	0	1	12
	Plastic		0	0	0	21
St. Paul unheated	Cloth	18%	0	0	7	13
	Plastic		73	0	1	70
St. Paul heated	Cloth	18%	0	0	0	22
	Plastic		30	0	1	94
Boise unheated	Cloth	18%	0	0	9	11
	Plastic		21	0	4	52

^aSeed treated with Captan at recommended rate, and seed inoculated with a mixture of *A. flavus*, *A. amstelodami*, *A. ruber*, *A. repens*, *A. ochraceus*, *A. candidus*, and *A. restrictus*.

^bRepresents 200 seeds.

moisture seed in this study showed a decrease in vigor when stored in a plastic bag and treated with a fungicide. It is apparent that high moisture seed should not be stored in sealed containers or containers that would maintain the high moisture for extended periods of time.

The influence of environment and treatment prior to storage on germination and vigor. There was no apparent change in germination due either to the storage location, moisture content before storage, treatment prior to storage, or the type of container in which the seed was

TABLE 2. Flow chart indicating number of seed samples, preparation, treatments, moisture content before storage, storage containers, and areas of storage of the seed dried at 28°C. in 1960 and 1961.

Storage container	m. c. before storage	treatment prior to storage ^a	place of storage ^b
Plastic bag	11%	non-treated 8 non-inoculated	Minn. Idaho
		treated 8 non-inoculated	Minn. Idaho
		treated 8 inoculated	Minn. Idaho
Cloth bag		non-treated 8 inoculated	Minn. Idaho
Plastic bag	18%	non-treated 8 non-inoculated	Minn. Idaho
		treated 8 non-inoculated	Minn. Idaho
		treated 8 inoculated	Minn. Idaho
Cloth bag		non-treated 8 inoculated	Minn. Idaho

^aSeed treated with Captan at recommended rate, and seed inoculated with a mixture of *A. flavus*, *A. amstelodami*, *A. ruber*, *A. ochraceus*, *A. candidus*, and *A. restrictus*.

^bSeed stored in two different buildings at St. Paul, Minnesota, one heated and the other unheated. The building at Boise, Idaho was unheated.

stored. All seeds lots, Table 3, averaged 94% germination or better after 8 months of storage in the three different environments.

The vigor of seed subjected to the different treatments and stored for 8 months is summarized in Table 3. Seed that was stored with an original moisture content of 11% showed no large differences in vigor when stored at the 3 sites. However, there was a serious reduction in vigor of seed with an original moisture content of 18% stored in plastic bags at the three locations. The greatest reduction in vigor was in seed that was treated, non-inoculated and stored in plastic bags in a heated building at St. Paul, Minnesota. It is apparent that seed treatment of high moisture seed stored in impermeable or semi-permeable containers such as plastic is not advisable. In general, the high moisture seed stored in plastic bags was low in vigor regardless of the treatment prior to storage. In the case of high moisture seed it should be artificially dried. If dryers are not available it would be advisable to store in cloth bags in low humidity areas until the moisture content of the seed decreased to a level where it would not be invaded by various microorganisms which attack grain in storage.

The influence of environment on the moisture content of stored pea seed. Table 4 shows the moisture content of seed stored for 8 months in 3 different environments. Seed with an original moisture content of 11% and stored in cloth bags remained about the same after 8 months of storage in an unheated building at St. Paul

TABLE 3. Per cent vigor of seed stored at three locations in cloth and plastic bags and the influence of treatment prior to storage after 8 months.

Storage area	Type of Storage Container	Original Moisture Content	Treatments ^a prior to storage			
			Non-treated non-inoc.	Treated non-inoc.	Treated inoc.	Non-treated inoc.
St. Paul unheated	Cloth	11%	88	92	90	93
	Plastic	11%	87	88	88	83
St. Paul heated	Cloth	11%	88	86	91	89
	Plastic	11%	86	84	88	87
Boise unheated	Cloth	11%	76	83	85	84
	Plastic	11%	81	88	84	90
St. Paul unheated	Cloth	18%	84	91	84	82
	Plastic	18%	63	67	74	51
St. Paul heated	Cloth	18%	87	92	91	81
	Plastic	18%	57	38	59	49
Boise unheated	Cloth	18%	85	79	89	87
	Plastic	18%	75	63	60	63

^aSeed treated with Captan at recommended rate, and seed inoculated with a mixture of *A. flavus*, *A. amstelodami*, *A. ruber*, *A. repens*, *A. ochraceus*, *A. candidus*, and *A. restrictus*.

NOTE: Four replicates of 50 seeds each were used in determining the vigor.

TABLE 4. Moisture content of seed stored for 8 months in three different environments.

Moisture content at harvest	% moisture content before storage	% moisture content at end of storage period					
		St. Paul unheated		St. Paul heated		Boise unheated	
		C ^a	P	C	P	C	P
25%	11%	10.9	11.3	8.8	9.4	8.0	10.3
25%	18%	12.4	16.8	8.9	12.2	8.2	14.5

^aC, cloth bags; P, plastic bags.

while the moisture decreased to 8.8% in a heated building at St. Paul and 8.0% in an unheated building at Boise, Idaho. Seed with an original moisture content of 11% and stored in plastic bags also decrease slightly in moisture content when stored in a heated building at St. Paul, while it remained approximately the same in the two other environments. Seed with an original moisture content of 18% and stored in cloth bags decreased drastically in moisture content at all three storage sites. The same seed stored in plastic bags also decreased after 8 months of storage in the different environments but the decrease was not as severe.

The results indicate that if pea seed is placed in storage with a high moisture content it will decrease whether it is stored in a plastic bag or a cloth bag. When the seed was stored in a heated building the reduction in moisture was greater than when stored in an unheated building. The data show that seed stored in plastic bags can lose moisture but in no cases did the seed gain an appreciable amount of moisture.

The low relative humidity existing in the western intermountain area explains why western grown pea seed has been stored for more than 2 years in cloth bags and still remained as quality seed. Pea seed stored in the midwest has not been of good quality after two years of storage in cloth bags. The pea seed stored in plastic bags would not absorb moisture in the high humidity areas and would, therefore, not be vulnerable to deterioration by storage fungi. As shown by the results, seed could be stored for a number of years in the midwest and still be quality seed.

DISCUSSION AND SUMMARY: Due to the fact that long-term storage of pea seed in the midwest was previously unsuccessful by canning companies, it would seem reasonable to assume that impermeable containers would make possible the storage of seed with low moisture contents in areas of high relative humidity and high temperature. Although the seed did not decrease in germination in any of the seed lots there is reason to believe that if the seed would have been stored through the summer

months when temperatures are optimum for mold growth a decrease in germination would have occurred. However, deterioration did occur in seed stored in plastic bags with an original moisture content of 18%.

Seeds stored at moisture contents below 13%, irrespective of type of container, yielded storage molds. It seems possible that the fluctuating relative humidity (often greater than 75%) occurring in the storage areas created a microenvironment suitable for fungus development around the seed without increasing the moisture content of the seed.

The effectiveness of a fungicide treatment of mold-free pea-seed in preventing invasion by fungi during storage indicates another possible method of maintaining high quality seed. These studies indicated also that the fungicide actually kept seed free from molds during storage, and did not act merely to suppress growth of fungi when seed were subsequently plated on nutrient agar. However, results indicate that caution should be exercised in treating seed of high moisture contents (18%) due to possible chemical toxicity. The treatment of artificially inoculated seed with a fungicide (Captan), greatly reduced the percentage of seed yielding storage molds as compared to non-treated seed. Invasion of non-treated, non-inoculated seed did occur, indicating the importance of a fungicide.

In general, the maintenance of quality pea seed for canning purposes in the midwest can be best obtained by storing seed with a moisture content of 11-12%, treating seed with a fungicide and storing in water impermeable containers.

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