Effect of atmospheric humidity during ripenning on the pod setting and seed germination in excised alsike clover (Trifolium hybridum L.)

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Effect of atmospheric humidity during ripenning on the pod setting and seed germination in excised alsike clover (*Trifolium hybridum* L.)

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Introduction

Certain regions of the world because of favorable climatic condition are better adapted to growing the legume seed than other sections are. One of the favorable climatic factors is dry weather. It is known that the soil and atmospheric humidity affect the seed setting of legumes and a few investigations have been done with legumes on this subject. It would appear that atmospheric humidity affects the hard seed percentage of legumes, however there are few reliable studies on this subject. This fact probably due to the difficulty of atmospheric humidity control within the cabinet for growing plants.

Recently, phytotrons are building in many countries including Japan, but few studies have been done with legumes on the effect of atmospheric humidity on the seed production and germination. In Japan, atmospheric humidity in season of cross-pollination and ripenning of legumes is high and the author is interesting in this subject.

Review of literature

There are a few reports on the effect of atmospheric humidity on the seed production of legumes. Alter (1920) stated that observations have indicated that the production of alfalfa seed is best in area which the air humidity is usually low and the soil moisture below optimum. Blinn (1920) concluded that dry climatic conditions with high temperatures seem to be among the most essential requirements for seed

production of alfalfa. Hollowell (1929) found in his experiment that atmospheric humidity does not affect the setting of red clover seed under greenhouse or field conditions.

Grandfield and Zink (1937) have used the chamber for the control of air temperature and relative humidity, in which sulfuric acid was used to control humidity. This equipment permitted the studies to be carried on in natural light under greenhouse conditions. According to Grandfield (1945) the number of flowers setting pods of alfalfa increased as the relative humidity decreased from 90 to 10 per cent. At 80°F., the difference between the percentage of pod set at 10 per cent relative humidity and the percentage at 90 per cent was 24.

There are few studies on the effect of atmospheric humidity during ripenning of seed on the germination percentage of legumes. According to Jones (1928), Hiltner (1903) thinks that crops ripenning in a dry climate or in dry seasons show a greater proportion of hard seed. Jones stated that the hard seed percentage in *Vicia villosa* can be distinctly influenced by the storage temperatures and humidities, high humidity usually decreasing the proportion of hard seed through its influence on the plant. The data reported in his paper shown considerable variation in the hard seed percentages in *Vicia villosa* from different sources. Wheeler (1950) stated that in certain forage seeds, hard seed percentage varies with (1) species or variety (2) stage of maturity, and (3) conditions under which produced.

Materials and methods

Stems bearing florets of alsike clover were gathered from plants growing in pots. They were then brought to the laboratory, any withered lower leaves were removed. The stems ranged from 15 to 16 cm. in length and each stem was bearing a head and two leaves.

In the first test, all wilted or unopened florets were removed and florets varied from 60 to 70 in number per head, and study was made in three replications using freshly open-pollinated florets. In the second test, wilted florets were used and in the third test, freshly opened florets were cross-pollinated by honey bees within bell jar without replication in each test. Analysis of variance was made in the first test.

The stems were placed in a glass container holding about 50 cc. of 1 per cent glucose solution. According to Battle (1949), 2 per cent sucrose solution was selected as the culture medium for red clover and about three-fourths of an inch of the cut end was charred in a Bunson flame. However, the author's preliminary trials indicated that 1 per cent glucose solution served as a satisfactory medium for keeping the

leaves of alsike clover alive and charring of the cut end was not necessary. Glucose solution was renewed every 3 days.

In the sampling of materials, the heterogeneity of alsike clover was under consideration, and the materials were gathered from same several plants in each test.

The glass containers were covered with bell jars, and dry or wet atmospheres which passed through the scrubbing bottles holding sulfuric acid or water were continually induced in bell jars at the rate of 700 to 1,400 cc. a minute. The apparatus was shown in Fig. 1.

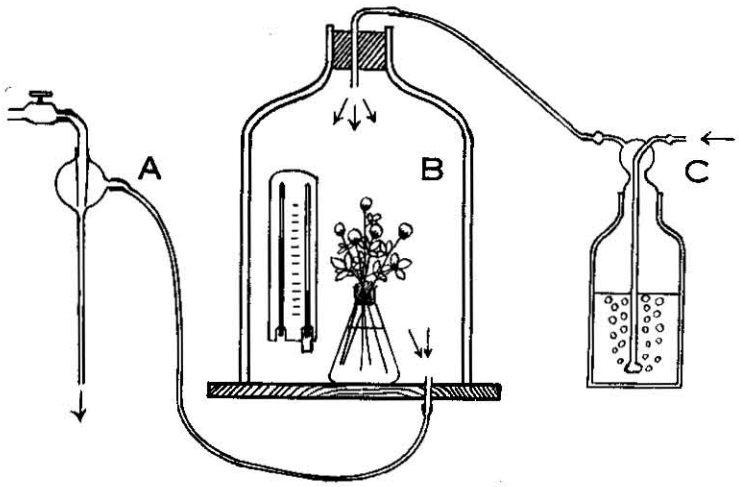

Fig. 1. Apparatus for control of atmospheric humidity.
A, Sucker; B, Bell jar; C, Scrubbing bottle.

Experiments were conducted in fields. To prevent the occurance of high temperatures within bell jar, it was necessary to place a shade over the apparatus and to dash water over bell jar when the sun was shining. In cold weather, it was necessary to pass air through ice blocks in order to obtain dry atmosphere.

The atmospheric humidity and the temperature within bell jar and field were measured every day. The season of experiments was from July 19 to August 8, 1956. The artificial control of humidity within bell jar was lasted for 2 weeks and from then glass containers were placed on a table in a moderately light laboratory.

When the heads were ripe, the seeds were removed and counted. The seeds were storaged in the decicators and germination tests with seeds obtained in the first test were made with three replications in 3, 8 and 11 months after harvesting. Apparent germination percentages are those calculated on the basis of the number of seeds planted. Apparent germination percentages are used in the results and discussion.

Results

1. *Atmospheric temperature and humidity*

One of the measurement results of atmospheric temperature and humidity within bell jars at 9 a.m., 2 p.m., and 6 p.m. was shown in Fig. 2. Atmospheric temperature was measured by mercury thermometer. Temperatures in field conditions during the experiment were not constant and therefore, as shown in Fig. 2, atmospheric temperature and humidity within apparatus were not constant. However, relative atmospheric humidity within apparatus varied from 85 to 95 per cent in the high humidity plot and those of the low humidity plot was from 50 to 80 per cent, and remarkable differences in relative humidity between in the high and low humidity plots were obtained. There were no marked differences in temperatures between in the two plots. It seemed that these differences in atmospheric humidity between in the high and low humidity plots served the purpose of the experiment. Temperature and humidity within other series of apparatus were approximately same as Fig. 2.

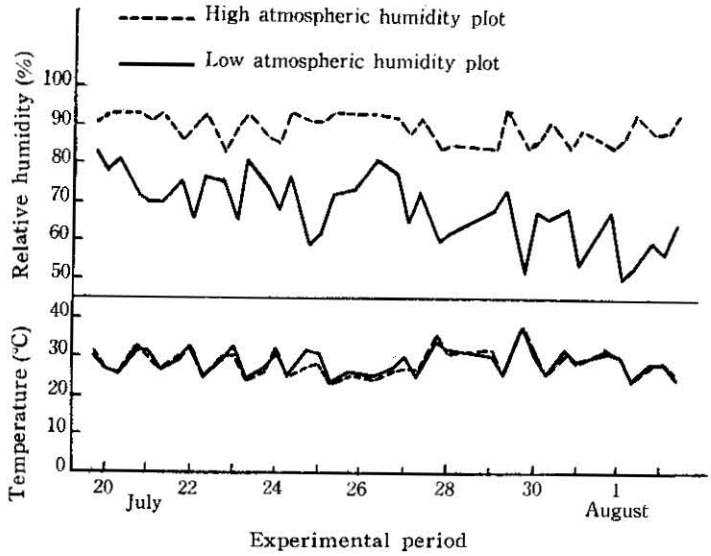

Fig. 2. Atmospheric temperature and humidity within bell jars (A-b).

2. *Observation during experiment*

The leaf color of alsike clover in the low humidity plot became paler green than that of the high humidity plot at 2 to 3 days after beginning of treatment, but at last of the experiment the latter lost the greeness and the margin parts of leaf were pale green. The florets

in the low humidity plot were of a pale brown color, while those of the high humidity plot were dark brown in color.

The transpiration was more remarkable in the low humidity plot than in the high one. At the beginning of experiment, the decreases in glucose solution were 35 to 40 cc. per glass container holding 5 stems every 3 days in the low humidity plot, whereas the corresponding numbers were 15 to 20 cc. for the high humidity plot. Transpiration of alsike clover decreased with the lapse of time.

3. Per cent pod set

The pod setting data for the first test are given in Table 1. Pod set of freshly open-pollinated florets on excised alsike clover varied from 34.1 to 61.3 per cent and general speaking, these percentages were somewhat lower than that of open-pollinated florets in the field conditions, whose value was 61.1 per cent. However, it seemed that the experiments were carried out successfully. As shown in Table 1, atmospheric humidity did not significantly affect the pod setting of freshly open-pollinated florets on excised alsike clover stem.

Table 1. Effect of atmospheric humidity on the pod setting of freshly open-pollinated alsike clover.

Apparatus	Treatment	Number of plants	Number of heads	Number of florets	Number of florets setting pods	Per cent pod set
A-a	Low humidity	4	15	543	329	60.5
	High humidity	4	20	1,104	677	61.3
A-b	Low humidity	4	30	1,924	799	41.3
	High humidity	4	26	1,518	605	39.9
A-c	Low humidity	7	46	2,675	911	34.1
	High humidity	7	54	2,822	1,207	42.8
Natural condition		12	117	7,888	4,816	61.1

Table 2 presents data on the pod set of the second test with the wilted florets, and the pod set on excised stems growing in the low

Table 2. Effect of atmospheric humidity on the pod setting of wilted florets of alsike clover.

Treatment	Number of plants	Number of heads	Number of florets	Number of florets setting pods	Per cent pod set
Low humidity	6	17	1,144	599	52.4
High humidity	6	18	1,189	633	53.2

humid atmosphere was 52.4 per cent and that of the high one was 53.2 per cent. Atmospheric humidity did not affect the pod setting in the second test.

Table 3 presents data on the pod set of the third test with florets which were cross-pollinated by honey bees in bell jar. The pod set of

Table 3. Effect of atmospheric humidity on the pod setting of alsike clover, cross-pollinated by honey bees in bell jar.

Treatment	Number of plants	Number of heads	Number of florets	Number of florets setting pods	Per cent pod set
Low humidity	3	25	958	210	21.9
High humidity	3	28	1,005	37	3.7

stems growing in the low humid atmosphere was 21.9 per cent, while the corresponding number for the high one was 3.7 per cent. The high atmospheric humidity significantly decreased the percentage of pod setting in the third test, and the percentages were lower than those of the first and second tests.

4. Germination of seeds

Table 4 presents data on the germination of alsike clover seed produced in the first test. In general, the germination percentages of alsike clover seeds obtained in the first test were very low when seeds

Table 4. Effect of atmospheric humidity on the germination of alsike clover seed produced in the first test (in per cent).

Apparatus	Replication of germination test	Season of germination test: First Oct. 26 to Nov. 5, 1956		Second Feb. 19 to Mar. 1, 1957		Third June 25 to July 5, 1957	
		Low humidity	High humidity	Low humidity	High humidity	Low humidity	High humidity
A-a	1	0	10	11	21	16	14
	2	1	8	7	18	18	12
	3	7	9	11	15	8	15
A-b	1	3	14	10	22	12	29
	2	0	18	13	23	15	29
	3	4	18	10	29	19	27
A-c	1	6	15	17	38	29	36
	2	0	30	30	41	21	41
	3	5	24	15	35	18	43
Average		2.9	16.2	13.8	28.0	17.3	27.3
Seed produced in natural condition		13.7		24.5		25.3	

were not scarified. However, the germination percentages of seeds produced in natural coditions were also low and those were 13.7, 24.5 and 25.3 per cent for the first, second and third germination tests respectively. The results of analysis of variance as applied to those data are presented in Table 5. As shown in Table 5, atmospheric humidity significantly affected the germination percentage of seeds produced in freshly open-pollinated florets on excised alsike clover stem and the germination percentages of seeds obtained in the high humid atmosphere were higher than those of the low one. The germination percentages of alsike clover seed significantly increased as the days after harvesting increased in the two cases.

Table 5. Analysis of variance of germination percentage.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	Ratio of variance
Humidity (H)	1,873	1	1,873.00	121.07**
Season of germination test (S)	1,803	2	901.50	58.27 **
Apparatus (A)	1,817	2	908.50	58.73**
Interaction H×S	56	2	28.00	1.81
H×A	354	2	177.00	11.44**
S×A	241	1	241.00	15.58 **
H×S×A	28	7	4.00	
Error (E)	557	36	15.47	
Total	6,729	53		

** Significant at the 1-per cent level.

Discussion

In this experiment, although the humidity was not constant, the control of atmospheric humidity within the plant growth chamber was successful. If this apparatus was placed in the temperature controled large chamber, the experimental condition would be more satisfactory.

It is interesting to note that the transpiration was more remarkable in the low humidity plot than in the high one. The decrease in glucose solution in glass container was more marked in the low humidity plot than in the high humidity plot, whereas the decreases in solution in the glass containers without excised alsike clover stems were very small in the two plots. Therefore, the following observations seem justified under conditions of the experiments that the difference in solution decreases in glass containers between the high and low humidity

plots probably was due to the difference in the transpiration between the two plots.

According to the results of this experiment, atmospheric humidity did not significantly affect the pod set percentage of freshly open-pollinated or wilted florets on excised alsike clover stems. The results in this study agree with those of Hollowell's study (7) on red clover. However, when the stems with unopened florets were excised and covered with bell jar and were cross-pollinated by honey bees in bell jar, the activity of the insect was more vivid in the low humid atmosphere than in the high humid atmosphere. Most of the honey bees in the high humid atmosphere died soon after beginning of experiment. In the case of cross-pollination by honey bees in bell jar, pod set was markedly lower in the high humid atmosphere than in the low humid atmosphere. It seems reasonable to conclude that the inactivity of honey bees accounts for the lower percentage of pod setting in the high humid atmosphere. There is a good reason that certain regions of the world because of dry climate are better adapted to growing the seed than other sections are, and in dry climate pollinators such as honey and bumble bees play their role vividly.

According to Battle's report (2) on red clover, the seeds produced on excised stems were uniformly impermeable, and there were many impermeable seeds in alsike clover seeds produced in this experiment, nevertheless the alsike clover seeds were not always impermeable and some of them were permeable.

Although the germination percentage of seeds obtained in this experiment was low, but not especially lower in percentege of germination than that of seeds produced in natural conditions.

In general, alsike clover seeds produced in Kyūsyū, warm area in Japan, are low in percentage of germination, and it appears that high temperature partly affect germination of this legume seed.

Seeds from the high humid atmosphere were higher in percentage of germination than those from the low humid atmosphere, and this result agrees with Hiltner's observation that crops ripenning in a dry climate or in dry seasons show a greater proportion of hard seed.

In this experiment, the effect of afterripenning on the germination of alsike clover seed was remarkable. It seems that it will be necessary to scarify the alsike clover seeds produced in Kyūshū.

Summary

1. This paper gives the results of study on effect of atmospheric humidity during ripenning on pod setting on excised alsike clover stems and germination of seed produced in this experiment.

2. Excised stems were placed in a glass container holding about 50 cc. 1 per cent glucose solution and the glass containers were covered with bell jar, and dry or wet atmospheres which passed through scrubbing bottles holding sulfuric acid or water were continually induced into bell jars.
3. The transpiration was more remarkable in the low humidity plot than in the high humidity plot.
4. Atmospheric humidity did not significantly affect the pod setting of freshly open-pollinated or wilted florets on excised alsike clover stems.
5. Excised stems bearing unopened florets were covered with bell jar, and when the florets opened they were cross-pollinated by honey bees in bell jar. In this test, the percentage of pod setting was lower than in the case using freshly open-pollinated florets. The percentage was significantly lower in the high humidity plot than in the low one.
6. In general, the germination percentage of alsike clover seeds obtained in this experiment were very low when seeds were not scarified. However, the seeds were not always impermeable. Atmospheric humidity significantly affected the germination percentage of seed produced in freshly open-pollinated florets on excised stems, and the germination percentage of seeds obtained in the high humidity plot was higher than that of the low one.
7. The germination percentage of alsike clover seed significantly increased as the days after harvesting increased.

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