# STIGMA REMOVAL STUDIES ON CERTAIN ACCESSIONS OF *Bothriochloa intermedia* AND *Dichanthium annulatum*

By

CHESTER L. DEWALD

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Thesis Approved:

Jack R. Harlan
Thesis Adviser

Wayne W. Huffine

Robert MacVicar
Dean of the Graduate School

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# INTRODUCTION

During the past several years, the forage section at this station has grown and maintained a large experimental garden of native and introduced grasses belonging to the tribe Andropogoneae (3).[1]. Accessions from many parts of the world have been received and surveyed in an attempt to bring new germ plasm into the pasture improvement program. Attempts to combine superior germ plasm by hybridization have resulted in varying degrees of maternal type progeny, and both facultative and obligate apomixis have been demonstrated in many accessions of these Old World Bluestems (4).

Very little can be said with certainty concerning the functions and requirements for pollen in apomictic species. It is not known why one source of pollen will stimulate seed set in a particular pseudogamous apomictic species and other sources do not, nor why more hybrids are produced by some facultative apomicts than by others.

In this study a stigma removal technique was applied to various accessions of *Dichanthium annulatum* and *Bothriochloa intermedia*. The primary purpose was to determine the relationship of pollination to seed set in some of the apomictic accessions (i.e. *D. annulatum* A-4099 and *B. intermedia* A-2655 and A-5450) and to compare this with other accessions which reproduce by a sexual mechanism (i.e. *D. annulatum* A-3242

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/1 Figures in parenthesis refer to Literature Cited.

and (55-X-98). The experiment concerns accessions at two ploidy levels and involves out-crossed and self pollinated infloresces.

## REVIEW OF LITERATURE

The various aspects of apomixis have been the subject of research by many investigators over a period of years. Extensive reviews concerning apomixis have been published including those by Gustaffson (5) (6) (7), Stebbins (16) (17), Powers (12), Nygren (10) (11), Maheshwari (9) and many others. The scope of these investigations is sufficiently broad that only a few general accounts pertinent to the present study will be reviewed here. More detail will be given to literature concerning the species used in this study and papers reporting the use of stigma removal as a means of studying the relationship of pollination to seed set in apomictic grasses.

Stebbins (17) defines apomixis in general as "all types of asexual reproduction which tend to replace or to act as substitutes for the sexual method." He states that production of a few hybrids does not eliminate the presence of apomixis, as facultative apomicts are capable of producing some hybrid progeny and that many apomicts require pollination to set seed, i.e. are pseudogamous.

Celarier and Harlan (4) in 1957 outlined the conditions necessary to demonstrate reproduction by apomictic means and showed that many species of Bothriochloa, Dichanthium and Capillipedium reproduce maternal type offspring by this mechanism. They concluded that pollen is probably necessary for seed production, because seed set varies greatly when different sources of pollen are applied to hand emasculated florets.

Stebbins (17) states that the only positive means of demonstrating apomixis are "studies of megaspore, embryo sac and embryo development." Brown and Emery (2) made such studies using Themeda trianda and Bothriochloa ischaemum, two members of the tribe Andropogoneae. They found only apomictic type sacs in this material.

Brooks (1) made studies of embryo sac development in selected Bothriochloa and Dichanthium species. She found that those species which reproduced by sexual means alone produced only one embryo sac per ovule. These sacs contained five nuclei plus antipodals, the five nuclei being two synergids, one egg nucleus and two polar nuclei. This five nucleate plus antipodal type sac was also found in certain facultative tetraploids, but was accompanied by a larger number of four nucleate sacs. The four nucleate sacs contained two synergids, one egg nucleus, one polar nucleus and no antipodals and were assumed to be of apomictic type. In these embryological studies Brooks (1) worked with all accessions used in the present study as well as with several others. She found only sexual type sacs in D. annulatum A-3242 and (55-X-98) and both apomictic type and sexual type sacs were found in ovaries of D. annulatum A-4099 and B. intermedia A-2655.

Warmke (18) appears to be the first investigator to use the stigma removal technique to study the relationship of pollination to seed set in apomictic grasses. He obtained an average of 1.7% seed set following stigma removal within two hours after anthesis in Panicum maximum, "common guinea grass". When stigmas were removed $3\frac{1}{2}$ to 12 hours after anthesis, 39.7% seed set was obtained, and 39.2% seed set was obtained when no stigmas were removed. Warmke (18) interpreted this to mean that pollination occurs and is necessary in guinea grass, an apomictic species.

Snyder, Hernandez and Warmke (15) performed similar experiments using <u>Pennisetum ciliare</u>, another apomictic species. Using two different accessions of <u>P. ciliare</u>, stigmas were removed 36 hours before anthesis and at hourly intervals up to 5½ hours after anthesis. Conclusions were drawn that stigma removal prior to 2½ hours after anthesis effectively eliminates seed production, whereas removal 3 to 3½ hours after anthesis gives seed set intermediate between the earlier results and normal seed set.

Snyder (14) applied this stigma removal technique to <u>Paspalum secans</u>, also an apomict, and obtained comparable results and drew similar conclusions.

## MATERIALS AND METHODS

Plant materials used in this study were selected from the Andropogoneae garden (3) at the Oklahoma Agricultural Experiment Station, located near Stillwater, Oklahoma. The species are listed below along with accession numbers used at this station, place of origin and approximate percent apomixis[1] (8).

| Species | Accession No. | Place of Origin | % Apomixis |
|---|---|---|---|
| Dichanthium annulatum | 3242 | Calcutta, India | 0 |
| Dichanthium annulatum | 4099 | Punjab, India | 82 |
| Dichanthium annulatum | (55-X-98)[2] | Stillwater, Okla. | 0 |
| Bothriochloa intermedia[3] gangetica type | 2655 | British Guiana | 85 |
| Bothriochloa intermedia gangetica type | 5450 | Delhi, India | 86 |

The plants were moved into the greenhouse in the fall of 1957 and 1958, and all data are from greenhouse material obtained during the late fall and winter months. Outside light was utilized and hence this factor varied from day to day; other environmental conditions were more constant.

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[1] The percent apomixis given here is the percent of the plant progeny identical to the maternal parent and is subject to change as more information is obtained (8).

[2] Dichanthium annulatum (55-X-98), a hybrid, is a tetraploid, exhibiting a high degree of sexuality. It was obtained at this station by crossing a diploid D. annulatum with a tetraploid D. annulatum, both of the tropical type (8).

[3] Bothriochloa intermedia, gangetica type, A-2655, is an introduction from British Guiana where it had been introduced (8).

In this study, florets were hand emasculated in a manner described by Richardson (13) and panicles were enclosed in glycine bags to prevent contamination. The day following emasculation, pollen was applied and the panicles remained in the bags until seed were harvested. D. annulatum (55-X-98) was not hand emasculated for reasons to be discussed later.

The combinations of female and male plants used in this study are listed below along with the Roman numeral which designates the table describing the results.

| Table Number | Female Parent | | Male Parent |
|---|---|---|---|
| I | B. intermedia A-5450 | X | B. intermedia A-5450 |
| II | B. intermedia A-2655 | X | B. intermedia A-2655 |
| III | D. annulatum (55-X-98) | X | D. annulatum A-4099 |
| IV | D. annulatum (55-X-98) | X | B. intermedia A-2655 |
| V | B. intermedia A-2655 | X | D. annulatum (55-X-98) |
| VI | B. intermedia A-2655 | X | D. annulatum A-4099 |
| VII | D. annulatum A-4099 | X | D. annulatum (55-X-98) |
| VIII | D. annulatum A-4099 | X | D. annulatum A-4099 |
| IX | D. annulatum A-3242 | X | D. annulatum A-3242 |

In studies to determine the time required for pollen to travel through the stigma and affect seed set, stigmas were removed at various intervals following pollination. Several techniques were tried to attempt stigma removal including clipping with small scissors, cutting with small sharp implements and pulling off with forceps. It was found that the use of forceps, described in 1954 by Warmke (18) for stigma removal purposes was faster, easier and less injurious to the ovary than the other methods. Forceps of surgical quality, with sharp needle-like points, were used in this study to remove the stigmas. Very little injury resulted to other female parts when stigmas were grasped near the ovary and slight pressure was applied to the forceps as the stigmas were removed.

Three to four weeks after pollination, the panicles were harvested by cutting the stem just below the bag which retained the emasculated florets. The contents were removed from the bag and seed set was determined by counting the florets which contained seed.

Seed set resulting from stigma removal after the various intervals up to four hours following pollination appeared to increase logarithmicly. All statistical analyses were calculated by the log of the precent seed to be set as expressed by the equation:

$$\log(100-Y) = \alpha + BX + E$$

Where:

Y = % seed set

X = time of stigma removal following pollination

$\alpha$ = intercept

B = rate of log seed to be set per unit of time

E = random error

The methods of statistical analysis will be explained further in the appendix.

## RESULTS AND DISCUSSION

In 1957 a preliminary study was conducted to determine the effect of stigma removal at intervals following hand pollination by determination of subsequent seed set of two accessions of *Bothriochloa intermedia* and a *Dichanthium annulatum* hybrid, designated (55-X-98). Although only limited information was obtained in this preliminary investigation, it was obvious that the two accessions of *B. intermedia* were behaving very similarly in regard to seed set following the stigma removal procedure. *B. intermedia* A-5450 was dropped from the study after obtaining the results shown in Table I. Investigations with *B. intermedia* A-2655 were continued the following year and more complete and accurate data were obtained as shown in Table II.

The *B. intermedia* accessions failed to set seed when stigmas were removed prior to two and one half hours following pollination. Intermediate to normal seed set was obtained when the interval between pollination and stigma removal was increased. The results are in close agreement with those of earlier workers (14) (15) (18) who studied selected species in the tribe *Paniceae*.

Pseudogamy, already suspected in these apomictic species of *Bothriochloa*, is also indicated here by the absence of seed set when stigmas were removed prior to two and one half hours following pollination. In the present study more direct evidence for pseudogamy was obtained when these species were hand emasculated and bagged to exclude pollen. From

TABLE I

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*B. intermedia* A-5450 X *B. intermedia* A-5450

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| Before 2.5 hours | 4 | 160 | 0 | 0 |
| 3.0 hours | 2 | 78 | 25 | 32.0 |
| 6.0 hours | 2 | 62 | 7 | 11.2 |
| 24.0 hours | 1 | 20 | 4 | 20.0 |
| Not removed | 2 | 60 | 3 | 5.0 |

TABLE II

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*B. intermedia* A-2655 X *B. intermedia* A-2655

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| Before 2.5 hours | 7 | 195 | 0 | 0 |
| at 2.5 hours | 5 | 150 | 31 | 20.6 |
| 3.0 hours | 4 | 102 | 24 | 23.5 |
| 3.5 to 6.0 hours | 6 | 136 | 34 | 25.0 |
| 24.0 hours | 4 | 124 | 38 | 30.6 |
| Not removed | 4 | 162 | 36 | 22.2 |

more than seven hundred florets thus treated, no seed were obtained as compared to fair seed set when pollen was applied as shown in Tables I and II. This is direct indication of pseudogamy and also shows reliability of the hand emasculation technique, developed by Richardson (13) and followed in this study.

The florets of eight panicles of D. annulatum (55-X-98) were hand emasculated and pollinated with its own pollen. Stigmas were removed at various intervals in five of the panicles and the other panicles remained with stigmas intact. No seeds were produced on these panicles whether stigmas were removed or not. A selfing experiment was initiated to determine if self sterility existed in this sexual tetraploid which was concurrently being hand emasculated for crossing studies in the breeding program. From twenty selfed heads (approximately 3000 florets) only .1% seed set was found. These few seed were believed to be the result of either a low degree of fertility or contamination. In the following season fourteen additional heads (approximately 2100 florets) were bagged for selfing before emergence from the boot and remained bagged until harvested. Only two seeds (.09%) were produced, and these were found in the glumes of an intact panicle, hence rendering the possibility of contamination improbable and suggesting that D. annulatum (55-X-98) is very highly, but not completely, self sterile. In the present study seed set was obtained when this plant was crossed with D. annulatum A-4099 and B. intermedia A-2655, both when used as the female parent or as the male pollen parent as shown in Tables III, IV, V and VII respectively.

The demonstration of self sterility was utilized in the present

TABLE III

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*D. annulatum* (55-X-98) X *B. intermedia* A-2655

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| .5 hours | 4 | 160 | 8 | 5.0 |
| 1.0 hours | 4 | 173 | 5 | 2.8 |
| 2.0 hours | 6 | 279 | 34 | 12.1 |
| 3.0 hours | 4 | 203 | 44 | 21.6 |
| 4.0 hours | 4 | 225 | 60 | 26.6 |
| 24.0 hours | 4 | 165 | 17 | 10.3 |
| Not removed | 4 | 133 | 35 | 26.3 |

TABLE IV

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*D. annulatum* (55-X-98) X *D. annulatum* A-4099

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| .5 hours | 4 | 182 | 6 | 3.2 |
| 1.0 hours | 4 | 169 | 44 | 26.0 |
| 2.0 hours | 4 | 180 | 89 | 49.4 |
| 3.0 hours | 4 | 154 | 96 | 62.3 |
| 4.0 hours | 4 | 200 | 127 | 63.5 |
| 24.0 hours | 4 | 171 | 105 | 61.4 |
| Not removed | 4 | 195 | 112 | 57.4 |

TABLE V

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*B. intermedia* A-2655 X *D. annulatum* (55-X-98)

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| 10 minutes | 1 | 26 | 0 | 0 |
| 20 minutes | 4 | 132 | 4 | 3.0 |
| .5 hours | 4 | 122 | 3 | 2.4 |
| 1.0 hours | 4 | 152 | 50 | 32.8 |
| 1.5 hours | 4 | 225 | 138 | 61.3 |
| 3.0 hours | 4 | 154 | 97 | 62.9 |
| 24.0 hours | 4 | 173 | 139 | 80.3 |
| Not removed | 4 | 185 | 144 | 77.8 |

TABLE VI

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*B. intermedia* A-2655 X *D. annulatum* A-4099

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| .5 hours | 4 | 158 | 2 | 1.2 |
| 1.0 hours | 4 | 168 | 32 | 19.0 |
| 1.5 hours | 4 | 176 | 79 | 44.8 |
| 3.0 hours | 4 | 168 | 112 | 66.6 |
| 24.0 hours | 4 | 174 | 122 | 70.1 |
| Not removed | 4 | 182 | 150 | 82.4 |

TABLE VII

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*D. annulatum* A-4099 X *D. annulatum* (55-X-98)

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| .5 hours | 3 | 60 | 0 | 0 |
| 1.0 hours | 4 | 93 | 6 | 6.4 |
| 2.0 hours | 4 | 96 | 38 | 39.5 |
| 3.0 hours | 4 | 98 | 35 | 35.7 |
| Not removed | 4 | 100 | 49 | 49.0 |

TABLE VIII

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
*D. annulatum* A-4099 X *D. annulatum* A-4099

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| .5 hours | 3 | 65 | 0 | 0 |
| 1.0 hours | 4 | 97 | 13 | 13.4 |
| 2.0 hours | 4 | 97 | 26 | 26.8 |
| 3.0 hours | 4 | 97 | 34 | 35.0 |
| Not removed | 4 | 125 | 72 | 57.6 |

experiment in which D. annulatum (55-X-98) was used as a female parent for stigma removal studies following cross pollination. Tables III and IV show data of D. annulatum (55-X-98) in crosses with B. intermedia A-2655 and D. annulatum A-4099 respectively. As would be expected, seed set was considerably higher in the intrageneric cross involving the same species, i.e. D. annulatum (55-X-98) X D. annulatum A-4099, than in the intergeneric cross, D. annulatum (55-X-98) X B. intermedia A-2655, as illustrated in Figure 1. A low degree of seed set was obtained when stigmas were removed as soon as thirty minutes following pollination in both crosses. Seed set usually increased as longer intervals were allowed between time of pollination and stigma removal. Seed set increased more rapidly at intervals up to four hours when D. annulatum A-4099 was used as the male than when pollen of B. intermedia A-2655 was used. In both crosses, seed set was highest when stigmas were removed at intervals of three and four hours following pollination, as illustrated in Figure 2.

The above cross of D. annulatum (55-X-98) X B. intermedia A-2655 resulted in the lowest percent seed set of all crosses in the study. The reciprocal of this cross resulted in one of the highest percent seed set obtained as shown in Table V. Very similar results were obtained from the cross B. intermedia A-2655 X D. annulatum A-4099 as shown in Table VI. A preference is shown by B. intermedia A-2655 for foreign pollen over its own, as nearly three times more seed were produced at each interval tested following cross pollination, Figures 3 and 4. Also stigma removal at intervals after cross pollination resulted in seed after much shorter intervals than those following self pollina-

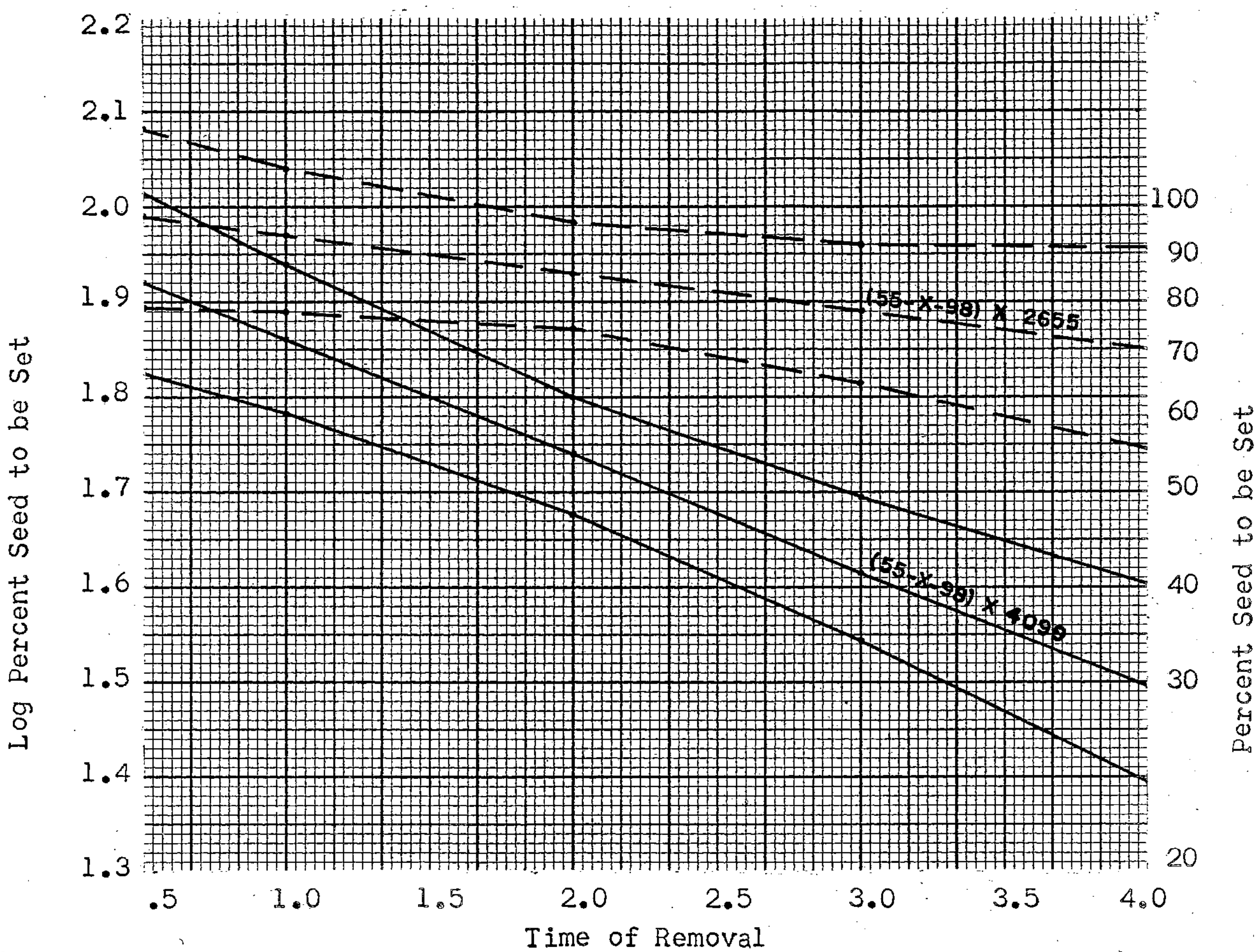


Figure 1. Sample Regression of D. annulatum (55-X-98) Treated with Two Sources of Pollen (4099 and 2655)

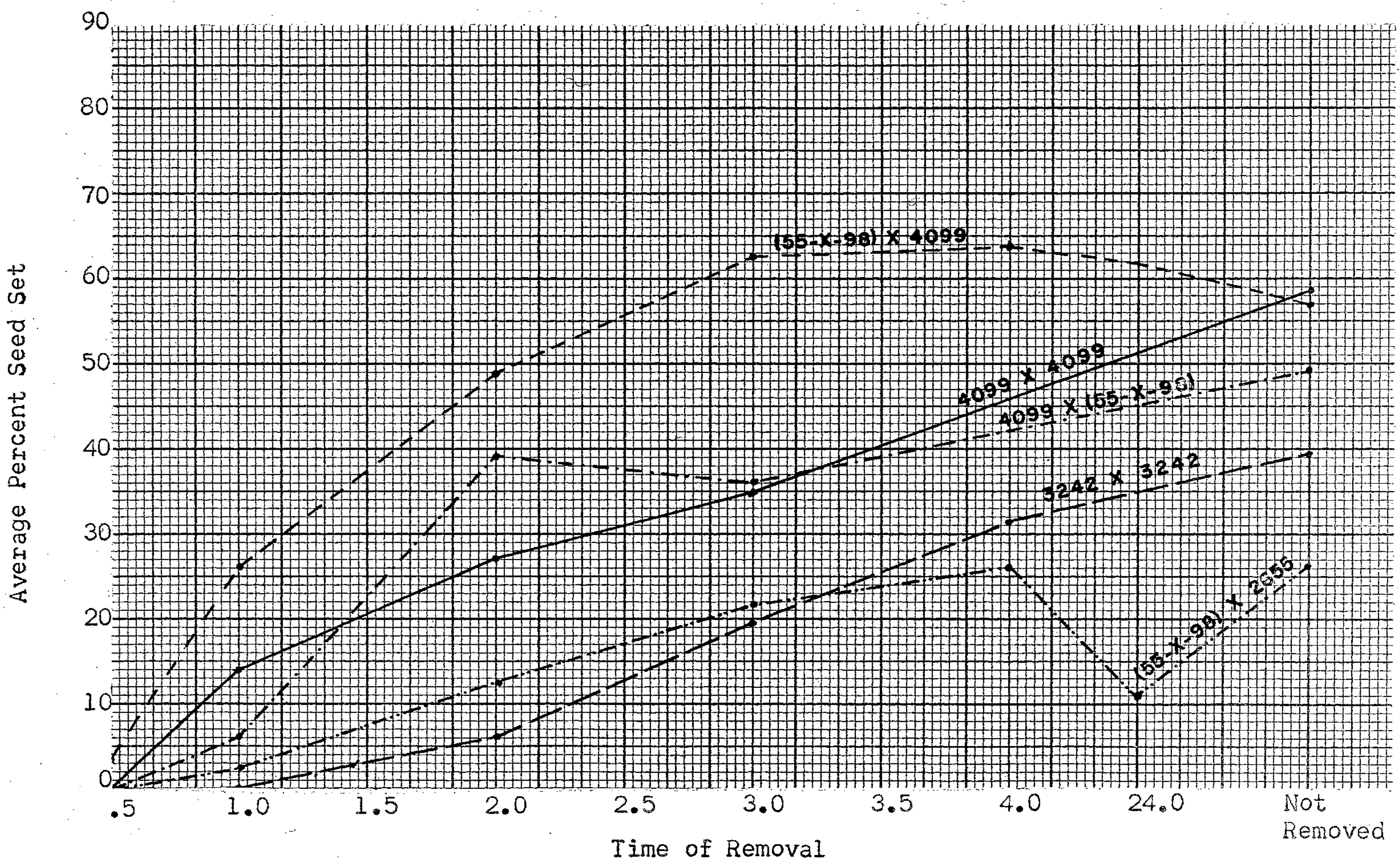


Figure 2. Average Percent Seed Set of the Selected D. annulatum Accessions Treated with Various Sources of Pollen

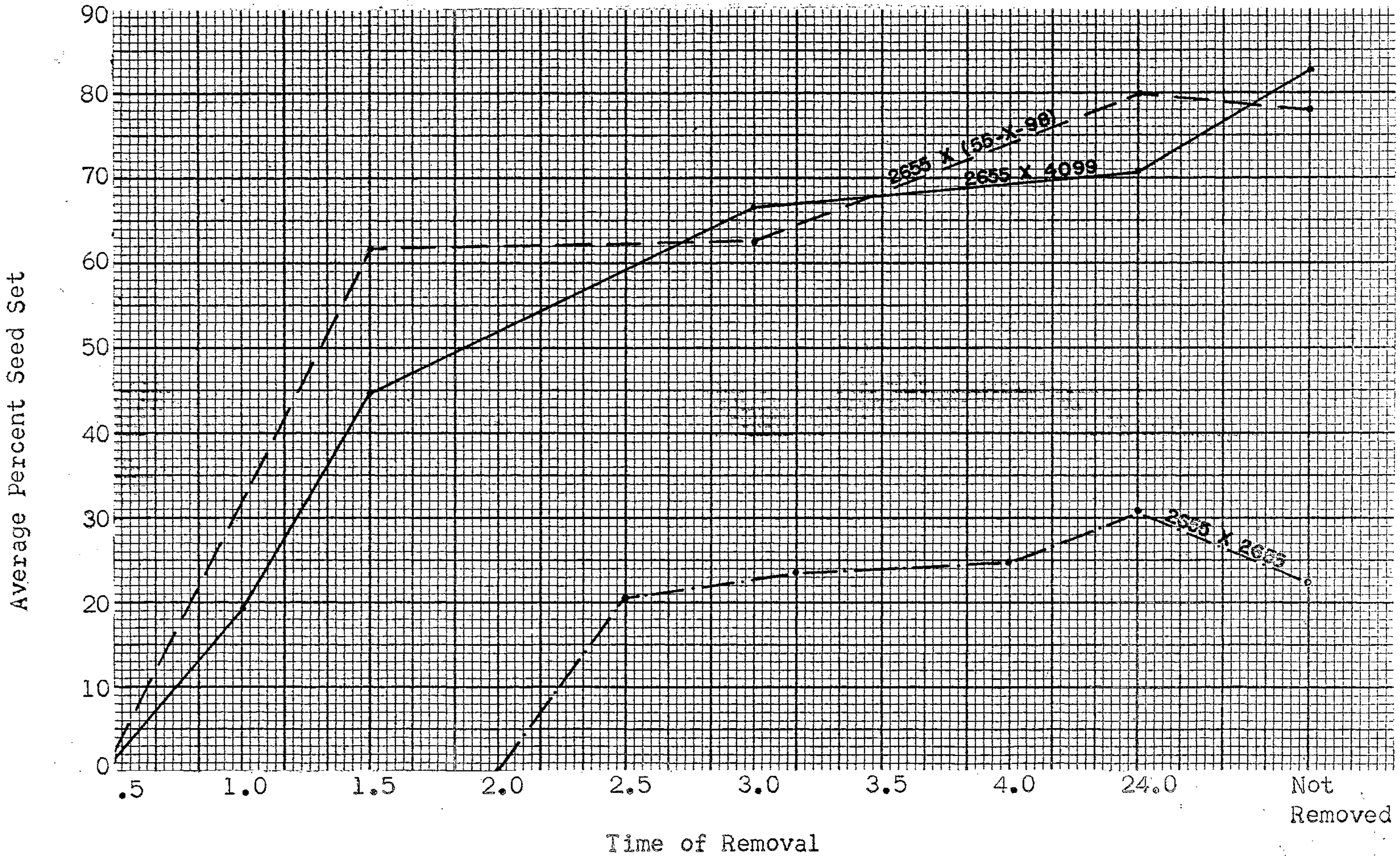


Figure 3. Average Percent Seed Set of B. intermedia A-2655 Treated with Three Sources of Pollen (2655, 4099 and 55-X-98)

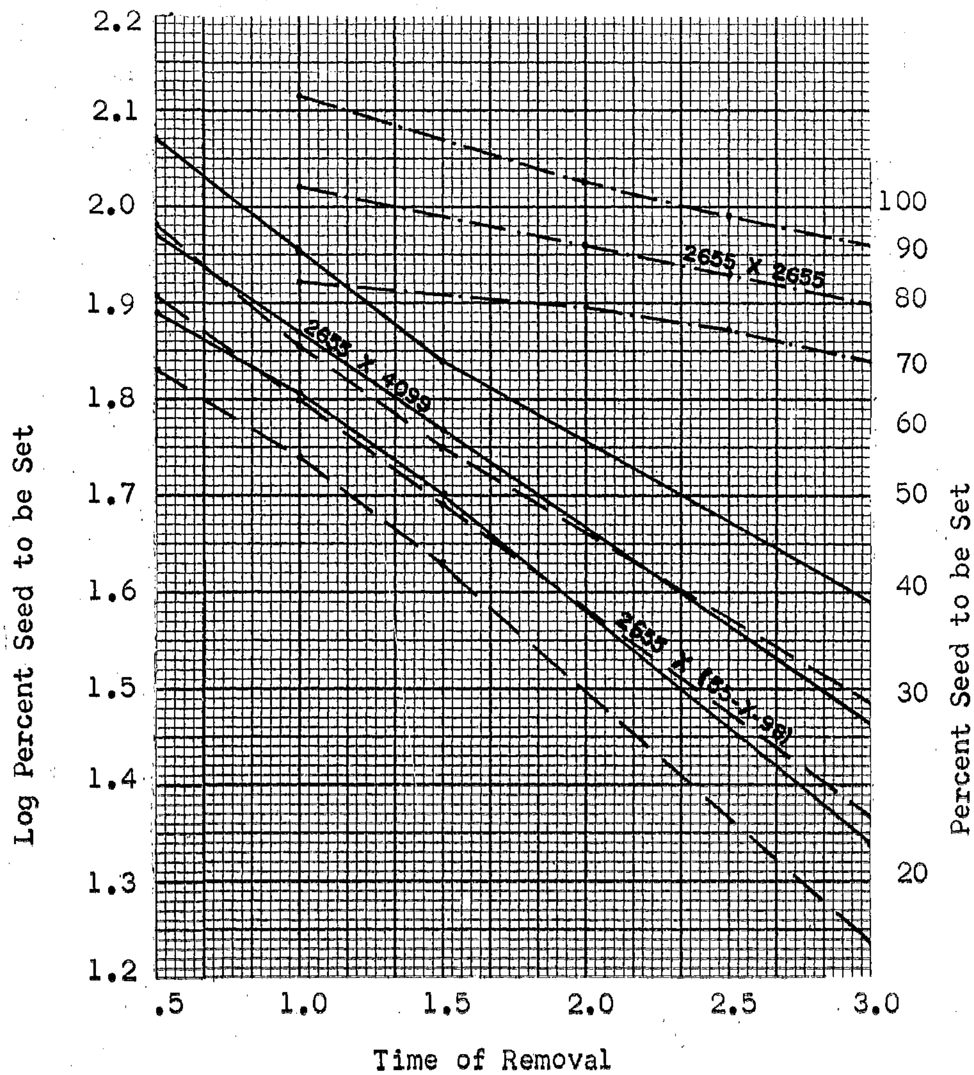


Figure 4. Sample Regression of B. intermedia A-2655 Treated with Three Sources of Pollen (2655, 4099 and 55-X-98)

tion. The data indicate that B. intermedia A-2655 has some degree of self sterility as expressed by slow movement of pollen through the stigmas. This pseudogamous apomictic species of Bothriochloa shows little or no preference as to pollen source whether it be from a species which reproduces by a sexual mechanism, e.g. D. annulatum (55-X-98), or by apomictic means, e.g. D. annulatum A-4099. This comparison can be made by studying Tables V and VI.

Tables VII and VIII show the results when D. annulatum A-4099, also an apomict, was pollinated with D. annulatum (55-X-98) and its own pollen respectively. Neither cross resulted in seed set after stigma removal thirty minutes following pollination, but some seed were produced after the one hour interval. Both crosses resulted in increased seed set as longer intervals were allowed between pollination and stigma removal. The highest average percent seed set was obtained when stigmas were left intact. D. annulatum A-4099 showed little preference for either source of pollen, setting as many seed with its own pollen as with pollen of D. annulatum (55-X-98), Figure 5.

Table IX shows data for the sexually reproducing diploid D. annulatum A-3242 with stigmas removed at intervals following self pollination. The data are limited by the few pollinations that could be made because of poor pollen development during October and November. A possible explanation of poor pollen development may be the high humidity during this period in the plastic greenhouse where the plants were grown. Light intensity was reduced by the film of moisture which condensed on the inner surface of the plastic. The few pollinations were made possible by clipping heads, inserting the stems in water, and moving them to

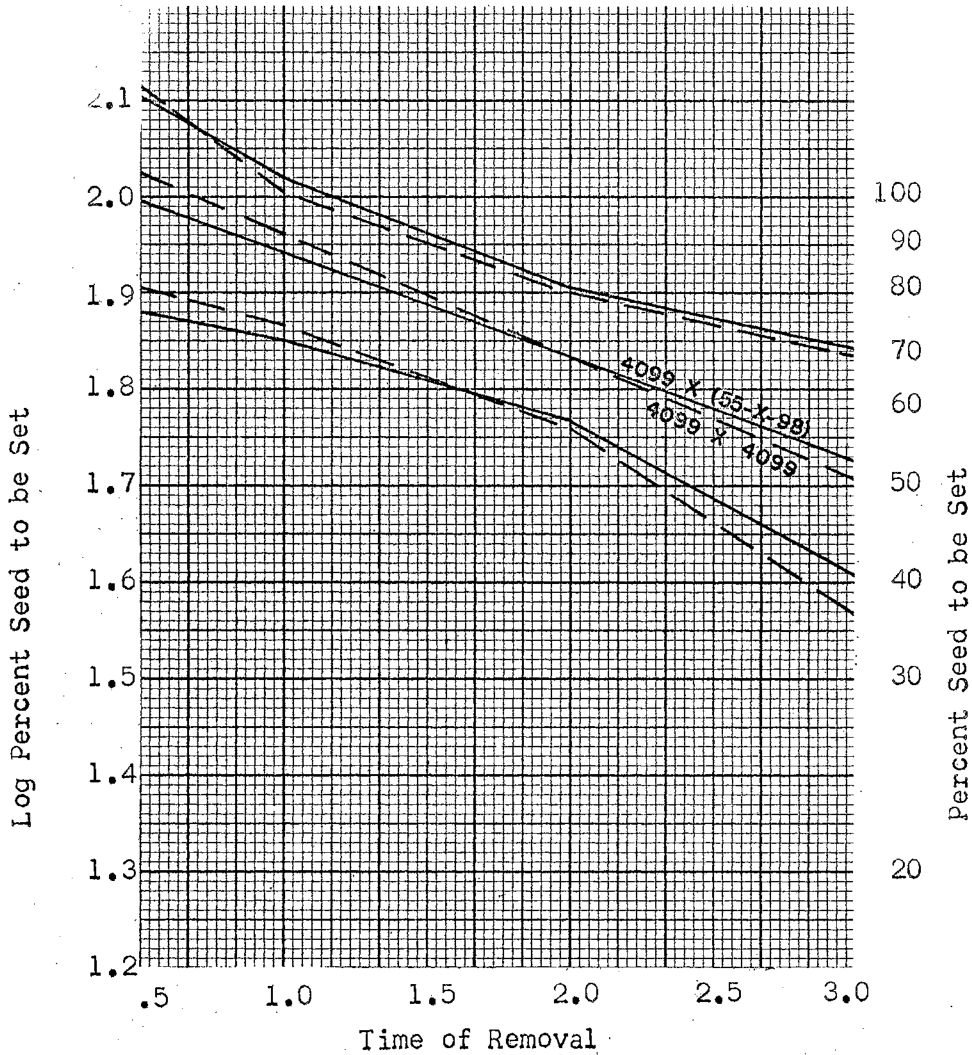


Figure 5. Sample Regression of D. annulatum A-4099 Treated with Two Sources of Pollen (55-X-98 and 4099)

TABLE IX

THE RELATIONSHIP BETWEEN STIGMA REMOVAL AT INTERVALS FOLLOWING POLLINATION AND SUBSEQUENT SEED SET
D. annulatum A-3242 X D. annulatum A-3242

| Time of Stigma Removal Following Pollination | Number of Panicles | Total No. of Florets | Total No. of Seed | Average % Seed Set |
|---|---|---|---|---|
| 1.0 hours | 1 | 28 | 0 | 0 |
| 2.0 hours | 2 | 48 | 3 | 6.2 |
| 3.0 hours | 5 | 196 | 38 | 19.3 |
| 4.0 hours | 2 | 86 | 27 | 31.3 |
| Not removed | 3 | 117 | 46 | 39.3 |

a glass greenhouse where light intensity and temperature were higher and humidity was lower. It is not unusual for environmental stress conditions to suppress pollen development in grasses of the Old World Bluestem group (Dewald in manuscript).

During this period seven hundred florets of A-3242 were selfed under bag and only .85% seed set resulted. It is quite evident that male sterility is exhibited in this species under stress conditions. A fair seed set was obtained when pollen could be provided but because of the limited pollinations, the story is not complete for this accession as indicated by the wide spread of the confidence belts illustrated in Figure 6.

From a standpoint of comparing pollen influence, the only instance in which rates of seed set were different when the same male was used on different females was when D. annulatum (55-X-98) was the pollinator. This difference can be observed by referring to Figure 7. With other pollinators no significant differences were obtained.

Table X shows analysis of covariance and multiple range test of the rates of seed set.

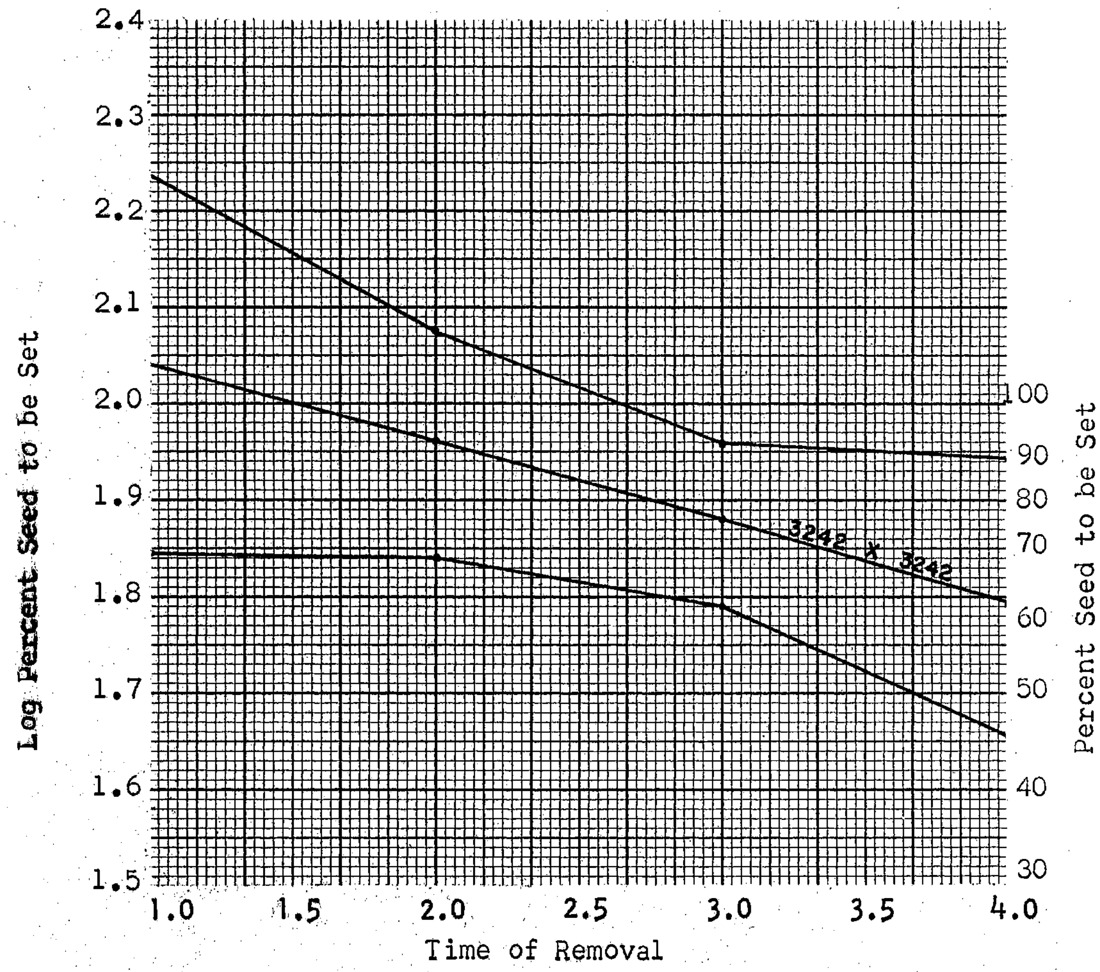


Figure 6. Sample Regression of D. annulatum A-3242 Treated with Its Own Pollen

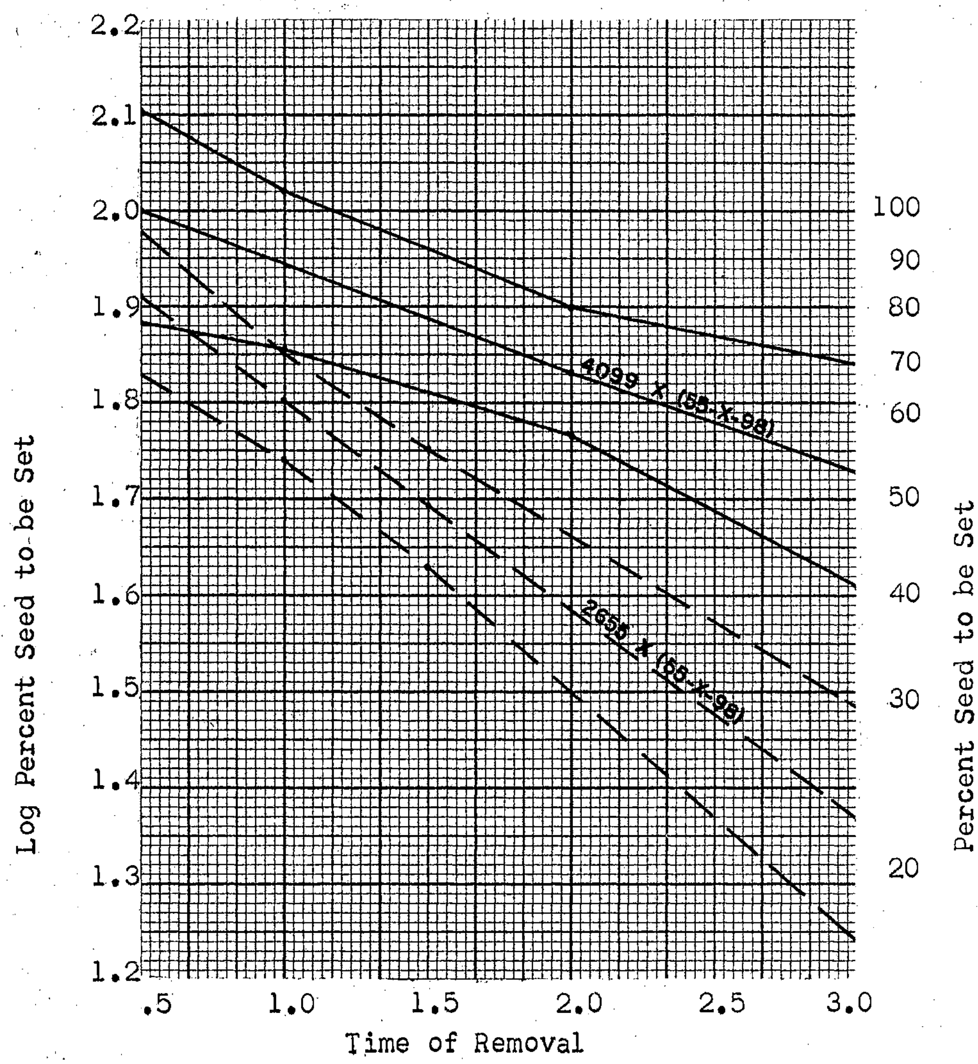


Figure 7. Sample Regression Comparing Two Females Pollinated with D. annulatum (55-X-98)

TABLE X

ANALYSIS OF COVARIANCE AND MULTIPLE RANGE TEST OF THE LOGARITHMIC RATES OF LOG. PERCENT SEED TO BE SET

| Crosses Female | | Male | N | $\sum x^2$ | $\sum xy$ | $\sum y^2$ | $\sum d^2 y.x$ | d.f. | Rate (b) | Multiple Range ∠x 5% |
|---|---|---|---|---|---|---|---|---|---|---|
| 2655 | X | (55-X-98) | 22 | 19.60 | -4.2838 | 1.9023 | 0.9663 | 20 | -0.218 | |
| 2655 | X | 4099 | 16 | 14.00 | -2.8576 | 0.8273 | 0.2440 | 14 | -0.204 | |
| 4099 | X | 4099 | 15 | 9.40 | -1.2196 | 0.4530 | 0.2948 | 13 | -0.129 | |
| (55-X-98) | X | 4099 | 20 | 32.80 | -3.9649 | 0.7529 | 0.2736 | 18 | -0.120 | |
| 4099 | X | (55-X-98) | 15 | 13.40 | -1.4327 | 0.4234 | 0.2702 | 13 | -0.106 | |
| 3242 | X | 3242 | 10 | 7.60 | -0.6231 | 0.1983 | 0.1472 | 8 | -0.081 | |
| 2655 | X | 2655 | 22 | 42.58 | -2.5202 | 0.1733 | 0.0241 | 20 | -0.059 | |
| (55-X-98) | X | 2655 | 22 | 32.82 | -1.3207 | 0.1670 | 0.1139 | 20 | -0.040 | |

Pooled $\sum d^2 y.x = 2.3345$

$$s^2 y.x \text{ (pooled)} = \frac{2.3345}{126} = 0.0185$$

∠x Any two means underscored by the same line are not significantly different.

## SUMMARY AND CONCLUSIONS

A stigma removal experiment was conducted in the greenhouse at the Oklahoma Agricultural Experiment Station Agronomy Farm in the fall and winter months of 1957 and 1958. The purpose of this investigation was to attempt to determine the relationship of pollination to seed set in selected grasses of the tribe *Andropogoneae* using two mating systems, with similar and different reproductive mechanisms, and at two ploidy levels. To study the various relationships, stigmas were removed at several predetermined intervals following pollination of hand emasculated florets in a manner described by Warmke (18) in 1954. The seed set resulting from the selected pollinations at the various intervals following pollination was used as an indication of the following relationships:

1. The time required for pollen to stimulate seed set as indicated by the shortest interval at which seed were set.
2. The rate of seed set as indicated by the increase in seed set as the time intervals increased.
3. The amount of seed set.

The apomictic *B. intermedia* accessions, A-2655 and A-5450, failed to set seed when stigmas were removed prior to two and one half hours following self pollination. Seed were set when stigmas were removed two and one half hours or more following pollination indicating pseudogamy. *B. intermedia* A-2655 shows a preference for foreign pollen, over its own, by increased rate and amount of seed set following cross pollination with D. annulatum A-4099 or D. annulatum (55-X-98). No detectable preference was shown for either source of foreign pollen and seed set resulted when stigmas were removed one half hour following pollination in both crosses.

A sexually reproducing D. annulatum hybrid designated (55-X-98) was treated as above with no seed being set whether stigmas were removed or not when treated with its own pollen. Selfing studies showed this hybrid to be very highly self sterile (99.9%). When florets of this hybrid were treated with pollen of B. intermedia A-2655 and D. annulatum A-4099 some seed were set at the one half hour interval in both crosses. Seed set increased at a faster rate and was greater in amount when pollen of D. annulatum A-4099 was used.

An apomictic D. annulatum accession, A-4099, was treated similarly using pollen of D. annulatum (55-X-98) and its own pollen. No preference was shown for either source of pollen. Rate of seed set was intermediate and was not significantly different from the other rates.

A diploid D. annulatum accession, A-3242, was also studied by the stigma removal technique. No seed were set following stigma removal prior to two hours after pollination. Seed were set as the intervals were increased; however, the limited pollinations made are insufficient from which to draw conclusions.

In all instances in which a female was treated with pollen of both D. annulatum (55-X-98) and A-4099, the rate of seed set was similar.

The apomictic accessions in this study only occasionally produce progeny which is not maternal. All accessions studied require pollen to stimulate seed set; however, the method of stimulation may be quite different between apomicts and sexually reproducing species.

In general, seed set increased as more time was allowed between pollination and stigma removal. Seed set was very low or absent when stigmas were removed at intervals less than one hour following pollination. The rate of seed set at intervals up to four hours appears to be directly correlated with the amount of seed set when stigmas were not removed.

When seed set was absent or very low at the one and two hour interval, it was also low when the intervals increased indefinitely. From this study it appears that the amount of seed which will be produced from a particular mating is directly correlated with the speed in which a pollen grain germinates and grows through the stigma.

Indications are that the growth rate of a pollen tube is not determined by the pollen source nor the female parent, but rather by an interaction of the two components.

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VITA

Chester L. Dewald

Candidate for the Degree of

Master of Science

Thesis: STIGMA REMOVAL STUDIES ON CERTAIN ACCESSIONS OF *Bothriochloa intermedia* and *Dichanthium annulatum*

Major Field: Field Crops

Biographical:

Personal data: Born in Vici, Oklahoma, August 2, 1934, the son of Chester Leroy and Ada Pearl Dewald.

Education: Attended elementary school at Tangier, Oklahoma. Received a high school diploma at Tangier High School, Tangier, Oklahoma in 1953. Received the Bachelor of Science degree from Oklahoma State University with a major in Field Crops in May, 1957. Attended Graduate School at Oklahoma State University 1957-1958.

Experience: Reared on farm; Employed by the Forage Section of the Agronomy Department at Oklahoma State University 1954-1959, the last two years as graduate assistant to Dr. Wayne W. Huffine.

Date of Final Examination: February, 1959

# APPENDIX

## STATISTICAL METHODS

The confidence belts plotted above and below the sample regression line in Figures 1, 4, 5, 6 and 7 are approximately at the 95% level. In order to test the rates (b) of log percent seeds to be set, it was assumed that the variances were homogeneous within each cross, thereby permitting pooling of sums of squares to arrive at an error of estimate (sy.x).

The multiple range test of the logarithmic rates (b) of log percent seed to be set, shown in Table X, was made as follows:

(1) The b values were arranged in ascending order.

(2) $t_{.05}(sy.x)\sqrt{\frac{1}{\sum x_i^2} + \frac{1}{\sum x_j^2}}$ was computed.

(3) To compute the first range line, the quantities $t_{.05}(sy.x)\sqrt{\frac{1}{\sum x_i^2} + \frac{1}{\sum x_j^2}}$ were computed for $j = 2, 3, \ldots 8$. The first significant difference was found to be between $b_1$ and $b_4$, hence the line was drawn under $b_1$ through $b_3$, thereby signifying these three b values could have come from populations in which the b's were the same.

(4) The above procedure was repeated for the second line by the same procedure except $\sum x_1^2$ was replaced by $\sum x_2^2$ and j set equal to 3, 4, 5, 6, 7 and 8.

(5) The procedure was repeated again for obtaining the third line. In this case $\sum x_3^2$ replaced $\sum x_1^2$ and j set equal to 4, 5, 6, 7 and 8.

(6) 1.980 was the 5% t value associated with the pooled degrees of freedom (126).

(7) $s^2_{y.x}$ was found by pooling the sum of squares of deviations and dividing this quantity by the pooled d.f.