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Successful boll development after ovary damage during emasculation of Upland cotton flowers

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Gossypium hirsutum **flowers are easily emasculated by splitting the staminal column with the fingernail and removing the corolla and androecium. However, any damage to the ovary is considered detrimental to successful boll formation and damaged flowers are typically discarded. This study evaluated boll retention after different emasculation treatments. Removal of the membrane surrounding the ovary reduces boll retention compared to self-pollinated flowers, and showed a similar response across 18 genotypes. Damage to the ovary wall reduced boll retention compared to emasculate flowers without ovary damage. Damaged flowers could be cross-pollinated to produce additional bolls as compared to discarding the flowers.**

Key words: Breeding, emasculation, *Gossypium hirsutum*, hybridization.

INTRODUCTION

Simple and rapid emasculation techniques to remove anthers, preventing self-pollination and allowing the introduction of foreign pollen to the stigma are essential for the development of new upland cotton (*Gossypium hirsutum* L.) varieties (Doak, 1934) and for hybrid seed production (Dong et al., 2004). Emasculation techniques can result in tissue damage and it is recommended that emasculated flowers with ovary damage be discarded as the frequency of boll retention under arid growing conditions is low (Wilson and Stapp, 1985). To assess the effects of ovary damage on boll retention under humid growing conditions, studies comparing four emasculation treatments were conducted. In addition, a study assessing two emasculation treatments applied to a set of 18 genotypes was conducted to determine if retention of bolls from damaged ovaries was genotype-dependent.

MATERIALS AND METHODS

Experiments were established on 25th April 2012 at the United States Department of Agriculture (USDA) in Stoneville, Mississippi. Seeds were planted in single-row plots 12 m in length with 1 m spacing between rows. The field included 24 rows of 'MD 25' used as the male parent for all crosses, female parents included 10 rows of 'Deltapine 90', and a single row each of 18 upland cotton genotypes. Each author conducted the same experiments individually and data from these three trials were combined for analysis. Trial was considered an additional source of random variation in the analysis.

In one experiment, 18 genotypes (Table 1) were evaluated for boll retention after damage during emasculation. The selected genotypes included both modern and obsolete conventional cotton maintained in-house at the USDA, Crop Genetics Research Unit, Stoneville, MS. Emasculation treatments were flowers allowed to naturally self-pollinate, and flowers with the corolla, stamina column, and membrane surrounding the ovary removed prior to cross-pollination on the morning of anthesis. The treatments were

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Figure 1. Flowers after application of emasculation damage treatments. Top left: not emasculated and allowed to self-pollinate, top right: corolla and staminal column removed prior to pollination, bottom left: corolla and staminal column removed and ovary wall penetrated with fingernail prior to pollination, bottom right: corolla, staminal column, and membrane surrounding ovary removed prior to pollination.

arranged in a factorial manner and evaluated in a randomized incomplete block design with 2 blocks and 5 replications within each treatment x block combination. The trials were conducted using five flowers (replications) per treatment for each cultivar and applied on two different days (blocks) over a 10 day period. The number of bolls for each treatment category retained on the 18 genotypes was counted 12 days after the final emasculation treatment was applied. The percentage of boll retention for each treatment was subjected to arcsine square root transformation prior to analysis to meet the assumption of normality required for analysis of variance (Little and Hills, 1978). The mixed models procedure in SAS (SAS v. 9.0 PROC MIXED, SAS Institute, Cary, NC) was used for analysis of variance and differences between treatment means were identified using differences of least square means ($P \leq 0.05$). Back transformed means are presented.

In a separate experiment, the genotype 'Deltapine 90' was used to evaluate 4 emasculation treatments that varied in the degree of ovary damage, Figure 1. Treatments were: naturally self-pollinated flowers that served as an undamaged control; flowers that were emasculated by removing the corolla and staminal column followed by cross-pollination; flowers that were emasculated by removing the corolla, staminal column, and membrane surrounding the ovary followed by cross-pollination; and flowers that were emasculated by removing the corolla and staminal column, and damaging the ovary wall with the fingernail followed by cross-pollination. Emasculations were done the morning of anthesis prior to pollen shed then cross-pollinated. The treatments were evaluated in a randomized complete block design with 4 blocks and 10 replications within each treatment x block combination. The trials were conducted using 10 flowers (replications) for each treatment category on each day and repeated on four different days (blocks) over an 18 day period. Percentages of boll retention for each treatment was determined 18 days after the last set of emasculation treatments were completed. Boll retention percentages were subjected to arcsine square root transformation prior to analysis to meet the assumption of normality required for analysis of variance (Little and Hills, 1978). The mixed models procedure in SAS (SAS v. 9.0 PROC MIXED, SAS Institute, Cary, NC) was used for analysis of variance and differences between treatment means were identified using differences of least square means ($P \leq 0.05$). Back transformed means are presented.

RESULTS AND DISCUSSION

Across the 18 upland cotton genotypes evaluated, removal of the membrane surrounding the ovary during emasculation significantly lowered boll retention compared to self-pollinated flowers shown in Table 1. No significant differences were observed between genotypes. The interaction between genotypes and emasculation treatments was not significant, indicating that genotypes responded similarly to the damage treatment. The membrane provides extra structure and protection to the style and removal of the membrane may have resulted in lower boll retention due to failed pollination from breakage or desiccation of the style prior to fertilization.

The removal of the corolla and staminal column during emasculation with no ovary damage did not significantly lower boll retention compared to self-pollinated 'Deltapine 90' flowers, Table 2. Successful boll development using the Doak (1934) emasculation technique was reported to

Table 1. Boll retention on 18 *Gossypium hirsutum* genotypes following damage to the ovary during emasculation.

Treatment	Emasculation treatment	Boll set (%)
Emasculation	No emasculation, self-pollinated	76.3
	Membrane removed during emasculation, cross-pollinated	45.1
	F	37.25
	P > F	<0.0001
Genotype	Coker 100A	67.0
	Coker 312	40.6
	Cook307-6	38.3
	DES 119	55.8
	Deltapine 16	64.5
	Deltapine 5690	71.0
	Deltapine 90*ne*	67.3
	Fox 4205-7139	58.7
	King, No Spot	52.8
	LA 887	69.2
	Lone Star	48.5
	MD 51*ne*	68.7
	MD 10-5	68.4
	MD 25-26*ne*	66.6
	Patty's Toole	76.3
	SG 747	64.8
	Stv7A	52.3
	Watson's Dixie Triumph	69.4
	F	0.89
	P>F	0.5855
Genotype × emasculation	F	0.82
	P>F	0.6659

Values are backtransformed means of 195 observations in three trials.

Table 2. Boll retention on *Gossypium hirsutum* 'Deltapine 90' following different levels of damage to the ovary during emasculation.

Emasculation treatment	Boll set (%)
No emasculation, self-pollinated	87.5^{a}
Emasculated, cross-pollinated	73.1^{ab}
Membrane removed during emasculation, cross-pollinated	51.5^{bc}
Ovary wall damaged during emasculation, cross-pollinated	25.0^{c}

Values are backtransformed means of 48 observations in three trials. Numbers followed by the same letter are not significantly different based on differences of least square means ($P \leq 0.05$).

range from 50 to 90% (Loden et al., 1950; Brown and Lee, 1976; Lee, 1980; Wilson and Stapp, 1984) and similar results were observed in this study. Removal of the membrane surrounding the ovary did not significantly lower boll retention compared to emasculated flowers that were cross-pollinated. Wounding of the ovary wall during emasculation significantly lowered boll retention compared to emasculated flowers that were cross-pollinated. Wilson and Stapp (1985) reported 6% boll retention for flowers with damage to the ovary wall compared to 25% boll retention observed in the present study. Boll development will depend on multiple factors, including the location, season, temperature, rainfall, and time of emasculation and pollination (Loden et al., 1950; Douglas and Adamson,

Figure 2. Immature bolls of *Gossypium hirsutum* 'Deltapine 90' in which the ovary wall was breached during emasculation showing the discoloration and deterioration of seeds and fiber.

1965; Lee, 1980; Wilson and Stapp, 1984; Dong et al., 2005). Climatic conditions in Mississippi could have been more favorable leading to the higher boll retention observed in the present study. Wilson and Stapp (1985) reported reduced seed production for ovary damaged flowers. Internal damage with discoloration and deterioration of ovule tissue was observed for bolls with ovary damage in the present study, Figure 2. This ovule damage was confined to the locule that was directly affected by the ovary wounding; however, the visual evaluation was destructive and no assessment was conducted on mature bolls to assess seed production and viability.

Results of this study indicated that cross-pollination of flowers where the membrane surrounding the ovary was removed during emasculation will still successfully produce bolls approximately 50% of the time. Pollinating these flowers should be considered, especially if flower number is a limiting factor. No genotype by emasculation treatment interaction was observed, suggesting boll retention should be similar for many diverse upland cotton cultivars. In cases where the ovary wall is damaged, pollination of these flowers should be considered based on the availability of flowers for crossing, pollen availability, and seed production requirements.

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