Seed and Seed Coat Characteristics in Six Seed Classes of Alfalfa

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ABSTRACT

Alfalfa, *Medicago sativa* L., seed losses in the southern Great Plains are extremely high and cannot be attributed to any single factor. Seed yield components must be determined in ascertaining seed losses. Yield components are complex, and it is often difficult to visually distinguish one seed damage class from another. Alfalfa seed were mechanically and visually separated into six seed classes: sound seed, obvious chalcid (*Bruchophagus roddi* Guss.) infested seed, suspected chalcid infested seed, obvious diseased seed, suspected diseased seed, and other damaged seed. The objectives were to describe and characterize the seed coat cuticle and hilum region of six yield component classes of alfalfa seed, and verify the validity of our separation procedures. Sound alfalfa seed were gold to golden-brown and well filled. The seed coat cuticles were continuous and void of microorganisms. Chalcid infested seed varied from light to dark brown. The seed coat cuticles were often cracked and lacked microorganisms. Obvious diseased seed were reddish-brown and powdery in appearance. The seed coat cuticles of this seed class were infested with fungal mycelia. Suspected diseased seed were similar to the previous class, but lacked the powdery appearance. Seed coat cuticles of this class were infested with fungal mycelia. Otherwise damaged seed comprised green immature seed, brown shriveled seed, and brown non-shriveled seed. Seed coat cuticles of this class lacked microorganisms.

Additional index words: *Medicago sativa*, **seed quality, germination**

INTRODUCTION

Field culture and management practices considered optimum for alfalfa, *Medicago sativa* L., seed production are often not reflected in terms of increased seed yield in the southern Great Plains (Ahring et al., 1985). The alfalfa seed chalcid and *Lygus* spp. as well as seed diseases are known contributors to seed losses in this region (Ahring et al., 1984). Laboratory analyses of seed yield components are necessary to determine the causes of alfalfa seed losses. In legumes, such as alfalfa, seed yield components are difficult to estimate and if determined by hand are time consuming. Mechanical methods of seed separation lack the precision for accurately making estimates of seed losses. Thus, mechanical separation along with visual evaluation offer the most rapid and reliable method for determining sound seed in alfalfa.

Many factors affect the number of sound seed set per pod, e.g., insect pest, age of flower, type of pollination, viability of pollen, moisture, soil fertility, ovule abortion (Armstrong and White, 1935; Brink and Cooper, 1936; and Tysdal, 1946). In determining the source of seed losses in alfalfa it is often difficult to visually distinguish one seed damage class from another. In our laboratory we mechanically and visually separated alfalfa seed into either well-filled sound, obvious and suspected chalcid infested, obvious and suspected diseased, or otherwise damaged seed. The objectives of this study were to describe and characterize the seed coat cuticles and hilum regions of the six yield component classes of alfalfa and to verify the validity of our separation procedures.

MATERIALS AND METHODS

Alfalfa pods were collected prior to harvest from a seed production field in northwestern Oklahoma. Seed were carefully extracted from the pods by gentle abrasive action on a rub-board. Care was taken to avoid crushing chalcid-infested seed. Inert material was removed from the hand-threshed samples with a seed blower South Dakota model B[3] set at an air valve opening of 20°. Coarse separation (based on relative density) of each seed class was made with the seed blower. The majority of obvious chalcid seed was removed at an air valve opening of 30°, suspected chalcid seed at an opening of 35°, and otherwise damaged seed at an opening of 55°. Seed remaining at the bottom of the separation column after the 55° blowing were classed as sound seed. Diseased seed occurred in all seed fractions, and were sepa

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rated by hand from other seed in each fraction. All fractions were examined with an illuminated magnifier for accuracy of class, and further separations were made by hand into six seed classes: 1) sound seed, 2) obvious chalcid infested seed, 3) suspected chalcid infested seed, 4) obvious diseased seed, 5) suspected diseased seed, and 6) otherwise damaged seed. The otherwise damaged seed class contained light immature seed, *Lygus*-damaged seed, and brown non-shriveled seed. The mean seed weight (mg 100^{-1} seed) for each seed class was determined by averaging 10 subsamples of 100 seed each. A seed germination test was conducted comparing four of the six seed classes: sound seed, obvious diseased seed, suspected diseased seed, and otherwise damaged seed. The germination environment consisted of cycled periods 8 hours light and 16 hours dark at a constant 20 C. Germination data were analyzed using PROC ANOVA (SAS Institute 1985) as a randomized block design consisting of four treatments (seed classes) and four replications each containing 50 seed.

Representative seed from each seed class were processed for viewing with a scanning electron microscope (SEM) (Rugenstein and Lersten, 1981). These were sonicated in 50% ethanol for 1 minute, air dried for 15 minutes, mounted on aluminum SEM stubs, and coated with gold-palladium. Seed were observed with a JEOL 35U[1] scanning electron microscope at 15 KV.

RESULTS AND DISCUSSION

Seed Coat Characteristics

Sound alfalfa seed were gold to golden-brown in color and well-filled. The seed coat cuticles were continuous, unbroken, and lacked microorganisms (Fig. 1). The hilum region and funicular tissue were unblemished and appeared normal (Fig. 2).

Chalcid-infested seed were grouped into two classes either as obvious or as suspected chalcid-infested seed. Of the six seed classes, the obvious chalcid-infested seed class was the easiest to recognize. The distinguishing characteristic of this class was an exit hole, left by the emerging adult seed chalcid (Fig. 3). The seed coats had a mottled appearance, and varied from light to dark brown in color. Except for the conspicuous exit hole, suspected chalcid-infested seed possessed similar characteristics as those of the obvious chalcid-infested seed. The seed coat cuticles of the suspected chalcid seed were often cracked (Fig. 4). The seed coat cuticles of the obvious and suspected chalcid infested seed classes lacked microorganisms.

Diseased seed were also grouped into two classes either obvious or suspected diseased seed. Obvious diseased seed were black to reddish-brown in color, misshapen, and had a powdery or fuzzy appearance under low magnification. Scanning electron micrographs showed the seed coat cuticle (Fig. 5) and the hilum region (Fig. 6) to be infected with fungal mycelia. Suspected diseased seed were usually similar to obvious diseased seed in size, shape, and color, but lacked the powdery appearance. Apparent 'sound seed' that had conspicuous blemishes, e.g., sunken black or discolored spots of various sizes, on the seed coat were considered as suspected diseased. The depressed and discolored areas were infested with fungal mycelia (Fig. 7). Also, the hilum region were infected with mycelia; however, mycelia are not as prevalent as in the obvious diseased class (Fig. 8). It appeared that the discolored areas were caused by the alfalfa seed chalcid in its attempt to penetrate the seed coat and parasitize the seed. Slight damage to the seed coat permitted infection by microorganisms. Consequently, the seed chalcid may render seed susceptible to a pathogen or serve as a vector of seed diseases in alfalfa.

Seed not classified into the previous five classes were classified as otherwise damaged seed. This class contained seed of three types, i.e., immature seed, *Lygus* damaged seed, and brown non-shriveled seed. Immature seed were green to yellow-green in color, often misshapened, and poorly filled (Fig. 9). *Lygus* damaged seed were brown in color, shriveled, and appeared to be collapsed (Fig. 10). Brown non-shriveled seed may have been damaged by *Lygus* or developed under unfavorable environmental conditions. The seed coat cuticles and hilum regions of seed in this class appeared normal and lacked mycelia.

Seed Weights and Seed Germination

The mean 100-seed weight of sound alfalfa seed was 185.3 mg and ranged from 142 to 248 mg (Table 1). The mean weight of the suspected chalcid infested seed (118.7 mg) was approximately twice that of the obvious chalcid infested seed (58.0 mg). Similarly, suspected diseased seed were approximately 1.5 times larger than obvious diseased seed.

Normal seed germination occurred only in seed of the sound seed class (Table 2). Abnormal seed germination was observed in all classes except the sound seed class. Suspected diseased seed class had the highest percent of abnormal seed germination. Those, otherwise sound suspected diseased seed with discolored spots germinated, but seedling development was abnormal. Like-

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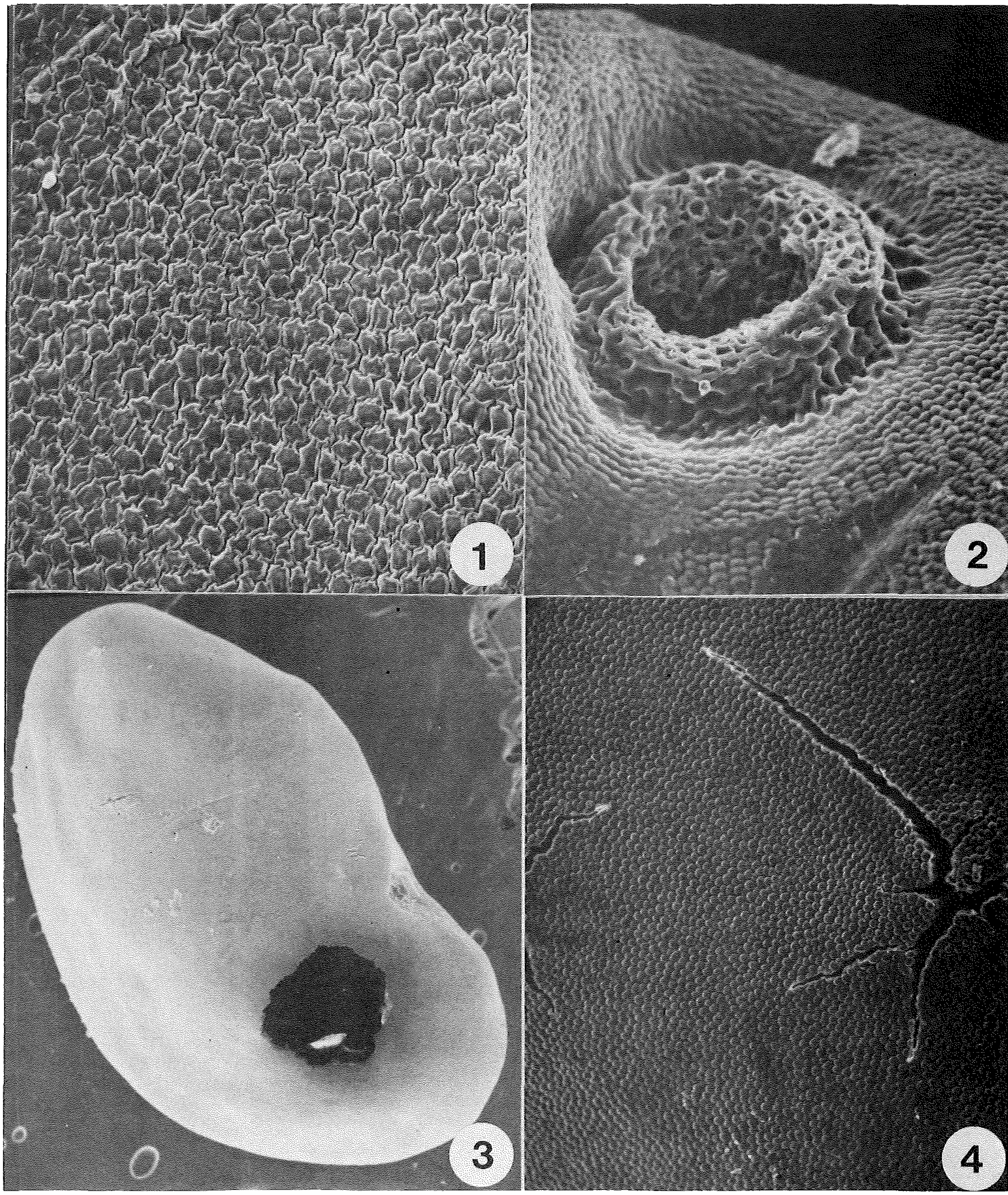

Fig. 1-4. Scanning electron micrographs of normal-healthy (sound) and chalcid infested seed. Fig. 1. Seed coat cuticle of sound seed x 720. Fig. 2. Hilum region of sound seed x320. Fig. 3. Obvious chalcid infested seed showing exit hole x40. Fig. 4. Cracks in seed coat cuticle of suspected chalcid infested seed x 20.

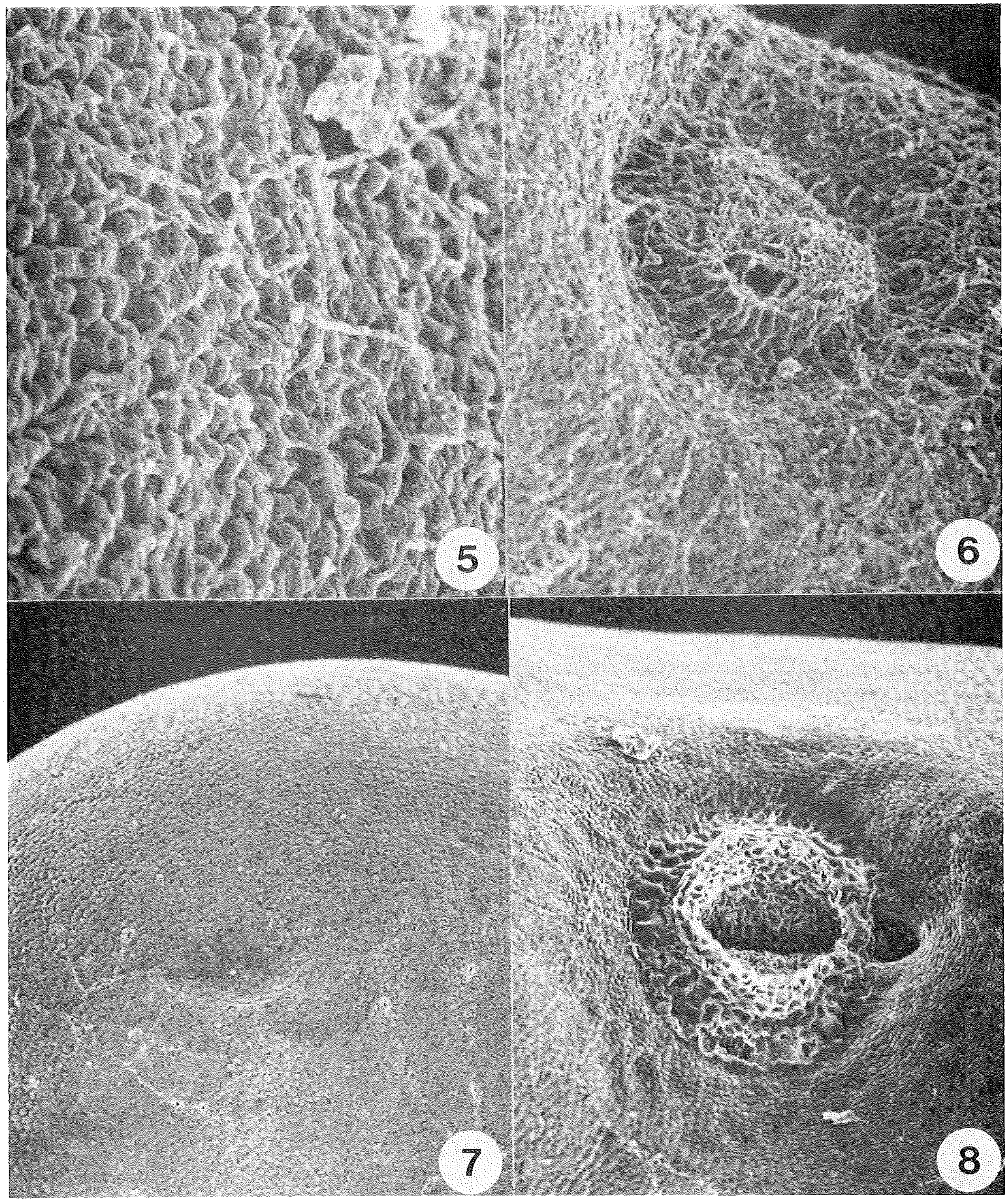

Fig. 5-8. Scanning electron micrographs of obvious and suspected diseased seed. Fig. 5. Seed coat cuticle of obvious diseased seed with fungal mycelia x860. Fig. 6. Hilum region of obvious diseased seed infested with fungal mycelia x300. Fig. 7. Suspected diseased seed with depression and fungal mycelia x200. Fig. 8. Hilum region of suspected diseased seed with fungal mycelia x200.

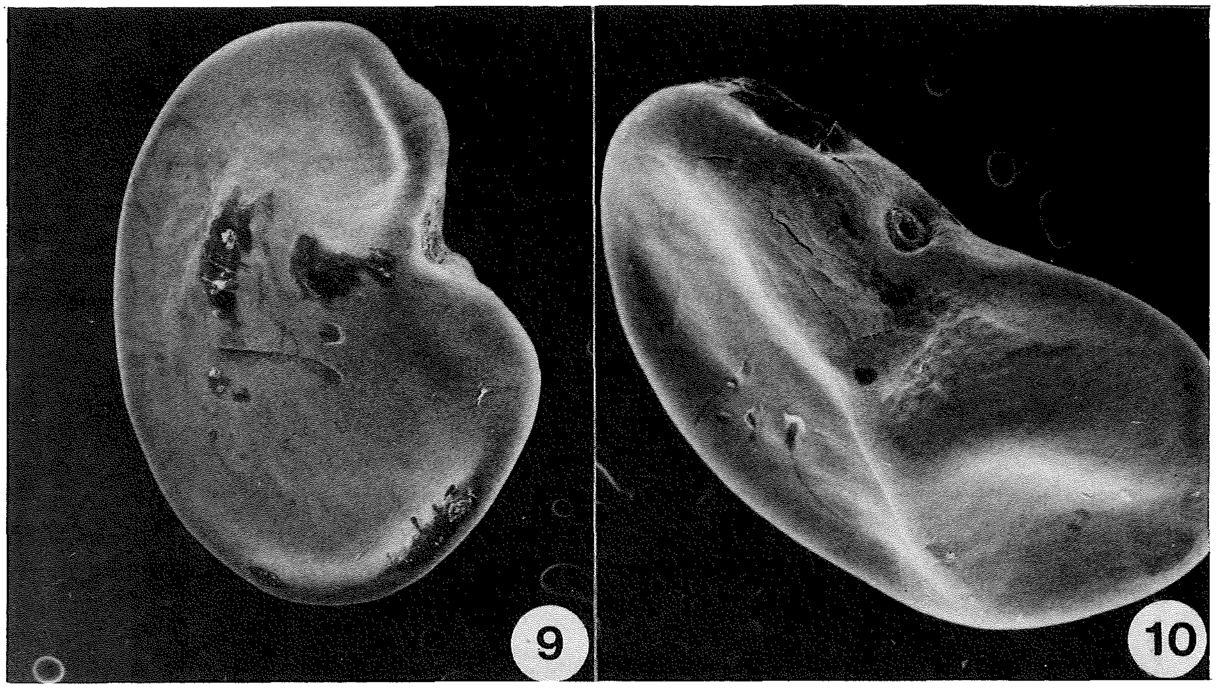

Fig. 9-10. Scanning electron micrographs of immature and suspected *Lygus* damaged seed. Fig. 9. Misshapen, poorly filled immature seed x40. Fig. 10. Collapsed suspected *Lygus* damaged seed x40.

Table 1. Hundred seed weight of alfalfa seed component classes.

Seed Class	Mean	SEM	Range	CV
	----------------	mg	-----------	-%-
Sound	185.3	26.9	142 - 248	14.5
Obvious chalcid	58.0	6.7	50 - 74	11.6
Suspected chalcid	118.7	15.7	101 - 146	13.2
Obvious diseased	62.8	13.8	40 - 83	22.0
Suspected diseased	95.3	22.4	63 - 131	23.5
Otherwise damaged	66.9	12.4	50 - 89	18.6

Table 2. Percent normal, abnormal, hard, and total seed germination of alfalfa by seed classes.

Seed Class	Normal	Abnormal	Hard	Total
	----------------	%	----------------	
Sound	80.0	0.0	20.0	100.0
Obvious diseased	0.0	2.0	0.0	0.0
Suspected diseased	0.0	14.5	6.5	6.5
Otherwise damaged	0.0	8.0	8.5	8.5
LSD (0.05)	6.9	4.1	8.0	3.4

wise, germination of immature seed gave rise to abnormal seedling growth. Hard seed at the end of a 21-day germination period favored the sound seed class, however firm seed occurred in the suspected diseased seed and otherwise damaged seed classes. Total germination (Table 2), i.e., normal germination plus hard seed, was 100% for the sound seed class, and 0% for the obvious diseased seed class. Only normal germination was observed for sound seed, thus the percent total germination for other classes is based strictly on its percent hard seed. Mold was not observed in germination boxes containing sound seed. A significant amount of mold was observed in germination boxes containing obvious diseased seed. Somewhat less mold occurred in the germination boxes containing suspected diseased seed and otherwise damaged seed.

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Growth Regulator Effects on Seed Production of *Bromus willdenowii* Kunth. cv. Grasslands Matua[1]

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ABSTRACT

The theoretical potential seed yield of prairie grass (*Bromus willdenowii* Kunth). cv. Grasslands Matua is 7 to 9 t ha^{-1}, but commercial seed yields are usually between 1.5 and 1.8 t ha^{-1}. Lodging, seed abortion, seed shedding and seed losses during harvest may all contribute to reducing seed yield.

The effects of three plant growth regulators (paclobutrazol, flurprimidol, chlormequat chloride) on seed yield of G. Matua were investigated in four trials at two sites in two seasons. All three products significantly increased seed yield, with the magnitude of the responses (0 to 42%) varying with different application rates. Paclobutrazol responses increased as application rate increased from 0.375 to 1.0 kg. a.i. ha^{-1}, but did not increase further at 2.0 kg. a.i. ha^{-1}. The maximum response to flurprimidol application occurred at 0.5 kg. a.i. ha^{-1} and yield was decreased at 2.0 kg. a.i. ha^{-1}. There were no yield differences with chlormequat chloride application rates between 0.75 and 3.0 kg. a.i. ha^{-1}.

Seed yield increases were associated primarily with increased seed number per spikelet, as other yield components did not differ. All three products shortened stem internode length and prevented lodging, which may have contributed to the reduction in seed abortion. However, data recorded for paclobutrazol and chlormequat chloride showed that both significantly delayed leaf tissue senescence, which may also have assisted in the retention of more seeds per spikelet.

Growth regulator application allowed direct combine harvesting of an erect crop which on a dry matter basis had 20% less plant material to pass through the threshing process.

Additional index words: **rescue grass; seed yield; paclobutrazol; flurprimidol; chlormequat chloride; lodging; seed abortion; leaf senescence.**

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INTRODUCTION

Prairie grass (also known as rescue grass) (*Bromus willdenowii* Kunth) cv. Grasslands Matua, described by Rumball (1974), is recommended for use in New Zealand dairy, sheep and cattle farming systems because of its potential to produce high quality feed, particularly in autumn and winter, and to persist under dry conditions