SOME FACTORS AFFECTING HYBRIDIZATION OF TALL FESCUE (*FESTUCA ARUNDINACEA*, SCHREB.) UNDER POLYETHYLENE COVERED ENCLOSURES

by

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SOME FACTORS AFFECTING HYBRIDIZATION OF TALL FESCUE (*FESTUCA ARUNDINACEA*, SCHREB.) UNDER POLYETHYLENE COVERED ENCLOSURES

Introduction

Forage crop breeders are in need of a method of isolation insuring maximum exclusion of foreign pollen, while facilitating the increase of sufficient seed for progeny testing. This isolating device must be of such a nature that it is adaptable to a variety of crops, substantial enough to withstand the elements, inexpensive to build, and easily stored. The plants enclosed should be subjected to a microclimate as nearly normal as possible, to avoid deleterious pathological and pollen viability problems arising from temperature and moisture difficulties.

Conventional techniques of making crosses between cross-pollinated plants are time consuming, laborious, and inadequate. Intercrossing may be accomplished by hand emasculation and pollination, and if the material is self-sterile, by mutual pollination, spatial, or shelter isolation. Hand emasculation is virtually impossible for most forage crop species. Mutual pollination of adjacent inflorescences in parchment paper bags, offers perfect isolation, but provides only a small quantity of seed. Spatial isolation, where two or more genotypes are grown in an area isolated from other plants of the same species with which they might cross, is limited by the availability

and suitability of natural isolation. Shelter isolation, where two or more entire plants are enclosed in a pollen-proof device, has been impracticable due to the lack of a satisfactory pollen-proof covering material which did not seriously impair the plant microclimate.

The objectives of this research were to study some of the factors (particularly temperature and humidity) affecting the yield and quality of seed from two self-sterile, but cross-fertile genotypes of tall fescue (Festuca arundinacea, Schreb.). The plants were grown in the field, under field conditions, but were isolated during the flowering period by polyethylene covered wood frame enclosures. Daily records were made regarding temperature maximums, and observations were taken with respect to humidity and condensation within the shelters. Data from the Oregon State Meteorological Station situated one half mile distant from the plots were used in comparing temperature, humidity, overcast, and precipitation records, as they related to seed production within the enclosures.

Review of Literature

Grass flowers are mostly small, delicate, and consequently difficult to manipulate individually (Smith, 17, p. 338). In addition several days are required for an inflorescence to complete flowering. Hence enclosure of the entire inflorescence at least, is essential. In this way, selfing of the enclosed inflorescence can be enforced.

Keller (12, p. 1614) reported that using self sterile plants, isolation for control of pollination could be obtained either by distance from plants with which they might cross, or by bagging. Jones (10, p. 25-40), Brewbaker (2, p. 323), and Griffiths (6, p. 23-29) have shown that rather large areas would be required to conduct a very extensive program using spatial isolation. Isolation under bags reported Keller, has some distinct advantages, such as "1. Isolations can be made any time prior to anthesis, 2. A large number of isolations can be made in a short time, and 3. Bags afford a high degree of protection. However, seed yields are generally lower under bags than by distance isolation of unbagged inflorescences."

Frandson (5, p. 307) discussed the difficulties and inadvisability of using single plant progenies in trying to evaluate new strains of forage species. Eventually the need for a progeny test becomes a significant part of a

forage breeding project, and adequate progeny tests are difficult to accomplish using the small populations peculiar to single plant origins.

Jenkin (9, p. 22-24) reported that by enclosing one or more inflorescences from each of two or more genotypes of a self-sterile species, "mutual pollination" of the enclosed inflorescences could be effected. Enough selfing probably occurs, except in highly self-sterile species, that "mutual pollination" techniques may not be suited to highly critical breeding programs.

Smith (17, p. 338) and Jenkin (9, p. 10) report that grass pollen is very small and may penetrate all but the finest muslin type cloth. This has forced breeders to consider other materials to afford isolation from unwanted pollen.

In techniques for obtaining selfed lines from naturally cross-pollinated species, isolation would not have to be so complete. Bateman (1, p. 243) points out that hybrids resulting from the entry of foreign pollen would be detectable by their increased vigour and size, and could safely be rogued on that basis. In a crossing program, entry of foreign pollen would be less readily detectable since these hybrids would likely show no evident difference in size and vigour, compared to the progeny of the desired cross. However, if reasonable care is taken very little pollen should penetrate the isolating medium

employed. Stapledon (18, p. 40) suggests that with several inflorescences actively shedding pollen inside the isolation device, the few pollen grains likely to gain entry would be in such relative minority, as to be rather highly selected against. Jenkin (9, p. 10) emphasizes the need of differentiating between a perfect but impracticable approach and an approach less than perfect, but nevertheless workable.

Pollen Isolation

To obtain large enough samples of seed for adequate progeny testing, various methods and materials have been used to effect controlled pollination. Much of this work has been devoted to structures and coverings which could enclose completely, one to several plants.

Waller and Thatcher (20, p. 192) suggest the ideal isolation device to be of a size and shape readily adaptable to various crops, and substantial enough to withstand the weather both during pollination and for some time afterward. Provision must be made for the escape of excess moisture from inside, prevention of entry of water from the outside, and adequate ventilation. Care must be taken to guard against the accumulation of excessive heat from the sun's radiation. In other words, as little interference as possible should be offered to the plant. In addition, these devices should be inexpensive to build and store, and

should be relatively maneuverable.

Hanson and Carnahan (7, p. 77) report that to date most attempts at controlling pollination by using an isolation device have been something less than successful. Consequently, most hybridization programs have had to rely on isolation by distance, or by parchment paper bags enclosing two or more inflorescences.

Stapledon (18, p. 39-40) described the need for choosing plants which will flower contemporaneously when enclosed or isolated together. That plants do so one year, does not necessarily mean they will another year, when under enclosure. Stapledon used shelters which were fabric covered, with glass tops. Shutters were installed at ground level and were opened after flowering had finished. It was believed that agitation of the panicles would be necessary to effect pollen transfer. To accomplish this, the leeward shutters were opened and a stick was inserted to agitate the panicle bearing stems. This was done each day of the blooming period at the time of maximum pollen dehiscence. Only during exceptional years were satisfactory results obtained using shelters at Aberystwyth, and most controlled pollination studies there have been conducted in the greenhouse.

Malte (15, p. 26) described shelters built having three sides and the top covered with heavy canvas, but the side to the lee of the prevailing wind covered with

cheesecloth. The shelters were large and roomy giving maximum air volume to better simulate a more normal atmosphere.

It is likely however, that in most areas the prevailing wind would not be consistent enough to chance leaving one side of the shelter so exposed to stray pollen.

Jenkin (9, p. 10) trying to enforce self-pollination in a naturally cross-pollinating grass species concluded that satisfactory results could be obtained using the finest weave muslin available. Even this material had pores large enough to permit grass pollen grains to pass through. However, these pores were almost completely filled with the tangled ends of the cotton fibres. Although not offering 100 percent protection against stray pollen, Jenkin feels that this extremely fine-weave cotton would probably suffice for any but the most critical experiment.

Smith (17, p. 338) mentions that grass pollen is small enough to penetrate all but the finest muslin, and that for this reason most breeders use a parchment paper bag enclosing a few inflorescences for mutual pollination. This type paper gives perfect isolation, has little effect on temperature, and does permit some air exchange.

Wells et al (21, p. 379-380) enclosed emasculated heads of Abruzzi Rye in cloth, and found that the closer the mesh, the lower the seed set. Various types of cloth

gave a seed set range of 0-57 percent of normal, depending on the mesh. It was concluded that cloth shelters would not be satisfactory due to leakage of stray pollen, and the high cost of construction.

Bateman (1, p. 243) reports that sugar beet breeders maintain inbred lines by using brother-sister matings. Pollination is controlled by enclosing the plants in glass cages which have ventilators at the top and bottom on the side to the lee of the prevailing wind. These cages are left on throughout the long flowering season without apparent injury to the plants. Although isolation is not perfect, hybrids resulting from stray pollen are easily detected by their vigour and size, and could be eliminated on that basis.

Fluorescence in Lolium Spp.

Foy (4, p. 389) described a discovery where "roots of seedlings of Italian ryegrass (Lolium multiflorum) grown on white filter paper would bring about a condition in the paper which, when examined under filtered ultra violet light, would render it fluorescent, while the seedlings of perennial ryegrass (L. perenne) would not." Foy found that this ability to exhibit fluorescence was peculiar not only to annual species of Lolium, but also to certain seedlings thought to be perennial ryegrass. When these fluorescent seedlings were grown on to a stage where vegetative

characters made identification possible, they were identified as the intermediate false perennials.

Corkill (3, p. 134) described results indicating this fluorescence to be dependent on a single dominant Mendelian factor.

Temperature

Pearson (17, p. 501-506) reports that temperature has to a large degree been the determining factor in successful controlled-pollination. Temperatures were measured in bags of glassine, cellophane, white bond paper, manila and muslin which were inverted over branches of tomato leaves. Four or five layers of leaves covered the bulb of the glass thermometer used. Air temperatures were taken by exposing the bulb of the thermometer directly in the rays of the sun. Temperatures thus secured could be compared with those registered in empty bags, and those registered by the thermometers wrapped in leaves but not enclosed in bags. Temperatures were read daily during August, and showed distinct differences between treatments.

Pearson found muslin to be much superior in avoiding temperature rise, while manila and bond paper were nearly as good. Their maximum temperatures approximated those of exposed leaf tissue. Glassine, and cellophane enclosures were much warmer since they offered no shade, and permitted heat loss through radiation rather than transpiration.

Temperatures registered inside empty bags were much higher than those of the outside air. Bags of muslin in this case registered the highest.

In conclusion, Pearson reports: "Distinct differences in temperature of tissue enclosed in bags of various materials, depending on their shading effect, porosity, and energy transformation, were noted." Bags of dark porous material (muslin) enclosing leaves registered a lower internal temperature, while transparent bags of cellophane registered a higher temperature. Pearson also states that since these experiments were done in the field, with environmental control impossible, other factors not studied may be involved in affecting the responses of the complex photo-chemical reaction within a plant, to a given set of conditions.

Maheshwari (14, p. 377) in reviewing pollen longevity studies, describes grass pollen as being "notoriously ephemeral." The data discussed indicated that the most important factor in pollen storage is temperature, and second is relative humidity.

Humidity

Stapledon et al (17, p. 17-18) described several pollen proof coverings used at Aberystwyth, Wales. Glazed bags, opaque paper, fabric cages and waxed cylinders have been used. Waxed cylinders withstood practically all

weather conditions, but since insufficient aeration was provided, the enclosed inflorescences tended to become mouldy. Calico-like fabric with pores larger than grass pollen but filled with a complete entanglement of cotton ends probably afforded absolute protection. The best results, as judged by fertile seed, have been obtained with glazed bags, and the least satisfactory with paper bags and waxed cylinders.

Malte (15, p. 26) in discussing breeding of wind pollinated grasses, stated that self-fertilization operations should be conducted under the best possible guarantees. The isolation of individual heads, or a few heads together in paper bags is satisfactory as to foreign pollen exclusion, but otherwise is unsatisfactory because the isolation is perfect and provides no escape of transpirational waters. This keeps the atmosphere in the bags overcharged with humidity which often renders grass pollen unviable. Cloth covered cages have been successful in providing large quantities of seed, but little assurance of freedom from foreign pollen is offered.

Knoll (13, p. 5-7) reported finding a distinct negative correlation between seed setting, and absolute air moisture. High temperatures may have been a factor, or there may have been an interaction between high moisture and high temperatures, since the dependence of seed setting on these two factors is complex. Knoll noted differences

in the effect of various isolating materials on absolute air moisture and seed set. The lowest absolute air moisture, and highest seed set occurred under parchment paper, whereas the highest moisture, and lowest seed set occurred under oiled and waxed isolation materials.

Knoll also found that absolute moisture and consequent seed set are influenced by the size of the isolation chamber, and the number of plants enclosed together. In general, the larger the chamber, and fewer the plants enclosed, the lower the absolute moisture. Knoll concludes that the direct cause of poor seed set in cages is the injury to pollen by high moisture and temperature.

Brewbaker (2, p. 328-336) measured relative humidity and temperature inside bags of various sizes and types of paper and cloth enclosing Beta vulgaris inflorescences. Larger bags of vegetable parchment gave the most promising results while seed set was poor in small parchment and cellophane bags. Wide differences in relative humidity, and in temperature were found between bags of different materials, but these differences exhibited no correlation with the observed differences in seed set.

Pollination Cycles in Tall Fescue

Jones et al (11, p. 139) reported much seasonal variation in size of pollen grains of several species tested, and little seasonal variation in size for others. For

instance, meadow fescue pollen grains were found to be 37.6 $\pm$ 0.7 microns in diameter. Jones et al (10, p. 9-10) reported pollen dehiscence in tall fescue to be restricted almost entirely to the hours between 1:30 and 3:30 p.m., with a maximum amount shed at 2:30 p.m. These hours coincide with what is usually the hottest time of the day.

Materials and Methods

Plant Material

In the summer of 1956, two self-sterile genotypes of tall fescue (Festuca arundinacea, Schreb.) numbered 224 and 230[1], were cloned into 136 propagules each. These propagules were planted in plant bands in the green house, where they were maintained until root and top growth were vigorously established.

On October 4, 1956, these well established propagules were transplanted to 17 plots in the field. Each plot consisted of 16 plants, 8 from each genotype, planted 8 inches apart, alternately in two rows one foot apart. (Figures 1 and 2, p. 15 and 16)

Enclosures

During the winter of 1956-57, 14 enclosures each six feet long, three feet wide, and six feet high were constructed to be used as isolation shelters. Finished 1x6 inch lumber was used for the frame of the 6x6 foot side panels, and 1x4 inch lumber for the 3x6 foot top and end panels. (Figure 5, p. 19) These panels were covered with 1.5 mm. thick sheets of polyethylene film manufactured by the Bakelite Company. The sides facing north, and the tops

[1] Self-sterility data and genotype numbers from Dr. J. R. Cowan: unpublished data.

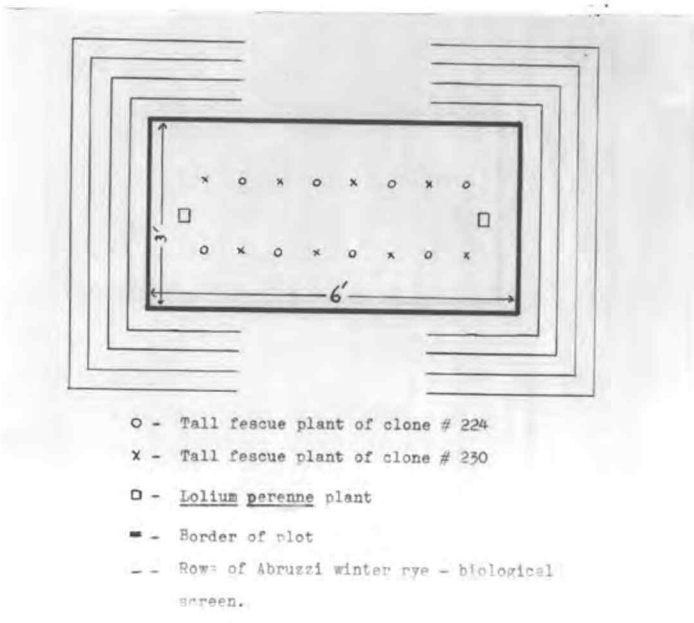
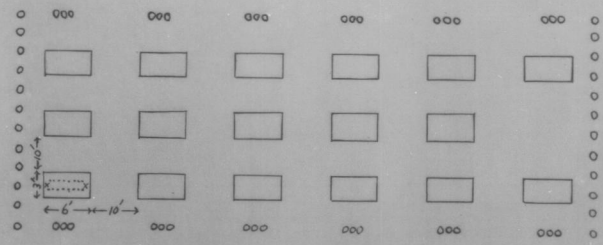


Figure 1. Single plot, showing dimensions, and arrangements of tall fescue plants, Lolium plants, and biological screen area.

Figure 2: Project area, showing plot arrangement, plant arrangement, and dimensions.



..... - Tall fescue plants inside the shelter

x x - Lolium perenne plants inside the shelter

ooo - Lolium multiflorum plants outside the shelter

Figure 3. General view of plot area - May 1957 showing rye used as biological screen.

Figure 4. General view of plot area - May 1958 showing oats used as biological screen.

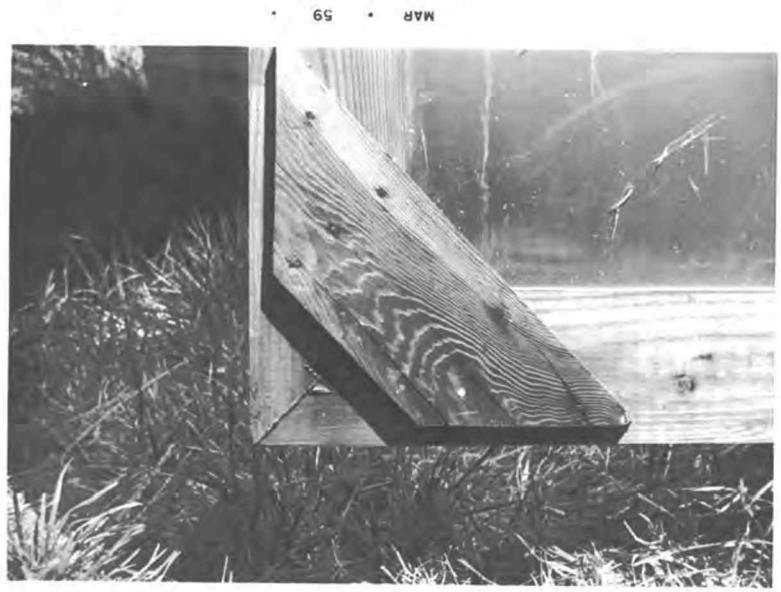


Figure 5. Detail of corner construction.

Figure 6. Detail of method used to hold plastic to frame. Note plastic rolled around lath, and nailed through lath. (Nails in lath in picture partly pulled out to show detail.)

were covered with a white opaque film, whereas the other sides were covered with a transparent film.

The polyethylene was fastened to the frames by rolling the ends of the sheet around a one inch lath and nailing through this roll using a shingle nail. (Figure 6, page 20)

In 1958, the frames were re-covered using 2.5 mm. thick transparent polyethylene film. To support the polyethylene film against wind damage, a 1x2 inch strip of wood was fitted across the large six foot square panels. The polyethylene was nailed to this through a lath. (Figure 7, p. 22)

The panels were fastened together with nails in 1957, as they were assembled over the plots of tall fescue on May 11. In 1958, the panels were secured together in the workshop by a system of bolts and wing nuts. (Figure 8, p. 23) A gasket of thick cotton twine was laid between all edges where the panels joined, and caulking compound was daubed into all corners from the inside of each assembled shelter. The finished shelters were then transported by truck to the field, where they were set down carefully over the tall fescue plots, on April 26.

In 1957, two one inch by three feet cores of soil were taken from under each type of shelter; from a pathway between the shelters; and from an adjacent fallowed field. These cores were weighed, dried at 102°C for three days, and reweighed to calculate soil moisture differences

Figure 7. Side of shelter showing addition of center support during 1958. Note transparency of polyethylene, and luxuriant growth inside shelter. Note stake at bottom, driven into ground at an angle. One of these at each side of each shelter was sufficient anchor against winds.

Figure 8. Detail of method of assembly of panels during 1958. Note eye bolts, wing nuts and steel plates.

between the areas sampled.

A Norwood Director light meter was used to determine the amount of light penetrating the polyethylene shelters. Readings were taken over several hours during one day each year, and compared with readings taken in direct sunlight outside the shelters.

Treatment and Randomization

The shelters were of four types in 1957. Four shelters were constructed with a six inch wide vent at ground level in each end of each of the shelters. (Figures 9, p. 25; and 10, p. 26) Similarly, four shelters were built with twelve inch wide vents (Figure 11, p. 27), four shelters had eighteen inch wide vents (Figure 12, p. 29), and two shelters were unvented, that is, completely enclosed on all sides. (Figure 13, p. 30) As a control, two plots were left exposed, with no shelters over the tall fescue. (Figure 14, p. 31)

In 1958, only the six inch wide vented shelters were compared with completely enclosed shelters, and with unsheltered, or exposed plots. Two additional treatments were included. Ventilators three inches in diameter were installed on the top of two shelters. (Figure 15, p. 32) One of these shelters was completely enclosed, while the other shelter had a six inch vent at ground level at each end. (Figure 9, p. 25) These vent pipes were loosely

Figure 9. End of shelter showing six inch vent, and biological screen.

Figure 10. Showing six inch vent and biological screen during 1958.

Figure 11. End of shelter showing twelve inch vent and biological screen.

packed with fibre-glass insulation material to filter out dust, insects, rain or any other material which might tend to blow in if the pipes were left open. An inspection door three feet square was made in each of these shelters.

To determine if the clones of tall fescue used were completely self-sterile, plants of clone 224 were removed from two plots which were to be covered by an unvented shelter. Thus, plants of clone 230 only, were isolated in these shelters. Similarly, plants of clone 230 were removed from two plots leaving plants of clone 224 only, enclosed together.

To lessen the possibility of wind-born pollen entering the shelters, a screen of plants was seeded across the ends of each plot. (Figure 1, p. 15) Four rows of Abruzzi winter rye were seeded using a Planet Jr. seeder, in 1957. In 1958, Grey winter oats were broadcast seeded by hand into a one foot wide strip around the ends of each plot. This strip was then raked lightly to cover the seeds with soil.

A preliminary examination of the uniformity and density of the biological screen around each plot determined the distribution of treatments each year. The vented shelters were alloted at random to the plots with the most uniform, dense stand of the plants composing the screen. The unvented shelters were alloted to those plots bearing a poor stand. In 1957, the five treatments were

Figure 12. End of shelter showing eighteen inch vent and biological screen.

Figure 13. Showing unvented shelter. Biological screen not required.

Figure 14. Showing plot with no shelter. Note height of rye biological screen.

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Figure 15. Showing vent at top of shelter. Note open inspection door.

Table 1. Treatments employed

Treatments - 1957	Number of shelters so treated
1. Plots with no shelters:	
a. All four rows of rye retained	2
2. Plots covered with shelters with six inch wide vents:	
a. All four rows of rye retained	2
b. Only two rows of rye retained	2
3. Plots covered with shelters with twelve inch wide vents:	
a. All four rows of rye retained	2
b. Only two rows of rye retained	2
4. Plots covered with shelters with eighteen inch wide vents:	
a. All four rows of rye retained	2
b. Only two rows of rye retained	2
5. Plots covered with shelters with no vents:	
a. All rows of rye removed	2
Treatments - 1958	
1. Plots with no shelters = control	3
2. Plots covered with shelters with six inch wide vents	4
3. Plots covered with shelters with no vents	4
4. Plots covered with shelters with three inch diameter top vents:	
a. Shelter with six inch ground level vents only	1
b. Shelters with no ground level vents	1
5. Single clone plots - Shelters with no vents:	
a. Plots with clone 230 only	2
b. Plots with clone 224 only	2

completely randomized in two replicates. In 1958, only one size of vent was studied, while two additional treatments were included. These were: ventilators installed on the tops of two shelters, in an attempt to obtain a more effective air exchange; and single clone plots to determine if the tall fescue clones in use were fully self-sterile.

Contamination Detection

Two plants of Lolium perenne were transplanted from the greenhouse to each tall fescue plot on May 7, 1957. (Figure 1, p. 15) Several plants of L. multiflorum were transplanted to locations around the perimeter of the project area. (Figure 2, p. 16)

Most of the L. perenne plants did not survive beyond one year of the tall fescue competition. As a check on pollen contamination during 1958, microscope slides lightly coated with vaseline were suspended inside the two shelters which were vented at top and bottom. (Figure 16, p. 35) Four slides were clipped to a string strung diagonally across the top of the shelter, approximately six inches from the top, four more were clipped to a string running diagonally from one top corner to the opposite bottom corner, and two slides were suspended directly in front of each of the ground-level vents. One slide was suspended inside each top vent pipe. These slides were removed at three day intervals, and checks were made to see

Figure 16. Taken looking through polyethylene film. Note string running diagonally from top right corner to far bottom corner. Also note clothespin holding greased microscope slide to string.

if any pollen was adhering.

To eliminate pollen production inside the shelters, all inflorescences were cut off, and kept trimmed back. Thus any pollen found on the slides should have been foreign pollen from outside of the shelter, indicating a leak in the isolation.

Temperature

To study the effect of the shelters on the temperature of the enclosed area, direct reading thermometers were read daily from May 21 to June 10, of 1957, during approximately the hottest part of the day. Thermometers were installed on a plot with no shelter, in a shelter with no vents, and in each of the 6-, 12-, and 18-inch vented shelters. The thermometers were installed in the shelters near the top on the shade side, and close enough to the polyethylene film to permit reading. (Figure 17, p. 37)

In 1958, Taylor maximum registering glass thermometers were installed on every plot. Each thermometer was fastened to one end of an 18 inch length of one quarter inch dowel. A one-hole rubber stopper was fitted to the other end. By inserting this stopper into a hole bored in the center support of each shelter (Figures 18, p. 38 and 19, p. 39), the thermometer was held in approximately the center of each shelter. A thermometer was installed on all unsheltered plots at approximately the same position as

Figure 17. Top left corner of shelter. Note thermometer. Read daily during 1957.

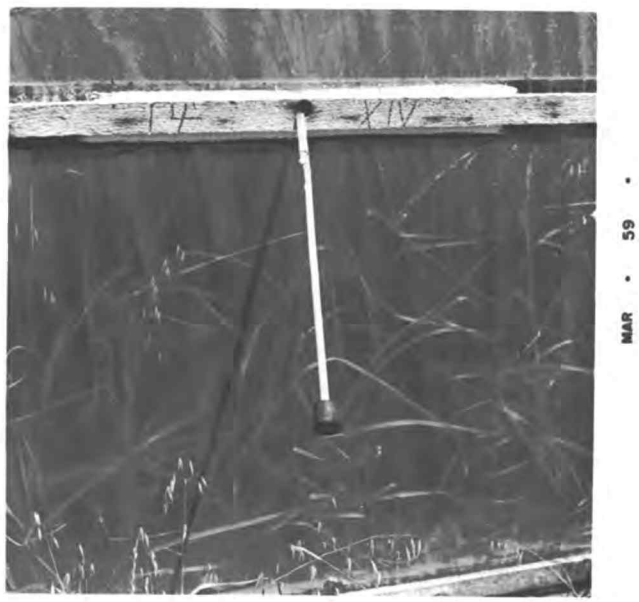

Figure 18. Installation of Maximum registering thermometers during 1958. Read and reset daily.

Figure 19. Looking down through open top of shelters, (1958). Note position of thermometers. Also note prolific development of inflorescences.

those in the shelters, but exposed directly to the sun.

These thermometers were read daily in the evening after the daily maximum had been recorded, and when danger of leakage of foreign pollen into the shelters through the thermometer hole was at a minimum. A wad of cotton was held over the hole while the thermometer was being read and reset.

Harvesting and Processing

The plots were harvested July 15 in 1957, although the tall fescue was mature somewhat earlier. In 1958, the check plots were harvested June 16, and the tops were removed from all the shelters to allow them to dry out prior to harvesting. The sheltered plots were then harvested June 19.

During both years, threshing was done with a cylinder type head thresher, the seed was cleaned using a South Dakota Blower, and was then weighed. Differences between yields due to treatment were tested for significance using the analysis of variance.

Representative samples of seed taken from each plot were subjected to a standard germination test to compare the effects of the treatments on germinability.

Experimental Results

Temperature

During 1957, daily maximum temperatures were recorded in each type of shelter, and on an unsheltered control plot during the period May 21 through June 10. Data in Table 2 were derived by subtracting the control maximums from those recorded within the shelters. In this way, the effect of each type of shelter on the temperature could be related to the control. Maximum temperatures inside the shelters showed only slight differences attributable to the different sizes of vents. Between the completely enclosed shelter, those vented, and the control, large differences in maximum temperature recorded, are shown to exist. Higher temperatures were recorded inside all shelters as compared to the control, except on June 5, 6, and 7, where the temperatures recorded inside the vented enclosures were from 20° to 24°F cooler. The rainfall of June 5, amounting to 0.22 inches, with lesser amounts on the 6 and 7, may have been a factor influencing this cooler temperature. The temperature recorded inside the completely enclosed shelter ran more nearly true to form at 17°F warmer than the control. As shown in the table, there was little variation between the daily maximums registered on the control plot over this period, whereas there was a 35° to 40°F difference between maximum temperatures recorded on two

Table 2. 1957 Daily maximum temperatures, as influenced by polyethylene covered shelters. Data presented to show degrees Fahrenheit above the control.

Date of observation	Degree of cloudiness	Daily rainfall (inches)	Temperature recorded on control plot	Temperature recorded inside the enclosures as they exceed (+) or are exceeded by (-) the temperature recorded on the control plot			
				6" vents	12" vents	18" vents	no vents
May 21	oc**	0.19	58	+10	+ 7	+ 4	+24
22	pc	0.02	63	+ 9	+ 6	+ 6	+15
23	pc	0	67	+18	+16	+15	+27
24	pc		70	+18	+20	+19	+28
25	c		74	+16	+15	+16	+22
26	c		76	+13	+12	+12	+20
27	c		79	+19	+11	+10	+18
28	c		83	+15	+13	+13	+22
29	c		88	+16	+14	+14	+22
30	pc		86	+10	+ 8	+ 9	+17
31	pc		77	+17	+ 6	+18	+17
June 1	sc		86	+19	+16	+15	+24
2	sc		86	+13	+10	+ 8	+19
3*	sc		82	+27	+25	+26	+28
4	bc		84	+17	+17	+12	+24
5	pc	0.22	86	-20	-23	-24	+17
6	oc	trace	72	- 2	- 2	- 1	+18
7	oc	trace	74	- 1	+ 2	+ 2	+11
8	pc		75	+15	+11	+11	+20
9	pc	0.02	77	+26	+19	+19	+27
10	pc		82	+14	+13	+14	+16
	Average		77.4	+12.3	+10.3	+10.4	+20.8

Table 2. (continued)

* Hottest temperature recorded under all shelters.

** c = clear, cloudless sky
sc = scattered clouds = 1/10 overcast
bc = broken clouds = 5/10 overcast
pc = partly cloudy = 7/10 overcast
oc = overcast = 10/10 overcast

consecutive days in three of the types of vented shelters.

In 1958, better thermometers and more replicates were used to record temperature differences which are reported in Table 4. In contrast to the 1957 data, little difference appeared to exist between maximum temperatures recorded in the vented, and completely enclosed shelters. It is interesting that during both years the vented shelters averaged approximately 10°F warmer than the controls. It should be noted, however, that the control in 1958 averaged approximately 20°F warmer than in 1957. The averages for the daily maximums during these periods both years, recorded by the Oregon State Meteorological Station located one-half mile distant from the plot area were approximately equal (1957 = 72.0°F, and 1958 = 71.1°F). The shelters vented at both top and bottom had an average daily maximum only 2.8°F above that of the control.

Table 3. 1958 daily maximum temperatures as influenced by polyethylene covered shelters. Data presented to show degrees Fahrenheit above the control.

Date of observation	Degree of cloudiness	Daily rainfall (inches)	Temperature recorded on control plot	Temperatures recorded inside the enclosures as they exceed (+) or are exceeded by (-) that recorded on the control plot		
				no vents	vents at ground level	vents at top and ground levels
May 1	c*		107	+ 1	+ 2	- 7
2	c		108	0	- 2	- 5
3	sc		103	+ 5	+ 7	- 4
4	sc		102	+ 3	+ 4	- 7
5	pc		96	+ 7	+10	- 2
6	pc		78	+24	+19	+16
7	pc	trace	96	+ 6	+ 6	+ 2
8	pc		97	+ 5	+ 5	+ 3
9	c		98	+ 3	+ 5	+ 3
10	sc		111	+ 1	+ 1	-11
11	oc		82	+13	+13	+ 8
12	pc	0.15	84	+16	+16	+10
13	c	0.04	99	+ 2	0	- 1
14	c	trace	101	+ 4	+13	- 4
15	c		109	+ 1	- 1	+ 1
16	c		110	+ 4	+ 1	- 4
17	pc		107	+ 4	+ 6	+ 1
18	sc		98	+12	+11	- 3
19	c		104	+ 8	+ 8	+ 1

Table 3. (continued)

Date of observation	Degree of cloudiness	Daily rainfall (inches)	Temperature recorded on control plot	Temperatures recorded inside the enclosures as they exceed (+) or are exceeded by (-) that recorded on the control plot		
				no vents	vents at ground level	vents at top and ground levels
May 20	c	0.07	109	+ 5	+ 5	+ 3
21	c		115	+ 5	+ 3	- 1
22	sc		92	+21	+21	+15
23	bc		111	- 5	- 5	- 2
24	pc	0.09	72	+20	+20	+24
25	pc	trace	107	+ 7	+ 3	- 2
26	oc	0.18	97	+15	+14	+13
27	pc	trace	92	+18	+17	+12
28	pc	0.01	102	+12	+12	+ 8
29	pc	0.22	104	+ 7	+ 6	0
30	c	0.12	100	+14	+11	+ 6
31	pc		76	+22	+20	+14
June 1	pc	0.24	86	+18	+24	+14
2	oc	0.13	82	+19	+26	+13
3	pc	0.42	107	+ 6	+ 5	- 2
4	sc	0.02	91	+11	+ 7	+ 6
5	pc		97	+12	+ 1	0
6	oc	0.41	63	+13	+13	+10
7	pc	0.42	105	+ 6	+ 4	+ 1
8	pc	0.68	89	+18	+17	+15
9	pc	trace	89	+12	+11	+ 7

Table 3. (continued)

Date of observation	Degree of cloudiness	Daily rainfall (inches)	Temperature recorded on control plot	Temperatures recorded inside the enclosures as they exceed (+) or are exceeded by (-) that recorded on the control plot		
				no vents	vents at ground level	vents at ground and top levels
June 10	oc	0.02	90	+16	+14	+14
11	oc	0.08	78	+16	+15	+ 7
12	pc		87	+15	+13	-24
13	c		88	+12	+ 9	-24
	Averages		95.2	+10.1	+ 9.6	+ 2.8

* c = clear, cloudless sky
sc = scattered clouds = 1/10 overcast
bc = broken clouds = 5/10 overcast
pc = partly cloudy = 7/10 overcast
oc = overcast = 10/10 overcast

Humidity

High humidity inside the enclosures was indicated by the condensation of moisture on the inside of the polyethylene. This condensation appeared within fifteen minutes after installation of a shelter on a tall fescue plot, and trickles of water running to the ground were observed within one hour. During the daytime all of the shelters but one, exhibited this profuse condensation of moisture, with trickles of water carrying off the excess to the ground. (Figure 20, p. 48) This condensation tended to decrease during the night, and during cloudy days. The one exception was the plot with a shelter having both top and ground level vents, where only rarely did even a slight misty condensation occur. In the shelters with ground level vents, the condensation seemed to evaporate at lower levels, even the trickles of water from above being dried off completely. (Figure 21, p. 49) There was not much discernible difference in amount or behaviour of condensation between the two years. Condensation did appear to be somewhat more profuse in 1958, although this was by observation only, since no accurate determination of humidity, relative or actual, could be made.

Soil moisture varied little between plots sampled (Table 4, p. 50), although the soil moisture content of the adjacent fallowed field was considerably higher.

Figure 20. Note condensation on walls of shelter. Note trickles of water running to bottom.

Figure 21. Note evaporation of condensation on lower part of shelter. Even that running down from above evaporates before it reaches the ground.

Table 4. Percentage soil moisture in different plot areas, July 22, 1957.

Area sampled	% moisture average of two samples
Fallowed field - 100 yards from plots	23.6
Plots with shelter completely enclosed	16.3
Plots with shelter with 12" vents	16.0
Plots with shelter with 6" vents	15.9
Pathway between plots	15.5
Plot with no shelter	14.8

Light

The opaque polyethylene film used on the tops, southern and western exposures in 1957, transmitted from one-third to one-half of the incident light. (Table 6, p. 51) In 1958, when all panels were covered with transparent film, approximately one-half of the light was transmitted.

Air Movement

In 1958, an infestation of aphids on the tall fescue was controlled by treating with "Parathion" in dust form. The nozzle of the duster was inserted through the thermometer hole of each shelter. At the time of dusting a strong wind was blowing lengthwise of the plots. When the cloud of insecticide dust was directed downward inside the shelters with ground level vents, the cloud of dust was

Table 5. Transmission of light through the polyethylene film covering the tall fescue plots. Readings made with a Norwood Director exposure meter, and expressed as foot candles of incidence.

Hour	Cloud conditions	Foot candles of incidence inside the shelter	Foot candles of incidence in direct sunlight	Ratio
July 16, 1957 - bright sunny day, scattered cumulus clouds				
8:30a.m.	cloudy	1350	3750	1 : 2.8
9:30	sunny	2400	9000	1 : 3.8
10:30	cloudy	3000	6000	1 : 2
11:30	sunny	3750	9000	1 : 2.4
12:30p.m.	sunny	3000	9000	1 : 3
1:30	hazy	3000	6000	1 : 2
2:30	sunny	3750	7500	1 : 2
3:30	sunny	3000	9000	1 : 3
4:30	sunny	2250	8250	1 : 3.7
5:30	sunny	2100	7500	1 : 3.6
June 25, 1958 - bright sunny day, no clouds				
10:30a.m.	sunny	4000	8000	1 : 2
11:00	sunny	4000	8000	1 : 2
11:30	sunny	3000	6000	1 : 2
2:00p.m.	sunny	3000	6000	1 : 2
2:30	sunny	3000	6000	1 : 2

immediately carried out through the vent at the leeward end of the shelter. When the dust was deflected upward inside the shelters, some few seconds elapsed before a much smaller amount of dust was carried out at the leeward end of the shelter. This suggests a strong movement of air through the shelter at lower levels, but little or no turbulence at the higher levels. These higher levels would correspond to the zone in which the inflorescences of tall fescue would be borne and pollen be dispersed.

Contamination Detection

Seed harvested in 1957 from the Lolium perenne plants in each plot was germinated and the seedlings were tested for fluorescence under ultra violet light. (Table 6, p. 53) Differences in numbers of seeds exhibiting fluorescence were inconclusive due to the low yield of L. perenne seed. However, trends suggested less contamination through smaller vents, and none where the shelters were completely enclosed. In 1958, the few surviving L. perenne plants failed to produce any seed, so testing for contamination could not be done.

The greased microscope slides used in two shelters in 1958 indicated the degree of contamination by foreign pollen occurring inside the shelters. (Table 7, p. 54) The table includes data from the enclosure vented at both top and bottom only. The shelter vented at the top only, showed no contamination on the slides within the enclosure, and amounts of contamination of the slide inserted in the top vent were similar to those recorded for the shelter vented both top and bottom.

Although some pollen grains were trapped at the ground level vents, only very few were caught at the lower levels of the interior, and none were found at the height which would be occupied by the tall fescue inflorescences in the other enclosures.

Table 6. Influence of type of shelter on germination of seed, and percentage of seedlings that exhibit fluorescence.

Treatment	Data reported as the average of two replicates	
	% germination	% of seedlings exhibiting fluorescence
No shelter = control	82	14
Shelter completely enclosed	74	0
6" vents, 2 rows of biological screen	26	12
6" vents, 4 rows of biolcgical screen	58	2
12" vents, 2 rows of biological screen	64*	6*
12" vents, 4 rows of biological screen	63	10
18" vents, 2 rows of biological screen	68*	22*
18" vents, 4 rows of biological screen	47	20

* One observation missing

Table 7. Variation in pollen count on greased microscope slides suspended at various locations inside a polyethylene covered enclosure vented at both top and bottom.

Dates when slides were in shelter	Slides strung across top				Slides strung on diagonal*				Slides at ground level immediately in front of open vents. Greased surface outwards				Top vents
									south end		north end		
	A	B	C	D	A	B	C	D	A	B	A	B	
May													
2-5	0	0	0	0	0	0	0	3	5	5	5	5	1
5-9	0	0	0	0	0	0	x	3	5	5	5	5	5
9-13	0	0	0	0	0	0	1	0	5	5	5	5	0
13-17	0	0	0	0	0	0	0	0	5	5	5	5	5
17-20	0	0	0	0	0	0	0	0	5	5	5	5	5
20-23	0	0	0	0	0	0	x	0	5	5	5	5	x
23-30	0	0	0	0	0	0	0	0	5	5	5	5	5
June													
1-7	0	0	0	0	0	0	0	0	5	5	5	5	5

* These slides were clipped to a string strung diagonally from one top corner to the opposite bottom corner. #A slide was nearest the top of the shelter, approximately one foot from the top; #B slide was lower, two feet from the top; #C slide was four feet from the top; and #D slide was five feet from the top, or one foot from the ground.

Code: 0 = no pollen grains
1 = few (1-4) pollen grains found on slide
3 = several (5-10) pollen grains found on slide
5 = many (11 or more) pollen grains found on slide
x = pollen grains other than tall fescue found present, but no tall fescue pollen present

Seed Yield

The analysis of variance indicated that differences between yields of seed from each plot in 1957 were not significant. (Table 8, p. 56) Seed yields varied widely between treatments, from 25.4 grams per plot in the control (no enclosure plots), to 8.6 grams in the completely enclosed plots. The shelters with the six inch vents reduced the yield only slightly as compared to the control plots. Seed from all plots germinated equally well as is shown in Table 8.

No seed was formed inside any shelter in 1958, although high yields were harvested from the control plots.

The self-fertility study involving the shelters enclosing single clones yielded no data, since no seed was formed inside any shelter.

Table 8. Relation of type of shelter to seed yield and germination - 1957.

Treatment	Average seed yield per plot	Average % germination per plot
No shelter = control	25.44 g.	97
Shelter completely enclosed	8.58	90
6" vents, 2 rows of biological screen	18.28	97
6" vents, 4 rows of biological screen	13.12	96
12" vents, 2 rows of biological screen	15.90	96
12" vents 4 rows of biological screen	14.89	98
18" vents, 2 rows of biological screen	21.33	96
18" vents, 4 rows of biological screen	16.51	98

Variation due to	Sums of squares	d.f.	Mean Squares	F
Treatments	367.90	7	52.56	0.90
Error	464.53	8	58.07	
Total	832.44	15		

Discussion

Hayes et al (8, p. 347) note that perennial forage crops are usually cross-pollinated, and often are self-sterile. Individuals within a species are highly variable both phenotypically and genotypically, and frequently a character will exhibit as much if not more variation within a variety, as between varieties. This variation provides a basis for the selection of specific characters for recombination into new more desirable varieties. Hayes emphasized that breeders of these crops are in need of a form of isolation that, while controlling pollination, provides quantities of hybrid seed sufficient for adequate progeny testing.

Hayes et al (8, p. 82) note that crossing different strains as compared to enforced selfing, usually involves the use of some special techniques appropriate for the particular crop and environment. Hayes discussed two general methods of exclusion of foreign pollen that may be used in controlling pollination. One method is the use of space isolation. That is, spacing the desired combination of plants far enough away from other plants with which they might cross to prevent contamination by wind or insect borne pollen. The distance needed for complete isolation will vary with the crop, weather, and other factors, but in general imposes physical limitations on the number of

crosses which can be made in the area available to the breeder, or suited to his needs. Another method is the use of some type of bag, either paper, parchment, or cloth to enclose one or more inflorescences of two or more genotypes to ensure isolation from foreign pollen. This method affords perfect isolation, but produces such small amounts of seed from each cross, that only limited progeny testing is possible.

The "poly-cross test" of Tysdal et al (8, p. 371) could be used as a means of producing hybrid seed of selected lines. There could be little assurance however, that any or all of the desired characters had been combined into the resultant seed. Neither is there any assurance of uniformity of combination of these characters through subsequent years.

There is then a need for a means of isolation combining the convenience, low-cost, dependability, etc., of parchment bags with the ability to provide sufficient seed for adequate progeny testing. This study was designed to investigate such a means of isolation. Several plants of each of two vegetatively propagated genotypes of tall fescue were enclosed together in a device constructed to offer a minimum of interference to the physiological processes of the plants, while affording a maximum of isolation.

The polyethylene film offered perfect isolation while

subjecting the plants to little or no shading effect. That temperature and humidity levels deleterious to seed production might occur was recognized, and to avoid this ventilators of various types were installed on several of the shelters. To avoid having contaminating foreign pollen blown in by too rapid movement of air, these vents were placed at ground level, and a screen of fall seeded rye (oats in 1958) was seeded across them.

There being no significant difference between yields attributable to the different sizes of vents used in 1957, only one size of vent was compared with no vent and with a control in 1958.

That high temperature, or high humidity, or both, are detrimental to pollen viability has been shown by earlier workers. Similar results are indicated by this study where the separate effects of high temperature and humidity appeared cumulative. In 1957 high temperature plus high humidity appeared not to reduce seed production significantly inside the shelters. In 1958 however, when the shelters were opened June 12 for examination of the degree of seed set, it was obvious immediately that little if any seed would be found. The tall fescue plants had such a damp, grey, unhealthy look and feel, that it was almost unnecessary to examine individual florets to find that fertilization had very likely never occurred.

There were two basic changes in construction of the

shelters used in 1958 compared to those used in 1957. A support across the large six foot square panels was added, and a more air-tight method of assembling the panels was used. The support largely stopped the wind induced bellows-like whipping action of the large expanse of polyethylene stretched across these panels. On warm days in 1957 when the polyethylene tended to become somewhat more lax, even a light breeze caused a considerable amount of action by these large expanses of film. This action could conceivably have aided in pollen transfer within the shelter, and have been a factor in keeping the temperature and humidity down.

The more air-tight assembly was achieved in 1958, by bolting the panels together in the shop, rather than nailing them together out in the field, enabling better matching of the edges of abutting panels. Also a cotton twine gasket was used between the adjoining panels and caulking compound was daubed into all the corners from the inside. This sticky non-setting material retained its perfect seal through the stress of moving each shelter by truck to its plot location in the field. When nailed together, the panels through expansion and contraction during wet and dry weather which loosened the nails, separated slightly. The separation was prevented in 1958 by fastening them together with bolts, and by using the gasket and caulking compound. The slight separation of

panels that occurred in 1957 could have provided for more air exchange than was realized, and thus could be a factor in the lower temperature and humidity observed. This leak may even have been the source of pollen effecting seed production assuming that inviable pollen was formed inside the shelters both years. It does not seem probable however, that sufficient pollen could have entered via this means to permit the degree of seed-set actually obtained in 1957.

The profuse condensation of moisture on the polyethylene film was ample evidence of high temperature and relative humidity inside the shelters during both years, while the temperatures recorded during 1958 were somewhat higher than those of 1957.

Normal extrusion of the tall fescue anthers could be observed through the polyethylene film, but whether the dehiscing pollen was viable or was clumped together was not determined. It is likely that clumps of non-viable pollen formed as a result of the high temperature and humidity.

The 1958 experiment provided evidence that there was little likelihood of serious contamination by foreign pollen in any of the shelters studied. The greased microscope slides in front of the ground level vents did trap many pollen grains as well as much dust and debris, but very little if any pollen was carried into the upper areas of the shelters to be trapped by the greased slides there.

The lack of turbulence in the upper areas of the shelters is further indicated by the evaporation at lower levels only, of the water of condensation on the walls, and by the effect of the wind on the insecticide dust applied inside the vented shelters. Occurrence of a small amount of contamination would be serious for extremely critical work only, and inconsequential for practically all other situations. If conditions inside the enclosure were such that the enclosed inflorescences were all actively shedding viable pollen, then the contaminating pollen would be in such a minority that it would be selected against rather severely.

Successful seed production in 1957 gives reason to believe that modification of present technique and construction will result in continued success. Results from 1958 show there are several factors which must be changed before satisfactory seed production will be obtained.

Of these factors, most important is the lowering of the temperature and humidity inside the enclosures. This is most easily done by an air exchange mechanism employing pollen-proof filters offering as little impedence as possible to the air flow. It is also evident that a means of agitation of the air inside the enclosure must be implemented since pollen transfer is dependent on this feature of the environment. If, at full anthesis, a jet of air were directed into the shelters, perhaps enough

turbulence would occur to assist in pollen transfer. It would be relatively easy to attach a pollen-proof filter to the air intake of the portable air compressor used to provide the air blast. Perhaps allowing the polyethylene sides the free bellows-like action noted in 1957 will suffice in this regard. Stapledon (18, p. 39-40) inserted a stick through the ground level apertures to agitate the panicle-bearing stems. It may be that a combination of two or more of these devices may be required.

To explore the air exchange possibilities further, there appears little to be gained through further study of shelters vented at ground level only. This study indicated that beneficial results from both top and ground-level vents, with only a small incidence of foreign pollen contamination, might be obtained.

A shelter with large filtered vents at top and bottom would be worthy of further study, if it were intensively and accurately investigated for temperature, humidity, and contamination by foreign pollen. Temperature and humidity are relatively easily measured if adequate mechanical equipment is available. Also, great care should be taken to remove as little as possible of the plant material when the inflorescences are removed. Removing too much of the plant material will greatly decrease the amount of transpiration and thus decrease the humidity trapped inside the shelter. Suspension of several greased microscope slides

inside such a shelter would be a suitable means of detecting contamination. Several strings each supporting several microscope slides greased on both sides, should be strung lengthwise in the shelter through the zone normally occupied by the tall fescue inflorescences. These slides should be examined for trapped pollen at least every other day.

Another possibility with regard to air exchange would be to have one side or end of an enclosure covered with a fine weave cloth which would be highly pollen-proof, yet would permit comparatively free air exchange. Perhaps a loosely woven but fluffy material such as terry cloth would be even better. The cloth covered panel would best be situated to the lee of the prevailing wind. Smith (17, p. 338) and Jenkin (9, p. 10) found that no cloth available had pores small enough to stop pollen. However, tangled ends of cotton fibres probably left few if any pores open to the passage of pollen grains when very fine muslin was used. These experiments were with shelters completely enclosed with cloth, so if only one side were cloth-covered the incidence of pollen contamination would be even lower. There would also be little or no shading effect with the three other sides and the top covered with transparent polyethylene.

It would be relatively easy to sample the pollen being dehisced inside a completely enclosed, tightly sealed

shelter, to determine if viable pollen is responsible for the lack of seed production. Whether viable pollen is formed, could be determined by trapping some during anthesis. This could be accomplished by inserting a greased microscope slide, or by removing a few florets for laboratory study, via a prepared trap-door arrangement. Also, by trapping normal pollen shed from a plant growing outside the shelter, then inserting it in the enclosed atmosphere for varying lengths of time before germinating it, the effect of high temperature and humidity could be determined. Also along this line of investigation, normal pollen from a plant growing outside the shelter could be dusted on a mature inflorescence inside the shelter to check on pollen germination in such an atmosphere.

To develop this study further, it would be advisable to enclose together several interfertile lines of tall fescue rather than just two. This would circumvent the possibility that the lines enclosed did not "nick" closely enough, under such an artificial environment, to permit fertilization to occur. Since this is only a technique developing approach, actual parentage of the resultant seed need not be a consideration, so long as contamination is eliminated, until perfection of this technique is attained.

In 1957 the plots enclosed were recovering from being transplanted the previous fall, and produced only limited

vegetative growth. By 1958, they were well established, and luxuriant vegetation literally filled the shelters with living transpiring material to a level near the mid-point of the interior of each shelter. For this reason, it would be better to have fewer plants enclosed together, since this would reduce the amount of transpiration, and help control the humidity. This could be effected by using larger shelters, but they would then become unwieldy for transport and storage. Perhaps they could be made somewhat higher allowing the hotter, more humid air to concentrate in a zone above that occupied by the plant inflorescences.

Summary

A study was undertaken to determine if hybrid seed of two tall fescue genotypes could be produced successfully from plants growing in the field while enclosed within a polyethylene covered framework.

Seed was obtained from all treatments in 1957 with no significant differences between treatments. High moisture and temperature inside the shelters were the most probable cause of failure to produce seed in 1958. Moisture and temperature effects were more pronounced than in 1957, due to the more luxuriant growth of foliage, and the tighter fit of the panels of the shelters.

The following recommendations are suggested for further study of the problem:

1. The moisture and temperature inside the shelters must be kept at lower, less harmful levels. This could be accomplished by a system of filtered ventilators, manual agitation of the panicle bearing stems, or a jet of air under pressure into the shelters for a short interval, daily during anthesis.
2. Vents at both ground level and at the top of the shelter should be critically examined.
3. If one side or end of the shelter were covered with a porous, fluffy type of cloth, perhaps

sufficient air exchange could be obtained without sacrificing any assurance of pollen isolation.

4. Pollen shed within the shelters should be sampled for viability.
5. Pollen produced on the control plots should be collected and inserted into several of the shelters for varying lengths of time, to study the duration of viability in the atmosphere of the interior.
6. Pollen produced on the control plot should be dusted on several inflorescences of the enclosed plants to determine if fertilization will occur if viable pollen is introduced.
7. Several genotypes should be enclosed together to ensure some degree of "nicking".
8. Fewer plants should be enclosed together to help reduce the transpiration, and consequently the humidity within the shelters. The effects of this humidity could be alleviated to some extent by using larger shelters, but maneuverability and ease of storage would be reduced accordingly.

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