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Title: Plant Growth Regulators and Shattering Control in Cool-Season Perennial grasses

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Shattering is a natural phenomenon of floret and spikelet disarticulation across abscission layers of the pedicel and rachilla, respectively, after seed maturation. Seed shattering in cool-season grasses and native grass species is an economic problem in seed production.

This study was conducted to determine whether the use of the plant growth regulators aminoethoxyvinylglycine (AVG) and trinexapac-ethyl (TE) could reduce the incidence and severity of seed shattering under greenhouse conditions, and increase harvested seed yield under field conditions in perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.).

In greenhouse experiments, the effect of several rates and application timings of aminoethoxyvinylglycine (AVG) and trinexapac-ethyl (TE) was measured in plant characteristics including chlorophyll content of flag leaves, days to harvest maturity, and floret and spikelet number. Shattering was evaluated on harvested spikes by using a

modified wrist action shaker.

Two field experiments were conducted for evaluation of harvest yield and shattering control. In field experiment 1, AVG at 75 ppm (135 g a.i. ha^{-1}) was applied once (T1) at peak anthesis and again (T2) 14 days after peak anthesis in perennial ryegrass. In field experiment 2, AVG was applied at the following rates: 100 ppm (180 g a.i. ha^{-1}), 200 ppm (360 g a.i. ha^{-1}), and 300 ppm (540 g a.i. ha^{-1}), and TE was applied at 200 g a.i. ha^{-1}, and 400 g a.i. ha^{-1} in perennial ryegrass and tall fescue.

Higher rates of both AVG and TE reduced flag leaf chlorophyll content. Although chlorophyll content increased, lower rates of AVG (50-100 ppm) are unable to detect differences using the statistical tests. The cumulated application of AVG increased chlorophyll content of perennial ryegrass flag leaves over the control 24 days after peak anthesis.

Neither AVG nor TE application affected the potential number of spikelets or florets formed in the spike. AVG treatments did not change spike length compared with the water treated control, but TE at 1.3 and 2.5 g a.i. L^{-1} shortened spikes by 1.6 cm (12.2%) and by 1.7 cm (13.0%), respectively. Application of AVG at the 100 ppm rate reduced shattering losses by 8.2 florets per spike when compared to the untreated control and the 50 ppm AVG rate treatment. This reduction in total shattering was attributable to less shattering of both spikelets and florets.

Seed yield in perennial ryegrass was reduced by the low rate of AVG (100 ppm) in the field experiment. Seed weight in perennial ryegrass was reduced by AVG in experiment 1, but not in experiment 2. No effect from AVG or TE on seed yield or seed weight of tall fescue was measured. PGR treatments had no effects on the level of

unutilized florets in perennial ryegrass, but differences among PGR treatments were evident in tall fescue. For both perennial ryegrass and tall fescue, the total number of seeds lost due to shattering (prior to and after swathing), was significantly reduced by the low rate of TE (200 g a.i. ha^{-1}). There were no significant differences in final germination percentage and mean germination time in perennial ryegrass and tall fescue over the various rates of AVG and TE application.

Despite the variable results, there is some evidence that both compounds reduced seed shattering in perennial ryegrass. While seed shattering was reduced in the trials, the lack of a positive effect from the late application of TE on seed yield does not warrant an expansion of its use for the purpose of seed shattering control.

Plant Growth Regulators and Shattering Control in Cool-Season Perennial grasses

by
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Suk-Kyun Lee, Author

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INTRODUCTION

The seed production industry in the Pacific Northwest has developed rapidly since 1940. The Willamette Valley is the center of seed production in the region, with a favorable climate and producers familiar with specialized agricultural practices. The success of the Willamette Valley as a seed production region results from extensive research, and cooperative development of seed production practices. The Willamette Valley's climate is ideally suited for seed production. Mild and wet winters assist the re-growth of grasses in the late fall to early spring. Dry summers allow two-step harvesting, which eliminates the need for drying and reduces the cost of production. Highly dependable yields can be expected every year in a variety of grass seed crops.

Shattering is a widespread natural phenomenon in plants and serves as a mechanism for dispersal of seed to favorable environments.

The development of seed retention has historically been a by-product of domestication and human selection (Kadkol et al., 1989). Unfortunately, many grass species utilized for forage and turf applications have a relative short history of domestication; thus, shattering is quite common in those species. Shattering of grass seeds occur before harvest and causes low and unpredictable seed yields because fewer seeds are harvested. Shattering can also lead to volunteer weed problems in following years. Control of the volunteers can increase the cost of seed production. Despite high seed yield potential, the actual seed yield harvested in perennial ryegrass (*Lolium*

perenne L.) can be much lower as a result of unproductive florets, seed shattering prior to and during harvest, and seed losses in cleaning processes (Anslow, 1964; Meijer, 1985).

Shattering is a major cause of seed yield loss for perennial ryegrass, comprising 10% of the total seed losses in this species (Griffith and Chastain, 1997; Warringa and Marinissen, 1997; Meijer, 1985). Less is known about shattering in tall fescue (*Festuca arundinacea* Schreb.), another important species.

Shattering is caused by abscission, which is the result of ethylene production. Ethylene also causes leaf and flower senescence (Yang and Hoffman, 1984). Ethylene is a gaseous plant hormone with a simple structure (C_2H_4), which is produced from the methionine in essentially all tissues of higher plants (McKeon et al., 1995; Salisbury and Ross, 1992).

Several compounds have been developed to block ethylene synthesis (Yang and Hoffman, 1984). AVG (aminoethoxyvinylglycine) inhibits ethylene synthesis, while STS (silver thiosulfate) inhibits ethylene action when incorporated into plant tissue. The compound 1-MCP (1-methylcyclopropene) binds ethylene receptors to block ethylene signal transduction in plant tissue (Sisler and Serek, 1997).Beltrano et al. (1994) reported that spraying ethephon, an ethylene releasing agent, accelerated maturation and senescence in wheat. The application of AVG delayed grain maturation and senescence of the chlorophyll containing organs of the inflorescence.

Shattering can occur by either active or passive mechanisms or a combination of the two (Kadkol et al., 1989). Active mechanisms involve the formation of abscission layers in the inflorescence, while passive mechanisms involve external factors that cause shattering. There are two abscission layers associated with seed disarticulation by

shattering (Burson et al., 1983). The primary abscission layer is located across the pedicel below the spikelet, and is common among many tropical grasses. The secondary abscission layer is formed across the rachilla below the fertile florets; many temperate grasses exhibit a secondary abscission layer. Secondary abscission layers are first visible at heading, while primary abscission layers are already initiated during the boot stage (Elgersma et al., 1988).

Two means of disarticulation of abscission layers are characteristic of the active mechanism (Burson et al., 1983, Elgersma et al., 1988). Biochemical changes take place at the primary abscission layer, which in turn forms lacunae starting from the cells between the vascular bundles and proceeds toward the epidermal cells. Mechanical tearing occurs at the secondary abscission layer, which forms cleavage starting at the epidermal cells and progresses toward the vascular bundles.

Uneven ripening contributes to shattering losses and requires much effort in the development of breeding (Falcinelli, 1987) and harvesting techniques (Kadkol et al., 1989). The goal of breeding programs for grasses and cereal crops are not for seed retention per se but rather focus on economic yield. Some genetic improvement of seed retention has been achieved since the late 1970s by breeding and genotype selection programs in some grass species (Weiser et al., 1979). Seed retention ability is often associated with the glume shape, flexibility, and silica content (Falcinelli et al., 1984). Mass selection appears to be superior for improving seed retention in wild rice (Everett and Stucker, 1983).

The traits most often associated with seed retention in the inflorescence are the non-brittle pedicel and rachilla (McWilliam, 1980). Seed yield and shattering were

strongly correlated in perennial ryegrass (Elgersma et al., 1988).

Since ethylene is involved in abscission layer formation, it might be possible to reduce seed shattering by blocking ethylene biosynthesis. Plant breeding programs have made some progress in reducing shattering (Everett and Stucker, 1983; Elgersma, 1990; Falcinelli et al., 1994; Gepts, 2002; Chen and Nelson, 2004), but multiple generations are required to achieve measurable results. PGR applications on the other hand, might provide an effective means to reduce shattering in the absence of genetically improved plant materials.

There is much interest in the use of many native grass species but seed shattering limits their adoption because of the high cost of seed. Application of PGRs to native grasses might facilitate economical seed production as a result of reduced shattering.

The objective of this study was to characterize seed shattering losses and to test the use of PGRs to decrease seed shattering in perennial grasses.

REVIEW OF LITERATURE

Seed shattering plays a critical role in determining yield losses of grass seed crops. Seed shattering starts as early as 15 days after anthesis in reed canarygrass (*Phalaris arundinacea* L.) (Baltensperger and Kalton, 1958). Shattering in economically valuable grasses leads to harvesting difficulties and results low yields with poor quality of the seed produced (McWilliam, 1980).

The terms 'shattering' and 'shedding' are often used interchangeably; however, 'shattering' has relevance in a physiological or anatomical context, while 'shedding' is used to denote seed loss in an agricultural practices context. In other words, shattering is the physical separation of the seeds from the mother plant, while shedding is the loss of shattered seeds causing yield losses as a consequence. For instance, phalaris (*Phalaris tuberose* L.) shattering takes place as soon as the seeds are mature. However, the fixed glumes hold seeds in place preventing seed loss. Shedding in *Phalaris* does not take place until an external force disperses seed away from the mother plant.

Agricultural practices have been developed to reduce shattering due to uneven ripening resulting from variable or prolonged timing of flowering. Baltensperger and Kalton (1958) observed that shattering of reed canarygrass occurs from the upper side of inflorescences, while the lower side is still immature. Several special harvest practices have been developed to reduce seed shattering. Repeated harvesting with specially-designed shaking machinery is costly. Spraying a water-soluble lacquer before seed maturity reduced shattering, but presents problems related to good coverage and additional costs (McWilliam and Schroeder, 1974).

Unproductive florets, seed shattering prior to harvest, seed losses during harvesting, and the seed cleaning process are factors leading to low seed yields in cool-season grasses (Elgersma et al., 1988).

Poor seed quality is one of the major impediments to the adoption of many grass species. Early harvesting of grass seed can result in impaired germination (Hill and Watkin, 1975) and produces many light seeds that will be discarded during the seed conditioning process.

Evolution of Resistance to Shattering

Shattering in cereals and other grasses occurs by abscission or by disarticulation of the dispersal unit as spikelets and single florets in inflorescences (Kadkol et al., 1989). Temperate cereals have spikes that are fragile shortly after maturation, thereby enabling the ease of harvesting these crops (McWilliam, 1980). Resistance to shattering in many crops appears to have evolved simultaneously under domestication and human selection. At maturity, cultivated tetraploid emmer wheat (*Triticum dicoccoides* L.) has a non-brittle spike. The reduced shattering trait in modern barley (*Hordeum vulgare* L.) and in rye (*Secale cereale* L.) results from the selection for recessive non-shattering mutants with free-threshing grain on a tough rachis at the time of harvesting.

While selection for reduced seed shattering made these crops less able to survive in the wild, they are now much better suited for agricultural production. The evolution of the seed retention trait in the inflorescence of cereals has been attained by development of the non-brittle pedicel and rachilla, which restrains shattering of the spikelets and florets in the inflorescence at maturity (McWilliam, 1980).

Mechanism of Shattering

Threshing is the removal of the seeds from within the glumes, whereas shattering occurs when both seeds and spikelets break away from the mother plant at disarticulation points located at the rachilla and pedicel (Bean, 1964). Disarticulation of individual seeds or spikelets is aided by the development of abscission layers, which are found in the middle lamella of parenchyma cells. The process is initiated in the early boot stage (Zadoks scale, 40-49).

Shattering occurs by either active or passive mechanisms (Kadkol et al., 1989). Active mechanism involves the formation of an abscission layer. In contrast, passive mechanism acts by external impact to increase the predisposition to shattering.

Burson et al. (1983) and Elgersma et al. (1988) reported that there are two general mechanisms of the active mechanism. Biochemical changes are responsible for disarticulating the middle lamella and cell wall by enzymatic lysis and mechanical tearing in weak tissue. Jagged cell walls are the result of enzymatic lysis, whereas mechanical tearing results in smooth rounded walls on abscission layers. Seed shattering in non-domesticated species of Poaceae shows disarticulation on the rachilla immediately above the glumes in many temperate species and on the pedicel immediately below the glumes in many tropical species.

In *Paspalum* spp. and *Panicum* spp., the primary abscission layer forms lacunae as a result of cell elongation and collapse, and disintegrates biochemically, starting from the cells between vascular bundles and proceeds toward the epidermal cells. The secondary abscission layer forms cleavage without cell walls thickening, starting epidermal cells toward the vascular bundles and disintegrates by mechanical forces

(Elgersma et al., 1988).

Elgersma et al. (1988) also reported that initial shattering in perennial ryegrass is observed 3.5 to 4 weeks after the beginning of anthesis depending on the weather conditions, i.e., wind and rainfall. Shattering begins with upper seeds of apical spikelets and continues with upper seeds of intermediate spikelets and central seeds of apical spikelets, and so on.

Shattering was found to be more severe on the windward side of a plot than on the leeward side and the strength of glumes should be the principal factor in determining shattering resistance in reed canarygrass (Bonin and Goplen, 1963).

Anatomical Basis

The abscission mechanism of perennial ryegrass is well known from anatomical and histological studies. Elgersma et al. (1988) reported that tearing of the florets from the rachilla starts from the epidermal sides, with only the central area between vascular bundles holding the floret in place. Seed shattering occurs following the tearing of vascular bundles.

A slight constriction in the region of the vascular bundle is the first visible sign of abscission layer development (Weiser et al., 1979). The non-shattering mutant of phalaris (*P. tuberose* L.) has a well-developed non-brittle rachilla below the fertile lemma (McWilliam, 1980).

The abscission layer cells with vascular bundles are small when compared to parenchyma cells in the rachilla of perennial ryegrass (Elgersma et al., 1988). The central area between the three vascular bundles is easily distinguishable from the outer area

between the vascular bundles and the epidermis.

Elgersma et al. (1988) also mentioned that cell walls in the abscission layer between the epidermis and the vascular tissue showed no evidence of cell wall thickening in perennial ryegrass. Cell wall thickening is the result of lignification of cell walls. In a non-shattering mutant obtained by irradiation, the abscission layer was present, but lignification of the walls did not occur.

Falcinelli et al. (1984) reported that seed shattering is associated with glume shape and flexibility, the level of silica content, strength of the rachilla, and densely packed panicles and spikelets appear to be the main causes of a high degree of seed retention. In three-dimensional view, the abscission zone on the tip of the rachilla was a circular trough of small, thin-walled cells and large, thick-walled cells surrounding the vascular bundle. The highest rate of seed retention has been found in plants with short rigid inflorescences containing a large number of densely packed spikelets (Falcinelli, 1987).

A primary abscission layer develops across the pedicel below the spikelet and a secondary abscission layer forms in the rachilla below the fertile floret. The primary layer accounts for 81% of seed abscission, while 19% occurs at the secondary layer (Burson et al., 1983).

The primary abscission layer is recognizable at the base of the spikelet, and when broken, causes the detachment of the entire spikelet. The secondary abscission layer is situated at the base of the floret and causes shattering of single fertile seed.

The primary abscission layer develops below the whole spikelet across the pedicel in tropical grasses like bahiagrass (*Paspalum notatum* Fluegge) and dallisgrass (*P.*

dilatatum Poir.). The secondary abscission layer develops below the fertile floret across the rachilla in guineagrass (*P. maximum* Jacq.) and perennial ryegrass, which are temperate grasses. Secondary abscission layers were first visible at heading, whereas primary abscission layers were already initiated during the boot stage (Elgersma et al., 1988).

In orchardgrass (*Dactylis glomerata* L.), one of the most important forage grasses of the temperate zone, seed retention should be a selection criterion in breeding programs to decrease seed loss (Piccirilli and Falcinelli, 1989). The typical inflorescence of orchardgrass is a branched panicle with spikelets arranged in small groups.

Genetic Control

Wild rice (*Zixania palustris* L.) exhibits high levels of shattering losses (Eiguchi and Sano, 1990), but the percentage of seed shattering losses varies in wild rice from 21% to 55%, depending on cultivar (Minnesota Agricultural Experiment Station, 1991). Seeds of non-domesticated wild rice are ready to shatter as soon as they are mature. In contrast, domestication in the development of wild rice cultivars has reduced shattering. Shattering in wild rice is controlled by two dominant genes and panicle spreading by one dominant gene, which was found to be independent in wild rice (Eiguchi and Sano, 1990).

Seed dispersal in the progenitors of wheat occurs at the rachis that is the disarticulation point of individual florets (Gepts, 2002). Reduced seed shattering is the result of the strengthening of the pedicel and the rachilla and/or the elimination of the abscission layers.

Rachis brittleness is controlled by the Q gene that is located on chromosome 5A in wheat species (Kadkol et al., 1989). The Q gene shows a dosage effect arisen from the multiplication of recessive allele q. A shattering resistance phenotype is in the recessive condition because rachis thickness depends on the dosage of recessive q alleles. Non-brittle rachilla in wheat (*Triticum aestivum* L.) is controlled by up to three recessive genes. Likewise, tetraploid oats (*Avena* spp.) in Ethiopia and wild rice are under control by two to few recessive genes (McWilliam, 1980).

Hexaploid oats have the opposite situation, in which shattering is controlled by a single dominant gene. A strong, non-brittle pedicel and rachilla that is the evolution of seed retention trait are under control of one or few dominant or recessive genes (McWilliam, 1980).

Breeding

Breeders of cool-season grasses have traditionally concentrated their efforts on improvement of desirable leaf characteristics and forage yield and quality, but selection for shattering resistance has not been a priority (Anslow, 1962). However, seed losses due to shattering have been effectively reduced by breeding and genotype selection in some grass species including timothy (*Phleum pratense* L.) and reed canarygrass (*Phalaris arundinacea* L.), but not in the genus *Panicum* (Weiser et al., 1979). Finding a natural mutant for shattering resistance is a good source of genetic materials to improve seed retention.

Everett and Stucker (1983) demonstrated that only one cycle per year is possible with half-sib family testing while mass selection appears to be superior for improving

seed retention in wild rice. The largest components of variability in wild rice breeding for seed retention are those of seed-to-seed and plant-to-plant variance. Seed-to-seed variance was reduced by multiple seed measurement per plant and at least equal gain per cycle was observed in comparison with half-sib family selection.

Seed yield and seed shattering were strongly correlated in perennial ryegrass (Elgersma et al., 1988). Despite this relationship to seed yield, the selection characteristics commonly considered by breeders of perennial ryegrass include leafiness traits, leaf color, disease resistance, vigor, and others, but not resistance to seed shattering. Selection for seed retention must be carried out simultaneously with selection for seed yield in perennial ryegrass.

Selection for better seed retention permits breeders to produce cultivars with acceptable seed ripening characteristic, which will allow these valuable ecotypes to be used commercially (Harun and Bean, 1979). Therefore, selection of inflorescence characteristics that favor reduced shattering should be done after anthesis to reduce the variation of selection (Baltensperger and Kalton, 1958).

Glume strength was important in determining the retention of the seed in the spike in timothy (*Phleum pretense* L.) (Bean, 1964). Ease of threshing of the spike depends upon the tenacity of glumes to the seeds, and shattering depends upon the firmness of glume's connection to the pedicel. In timothy, the characters of seed retention and shattering are separate. It should be possible to select for both seed retention and ease of threshing for breeding programs.

In orchardgrass, shattering results from the disarticulation of caryopsis from the rachilla or rachilla from the rachis followed by subsequent release of seed from the

glumes (Falcinelli, 1987).

Plant Growth Regulators

Seed shattering occurs at two abscission layers which are triggered by the phytohormone ethylene (Weiser et al., 1979; Reid, 1985; Beltrano et al., 1994; Smalle and Straeten, 1997; Balota et al., 2004). Ethylene has been implicated in aging processes in plants. Ethylene is a gaseous hormone with simple structure (C_2H_4), which is produced from methionine in essentially all plant tissues (Salisbury and Ross, 1992; McKeon et al., 1995). Since ethylene is involved in abscission layer formation, it might be possible to reduce shattering by blocking ethylene biosynthesis.

Ethylene plays a role in grain maturation in the wheat spike and in the senescence of the spikes where experimental manipulation of ethylene production or action was conducted. On the other hand, accelerated maturation and senescence of spikes were obtained by spraying ethephon, an exogenous source of ethylene. Abscisic acid is a stress response hormone. Mevalonic acid is the precursor of ABA as well as gibberellic acid (GA) (Halmann, 1990). Paclobutrazol influences ABA biosynthesis, which might influence floret and seed abscission in perennial ryegrass (Mares Martins and Gamble, 1993).

Weiser et al. (1979) reported that IAA application on guineagrass (*Panicum maximum* Jacq.) at three-fourths spike emergence reduced shattering up to 40%, but gibberellin application either reduced shattering or had no effect. IAA acts as an ethylene biosynthesis trigger by increasing the levels of mRNA which encodes ACC (1-aminocyclopropane-1-carboxylic acid) synthase (Yang et al., 1982; Nakagawa et al.,

1991).

Plant growth regulator (PGR) treatment effects vary by genotype. IAA treatment on short, fine leaved genotypes reduced shattering whereas no effect on tall, robust, and broad-leafed genotypes. The abscission layer treated with IAA showed no morphological or histological alterations but delayed abscission.

Aminoethoxyvinylglycine (AVG) and Aminooxyacetic acid (AOA) inhibits ACC synthase, which converts from S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC). Beltrano et al. (1994) and Reid (1985) reported that silver ion (Ag^{+}) inhibits ethylene action to decrease its emission from the spike in wheat. Little is known about the effect of shattering by application on perennial ryegrass.

AVG and STS prolonged the metabolism of florets by permitting greater accumulation of photoassimilates in the grains and resulting in an increase in dry weight (Beltrano et al., 1994). Aminoethoxyvinylglycine (AVG) is an ethylene synthesis inhibitor, likewise silver thiosulfate (STS, silver ions) acts as a non-specific anti-ethylene agent, inhibiting its action or incorporation into plant tissue.

Recently, 1-methylcyclopropene (1-MCP) was developed, which can block ethylene to its binding receptors on the plant cell surfaces. Zhong et al. (2001) reported that I-MCP delayed fruit ripening and leaf abscission.

Application of AVG and STS delayed grain maturation and increased final grain size (Beltrano et al., 1994). In contrast 1-MCP (1-methylcyclopropene) blocks ethylene binding to its receptors in plant tissues, because I-MCP has similar molecular structure to ethylene (Sisler and Serek, 1997).

Polyamines are related to the senescence of many organs and these compounds

are inversely correlated with ethylene production (Beltrano et al., 1994), because ethylene and polyamines share SAM as a common precursor.

Gibberellin (GA) does not seem to have a direct effect on seed shattering. Weiser et al. (1979) reported that the GA application to two guineagrass lines in a field study had no beneficial effect. However, the application of GA synthesis inhibitor may change morphological factors related to shattering or the balance of hormones within the plant. ABA and GAs are synthesized from mevalonic acid (Sponsel, 1995). The use of GAs inhibitors might result in more mevalonic acids converted to ABA, which is an important factor in ethylene synthesis. The application of GA inhibitors might also prevent shattering, in that disarticulation of florets and spikelets still takes place, florets and seeds are not lost because inhibitor-induced morphological changes in spikelets and florets result in a compact shape.

GA constitutes a large family of diterpenoids composed of 19-20 carbons. GAs biosynthesis has three stages (Yabuta and Sumiki, 1938; Helliwell et al., 1999). The first stage is the formation of ent-kaurene from mevalonic acid (MVA). This stage takes place in cytosol. ATP phosphorylates MVA to MVA-5-pyrophosphate. Next, MVA-5-pyrophosphate is decarboxylated to isopentenyl pyrophosphate (IPP) (Sponsel, 1995). Isomerasecatalyze of IPP gives dimethylallyl pyrophosphate (DMAPP). Series of head-to-tail condensation of IPP from DMAPP gives geranyl pylophosphate (GPP), and in turn condenses of another IPP to give farnesyl pyrophosphate (FPP). Further condensation of FPP gives geranylgeranyl pyrophosphate (GGPP). The final step in the first stage is the formation of ent-kaurene from GGPP catalyzed by ent-kaurene synthetase A, B.

The second step is oxidations to form GA_{12}-aldehyde. This stage catalyzed by

membrane-bound mono-oxygenases which requires molecular oxygen and a reduced pyridine nucleotide, NADPH. Next, cascade oxidations at carbon-19 of ent-kaurene gives ent-kaurenol, ent-kaurenal, ent-kaurenoic acid. A hydroxylation at carbon-7 of ent-kaurenoic acid gives ent-7α-hydroxykaurenoic acid. GA_{12}-aldehyde is formed by contraction of a ring, which is considered to be first GAs precursor.

The last stage of GA biosynthesis is formation of active GAs by GA_{12}-aldehyde. GA_{12}-aldehyde is oxidized to GA_{12} catalyzed either by a monooxygenase or a soluble dioxygenase (Hedden and Kamiya, 1997). GA_{12} is first converted to GA_{53} by hydroxylation of carbon-13, and then converted to GA_{19} by successive oxidations at carbon-20. The formation of GA_{20} is followed by the elimination of carbon-20 as CO_2. GA_{20} is then converted to the biologically active form, GA_1, by the enzyme 3β-hydroxylase.

GA retardants have also been utilized for lodging control in grasses and forage crops. Lodged crops have limited opportunities for pollination and shading reduces photosynthesis in the lodged crop canopy. Silberstein et al. (1996) reported that paclobutrazol had a more positive effect than uniconazol on seed yield and harvest index in red clover.

Trinexapac-ethyl (TE) is a widely used plant growth retardant in cool-season grass seed production (Zapiola et al., 2006). TE can be applied to crop foliage, blocking the activity of 3β-hydroxylase. Chastain et al. (2001) reported that the application of TE increased seed yield 25% in the 1st year and 41% in the second year over the untreated control with increased floret number and floret conversion to seeds.

Several investigators have cited the relationship of GA production and GA-

inhibitors, and other hormone production, and the collective impact on seed shattering (Kazuo et al., 1988; McKeon et al., 1995). The primary hormone related to floret and/or spikelet shattering is ethylene. It does not seem to be that GAs cannot directly regulate the ethylene production (Yang and Hoffman, 1984; Reid, 1985). However, cytokinin has long been considered as an inhibitor of senescence (Goldthwaite, 1987). Mares Martins and Gamble (1993) reported that GAs retardants might influence cytokinin biosynthesis. Both GAs and cytokinin biosynthesis start from mevalonic acid pyrophosphate (Halmann, 1990). The application of GAs retardants might influence floret and seed abscission by affecting cytokinin biosynthesis.

Seed Yield and Yield Components

There are three stages of seed development in cool-season grasses: seed growth, reserve food accumulation and seed ripening (Hill and Watkin, 1975). Important attributes of seed quality are seed viability, seedling vigor, and storage life. Direct threshing should be made early enough to avoid shattering losses without affecting seed viability.

Anslow (1964) and Meijer (1985) reported that 10% of the initial florets in perennial ryegrass were lost mainly due to shattering and the final weight of an individual seed depends mainly on its position within a spikelet.

Seed yield is determined by its components: spike number per unit area, spikelet number per spike, floret number per spikelet, floret site utilization (the percentage of the florets containing a seed) and seed weight (Elgersma, 1990).

Seed yield was positively correlated with the number of seeds per spike

(Elgersma, 1990). Seed yield can be considered the product of the number of seeds and the average seed weight. Therefore, a possible way to improve seed yield is to reduce shattering of spikelets and florets.

The number of seeds per unit area calculated as: number of spikes X number of spikelets per spike X number of seeds per spikelet, was three to four times higher than the seed number calculated afterwards from seed yield and 1000 grain weight (Elgersma, 1990).

Seed weight (1000 grain weight) and seed number are components of yield, while harvest index (ratio of seed dry matter to total dry matter, HI) is seed dry matter auto-correlated to the seed yield (Elgersma, 1990).

Anslow (1962) found that late application of nitrogen fertilizer induces late inflorescence emergence, which shows lower seed set. Late nitrogen application does not contribute substantially to seed yield in perennial ryegrass.

Spike length was also positively correlated with the number of spikelets (Elgersma, 1990). Compact spikes were associated with low numbers of seeds and florets per spikelet, but FSU (floret site utilization) was not correlated with other characters.

Harvest Timing

Hebblethwaite et al. (1980) reported that seed lost due to shattering reaches around 30% when the moisture content declined from 40% to 30% in perennial ryegrass. Early harvesting can result in overproduction of light seeds, which will be removed during seed cleaning. On the other hand, delayed harvesting may result in high seed losses because of shattering (Falcinelli, 1987).

Viable seed yield following swathing and combining was superior to direct harvesting at all harvest dates prior to peak yield (Hill and Watkin, 1975).

Early shattering cultivars have a significantly lower mean moisture concentration than high seed retention cultivars at ripening (Harun and Bean, 1979). Therefore moisture level is critical to determine optimum harvest timing. Seed yield with the best quality can be obtained at the time that 5% of the seed has shattered. The moisture level determinations can be useful for determining ripeness in many grass seed crops (Falcinelli et al., 1984). Many grass seed crops begin shattering at 40% seed moisture content. At this point, swathing should be done for maximum seed yield and seed germination.

Irrigation treatment affects the amount of shattered seeds. Garcia-Diaz and Steiner (2000) reported that water irrigation increased the percentage of total seed loss by shattering in birdsfoot trefoil (*Lotus corniculatus* L.). Therefore, high amounts of irrigation water increased the percentage of the potential seed losses that would be shattered at harvest time.

MANUSCRIPT I: THE USE OF PLANT GROWTH REGULATORS AND SEED SHATTERING IN PERENNIAL RYEGRASS (*Lolium perenne* L.): GREENHOUSE INVESTIGATION

ABSTRACT

Seed shattering is an economic problem limiting the seed production of cultivated and native grasses. This study was conducted to characterize the nature of seed loss by shattering and to determine whether plant growth regulators (PGRs) may reduce the incidence and severity of seed shattering in perennial ryegrasses (*Lolium perenne* L.).

Plant characteristics measured included chlorophyll content, days to harvest maturity, and floret and spikelet number. PGR treatments were several rates and application timings of aminoethoxyvinylglycine (AVG) and trinexapac-ethyl (TE). Shattering was evaluated on harvested spikes by using a modified wrist action shaker.

Inconsistent and variable effects of both AVG and TE were noted for several physiological and morphological characteristics of perennial ryegrass. Higher rates of both AVG and TE reduced flag leaf chlorophyll content. Although chlorophyll content increased in lower rates of AVG (50-100 ppm), it is inability to detect differences using the statistical tests. Neither AVG nor TE application affected the number of spikelets or florets formed in the spike. AVG treatments did not change spike length compared with the water treated control, but TE at 1.3 and 2.5 g a.i. L^{-1} treatment shortened spikes by 1.6 cm (12.2%) and by 1.7 cm (13.0%), respectively. Shorter spikes had lower shattering losses than longer spikes.

Application of AVG at the 100 ppm rate reduced shattering losses by 8.2 florets per spike when compared to the untreated control and the 50 ppm AVG rate treatment. This reduction in total shattering was attributable to less shattering in both spikelets and florets. A second experiment with both AVG and TE showed that although shattering was lower than the untreated control, these differences were not statistically significant. Despite the variable results, there is some evidence of activity of both compounds in reducing seed shattering in perennial ryegrass, thereby warranting further work.

Keywords: Shattering, Perennial ryegrass, Plant growth regulators (PGRs), Aminoethoxyvinylglycine (AVG), Trinexapac-ethyl (TE)

INTRODUCTION

Shattering is a widespread natural phenomenon in plants and serves as a mechanism for dispersal of seed to favorable environments. Shattering is a major cause of seed yield loss for perennial ryegrass, comprising 10% of the total seed losses in this species (Griffith and Chastain, 1997; Warringa and Marinissen, 1997; Meijer, 1985). Less is known about shattering in tall fescue (*Festuca arundinacea* Schreb.), another important species of cool-season grass.

Uneven ripening contributes to shattering losses and requires much effort in the development of breeding (Falcinelli, 1987) and harvesting techniques (Kadkol et al., 1989). The goal of breeding programs for grasses and cereal crops are not for seed retention per se but rather focus on economic yield. Some genetic improvement of seed retention has been achieved since the late 1970s by breeding and genotype selection programs in some grass species (Weiser et al., 1979). Seed retention ability is often associated with the glume shape, flexibility, and silica content (Falcinelli et al., 1984). Mass selection appears to be superior for improving seed retention in wild rice (Everett and Stucker, 1983). The traits most often associated with seed retention in the inflorescence are the non-brittle pedicel and rachilla (McWilliam, 1980). Seed yield and shattering were strongly correlated in perennial ryegrass (Elgersma et al., 1988).

The plant hormones mainly concerned with abscission layer development are auxin (IAA), cytokinin, abscission acid (ABA), while ethylene is thought to have the greatest involvement (Reid, 1985). Reduced auxin or low concentration promotes the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), which is the direct ethylene

precursor (McKeon et al., 1995). By comparison, increased exogenous auxin or high concentration decreases ethylene production (Gianfagna, 1995). Likewise, increased ABA is induced by wounding, chilling injury, drought stress, and flooding, which promotes ACC synthesis. Cytokinin cannot promote ACC accumulation by itself, but cytokinin with auxin, light, and good nutritional condition tends to reduce or delay abscission.

Shattering is caused by abscission, which is the result of ethylene production that also increases leaf and flower senescence (Yang and Hoffman, 1984). Ethylene is a gaseous plant hormone with a simple structure (C_2H_4), which is produced from methionine in essentially all tissues of higher plants (McKeon et al., 1995; Salisbury and Ross, 1992).

A possible way to reduce shattering losses in grass seed yield is the use of plant growth regulators (PGRs). Several compounds have been developed to block ethylene synthesis (Yang and Hoffman, 1984). AVG (aminoethoxyvinylglycine) inhibits ethylene synthesis, while STS (silver thiosulfate) inhibits ethylene action when incorporated into plant tissue. Beltrano et al. (1994) reported that spraying ethephon, an ethylene releasing agent, accelerated maturation and senescence in wheat. However, the application of AVG delayed grain maturation and senescence of the chlorophyll containing organs of the inflorescence. Commercial AVG (Retain®, Valent BioSciences Corp.) is an EPA approved agent (EPA Reg. No. 73049-45, EPA Est. No. 33967-NJ-1). The compound 1-MCP (1-methylcyclopropene) binds ethylene receptors to block ethylene signal transduction in plant tissue (Sisler and Serek, 1997). Polyamines are inversely correlated with ethylene production (Beltrano et al., 1994).

Gibberellin (GA) does not seem to have a direct effect on seed shattering. Weiser et al. (1979) reported that the GA application to two guineagrass (*Panicum maximum* Jacq.) lines in a field study had no beneficial effect. However, the application of GA synthesis inhibitor might change morphological factors related to shattering or the balance of hormones within the plant. ABA and GAs are synthesized from mevalonic acid (Sponsel, 1995). The use of GAs inhibitors might result in more mevalonic acids converted to ABA, which is an important factor in ethylene synthesis. The application of GA inhibitors might also prevent shattering, in that disarticulation of florets and spikelets still takes place. Florets and seeds are not lost because inhibitor-induced morphological changes in spikelets and florets result in a compact shape.

Another possible way to reduce seed shattering by GA retardant application is the modification of inflorescence morphology. GA retardants reduce rachilla length; shortened rachilla length reduces the distances between each floret and/or spikelet within the inflorescence, thereby restricting seed loss from the inflorescence.

The last stage of GA biosynthesis is formation of active GAs by GA_{12}-aldehyde. GA_{12}-aldehyde is oxidized to GA_{12} catalyzed either by a monooxygenase or a soluble dioxygenase (Hedden and Kamiya, 1997). GA_{12} is first converted to GA_{53} by hydroxylation of carbon-13, and then converted to GA_{19} by successive oxidations at carbon-20. The formation of GA_{20} is followed by the elimination of carbon-20 as CO_2. GA_{20} is then converted to the biologically active form, GA_1, by the enzyme 3β-hydroxylase. There are three different classes of such GAs inhibitors: (a) onium compounds, such as chlormequat chloride, mepiquat chloride, chlorphonium, and AMO-1618, which block the cyclases, ent-copalyldiphosphate synthase and ent-kaurene

synthase involved in the early steps of GA metabolism. (b) compounds with an N-containing heterocycle, such as ancymidol, flurprimidol, tetcyclacis, paclobutrazol, uniconazole-P, and inabenfide. These retardants inhibit oxidation of ent-kaurene into ent-kaurenoic acid. (c) structural mimics of 2-oxoglutaric acid, which is the co-substrate of dioxygenases that catalyze late steps of GAs formation, such as prohexadione-Ca and trinexapac-ethyl and daminozide, inhibits the formation of highly active GAs from inactive precursors. Trinexapac-ethyl (TE) is a widely used plant growth retardant in cool-season grass seed production (Zapiola et al., 2006). TE can be applied to crop foliage, blocking the 3β-hydroxylase activity.

Several investigators have cited the relationship of GA production and GA-inhibitors, and other hormone production, and the collective impact on seed shattering (Kazuo et al., 1988; McKeon et al., 1995). The primary hormone related to floret and/or spikelet shattering is ethylene. It does not seem to be that GAs cannot directly regulate the ethylene production, but cytokinin has long been considered as an inhibitor of senescence (Yang and Hoffman, 1984; Reid, 1985; Goldthwaite, 1987). Mares Martins and Gamble (1993) reported that GAs retardants might influence cytokinin biosynthesis. Both GAs and cytokinin biosynthesis start from mevalonic acid pyrophosphate (Halmann, 1990). The application of GAs retardants might influence floret and seed abscission by affecting cytokinin biosynthesis.

The objectives of this study were to (1) investigate the use of plant growth regulators (PGRs) to decrease seed shattering, (2) determine the timing and rates of PGRs, and (3) identify potential relationships between shattering and seed yield in perennial ryegrass.

MATERIALS and METHODS

Plant Materials and PGR Treatments

Greenhouse trials were conducted using a non-vernalizing clonal line of perennial ryegrass. The clone was selected by T. G. Chastain from individual plants of Cutter perennial ryegrass. The clonal line does not require vernalization and therefore will flower under greenhouse conditions without a cold treatment.

Clonal propagation of the non-vernalizing genotype was accomplished by removing individual tillers from stock plants maintained in the greenhouse and transplanting tillers into 1.5 L opaque plastic containers. Tillers with 3 leaves were selected to insure survival after transplanting. Equal amount of Sunshine SB40 (SunGrow Inc.) potting soil was mixed with osmocote (Controlled Release Fertilizer for Greenhouse Crops, 14-14-14) in each pot. An appropriate amount of water was used to wet the potting medium before planting the clones and watered twice a day after planting. Clones were grown in a greenhouse at 24°C for 16 h of light and at 16°C for 8 h of darkness. Illumination was provided by high pressure sodium lamp fixtures, with 400W bulbs. The clones were grown until the stem elongation stage (Zadoks scale, 30 to 39) when treatments were initiated.

PGR treatments were applied by spraying randomly selected tillers in plants every 5 days beginning with the appearance of tillers in the stem elongation stage (Fig. 1-1). The first of two greenhouse experiments was conducted from June 22, 2002 to Oct 20, 2002. The Zadoks stage of development of selected tillers was determined at the time of treatment, marked with a plastic band and recorded. AVG was applied by using a hand

sprayer to the leaf surface at 50 ppm (0.0033 g a.i./pot) and 100 ppm (0.0066 g a.i./pot), and a water control treatment was applied at each application date. Every treatment was applied with nonionic silicone surfactant (Sylgard 309, Norac Concepts Inc.) at 0.1% (v/v). Each treatment was replicated over 9 application dates.

The second greenhouse experiment for AVG and TE treatment was conducted from April 1, 2003 to Sep. 25, 2003. Three rates of AVGs were applied: 100 ppm (0.0066 g a.i./pot), 150 ppm (0.01 g a.i./pot), and 200 ppm (0.0132 g a.i./pot). TE was applied at 1.3 g L^{-1} and 2.5 g L^{-1}. A water control treatment was applied at each application date. Each treatment was replicated over 15 application dates. The Zadoks stage of tillers was determined at the time of treatment, marked with a plastic band, and recorded.

Inflorescences were placed in pollination bags (Lawson Bags #117) to prevent unexpected shattering by watering or touching. Tillers were considered to be matured for the purposes of harvesting when flag leaf color changed from dark green to yellow and when all parts of inflorescence had turned to yellow.

Harvested inflorescences of fully matured seeds were dried at 70°C for 72 hours in electrically operated dryer (MO1040A1-1, Lindberg/Blue M Inc.). The number of days to harvest maturity, number of florets, number of spikelets, and spikelet length, were for each treatment in the two experiments.

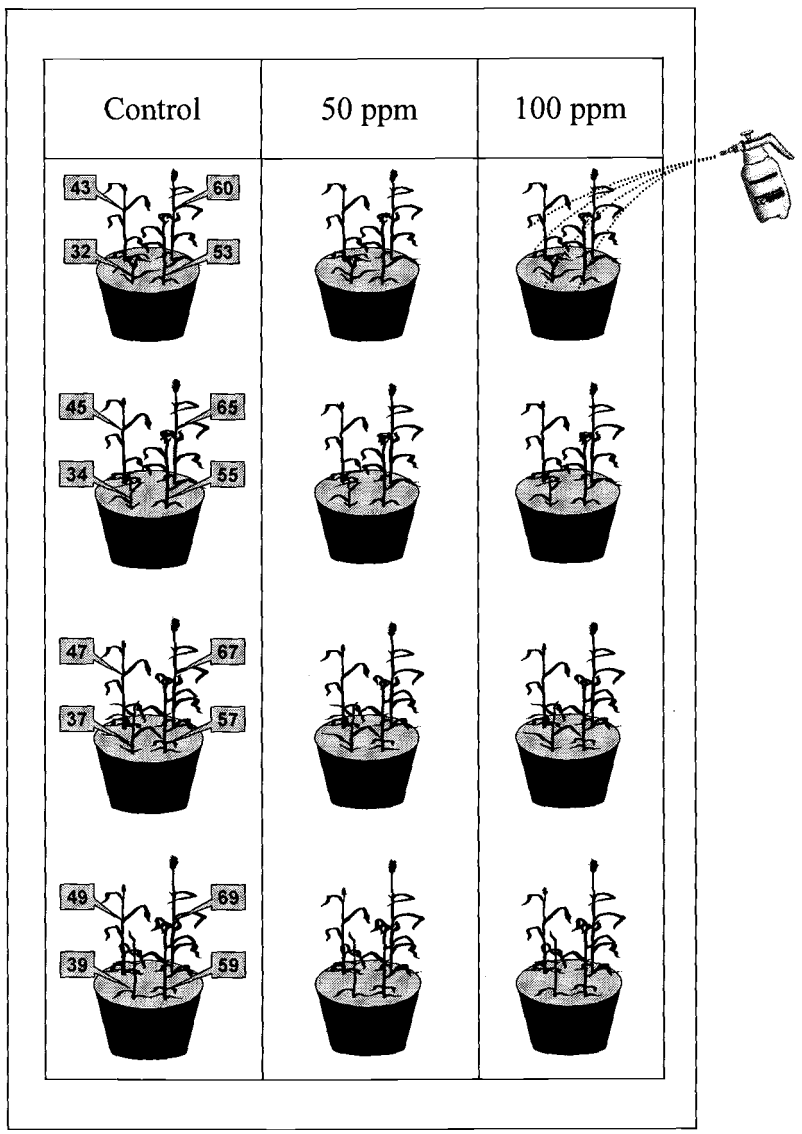

Fig. 1-1. Illustration of tiller selection process for PGR treatment of potted perennial ryegrass plants. The Zadoks stage of each tiller was determined and then the planted was with 0 (control), 50, or 100 ppm AVG, on each treatment application date. Numbers indicate Zadoks stage of tillers marked with a plastic band.

Chlorophyll Meter Calibration and Measurement

The Minolta chlorophyll meter SPAD-502 (Minolta Camera Co., Ltd., 1989) is an ideal instrument for obtaining large amounts of chlorophyll data without destruction of leaves. The SPAD-502 readings represent the amount of chlorophyll in the leaf by measurement of radiation at two wavelengths, 650 nm and 940 nm. The SPAD-502 is commonly used for agricultural purposes for the measurement of the effect of N-fertilizer on plant greenness and leaf senescence with nondestructive method in vivo (Peng et al., 1996; Shapiro 1999).

In spite of its convenient use and lightweight, SPAD-502 measurements only provide the trend of chlorophyll content change over time. The chlorophyll meter measures leaf greenness, which is related in a linear manner to extractable leaf chlorophyll concentrations for many crops. However, the chlorophyll concentrations can vary with the time of day and are influenced by environment, plant growth stages, species, and even varieties (Wood et al., 1993).

For this reason, the relationship between SPAD-502 meter readings at various leaf developmental stages and the extracted foliar chlorophyll concentration from the flag leaf of perennial ryegrass needed to be determined for the purposes of instrument calibration. Meter readings were obtained from three points and averaged on randomly selected 15 flag leaves of greenhouse-grown perennial ryegrass. The selected flag leaves represented a range in leaf ages.

The same 15 flag leaves used for meter readings were immediately harvested and chlorophyll concentration was determined. The fresh leaf tissue (0.1 g) was homogenized by placement in liquid nitrogen for 10 sec. Leaf chlorophyll was then

extracted from homogenized leaf tissue by 80% aqueous acetone (v/v) (Arnon, 1949). The homogenate was centrifuged at 50,000 X g for 5 min at 4°C, and the filtered aqueous acetone supernatant was measured at 645 nm and 663 nm with a spectrophotometer (UV-2101PC, SHIMADZU, Kyoto, Japan). Chlorophyll a and b concentrations were calculated as follows:

Chlorophyll a = ((45.6 X OD663) - (9.27 X OD645)) / 3585.75

Chlorophyll b = ((82.04 X OD645) - (16.75 X OD663)) / 3585.75

Chlorophyll a + b = ((20.2 X OD645) + (8.02 X OD663)) / 1000

All chlorophyll concentration values were normalized to sample fresh weight (mg).

The results were adequate for the degree of precision necessary to use the SPAD as a reliable estimator of leaf chlorophyll content, yielding an r^2 value of 0.95 (97.4% correlation, $p \leq 0.01$) for chlorophyll concentration vs. SPAD readings (Fig. 1-2).

The estimate equation is

Chlorophyll a + b (mg L^{-1}) = -0.409 + 0.054 X SPAD

The equation provides reliable, direct conversion of SPAD meter readings to foliar chlorophyll concentration with the desired accuracy in perennial ryegrass. Flag leaf chlorophyll content was measured with the Minolta chlorophyll meter (SPAD-502) to determine AVG and TE effects on crop maturity at the time of treatment and again every week until harvest. These values were converted to foliar chlorophyll concentrations.

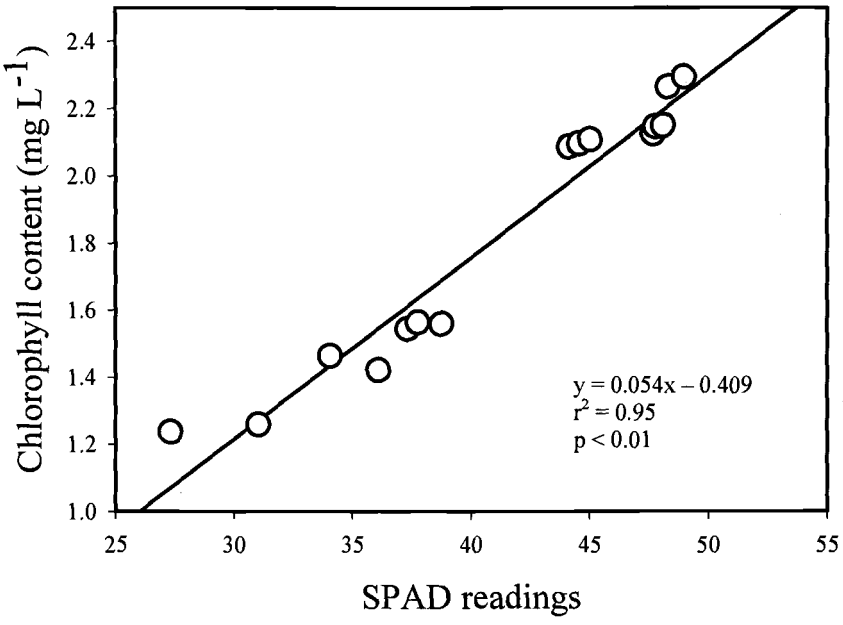

Fig. 1-2. Relationship of chlorophyll concentration and SPAD readings (Chlorophyll meter) in perennial ryegrass.

Evaluation of Shattering

When each spike reached physiological maturity, it was wrapped by pollination envelopes to prevent premature shattering by water or touch in the greenhouse until seed was filled. Each harvested spike was clamped in a modified wrist action shaker and subjected to a standardized shaking time (20 sec) and rate (127 rpm) of shaking. The number of filled seed lost as spikelets and florets from the spike were caught in a tray and recorded. The remaining seed were stripped from the spike and the weight and number of those seeds were determined. The length of the spike was measured from the upper most to bottom spikelet prior to shattering test.

Experimental Design and Analysis

The experimental design was a randomized complete block for both experiments. The first experiment had 3 treatments and 9 replications, while the second experiment had 5 treatments and 15 replications. Analysis of variance was performed on all experimental results. Differences among means were tested and LSD values are reported to indicate the significance of differences at $p<0.1$ and 0.05.

The regression data were fitted to linear (chlorophyll vs. SPAD reading) and to second-order polynomial curves to treatments over yield along with the accompanying 95% confidence intervals, using Statistical Analysis Systems (SAS Institute, 2002).

RESULTS and DISCUSSION

Physiological and Morphological Components

Chlorophyll

There was no significant effect of AVG on chlorophyll content of perennial ryegrass flag leaves in 2002 (p=0.12) nor did chlorophyll content of the flag leaves vary over growth stages (p=0.32) (Table 1-1, Fig. 1-3 and Fig. 1-4). In 2002, chlorophyll contents varied from 2.30 mg L^{-1} to 2.41 mg L^{-1}. No interactions of PGRs and growth stage were evident in either experiment.

Chlorophyll content was significantly affected by PGR treatments in 2003 ($p \leq 0.01$) (Table 1-1). Flag leaf chlorophyll concentrations were increased over the untreated control by the low rate of TE (1.3 g L^{-1}) (Fig. 1-3). While not statistically significant, the higher rates of AVG (150 ppm, 200 ppm) and of TE (2.5 g L^{-1}) decrease chlorophyll content when compared to the control. However, these three PGR treatments did reduce chlorophyll content over the low rates of both PGRs, AVG (100 ppm) and TE (1.3 g L^{-1}). AVG may increase chlorophyll content by inhibition of ACC synthase, which converts SAM (S-adenosylmethionine) to ACC (1-aminocyclopropane-1-carboxylic acid), the precursor of ethylene. Wheat flag leaves had higher chlorophyll concentrations during seed development when treated with AVG than the untreated control and ethephon-treated (ethylene) plants (Beltrano et al., 1994). The effect of AVG was different in perennial ryegrass flag leaves than in wheat in that high rates of AVG reduced rather than increased the chlorophyll content of perennial ryegrass flag leaves. At lower AVG rates 50-100 ppm, similar to those used in the Beltrano et al. (1994) study,

Table 1-1. ANOVA table for chlorophyll content, days to harvest maturity, and spike characteristic of Cutter perennial ryegrass under treatment with AVG only in 2002, AVG and TE in 2003.

Characteristic	2002†			2003‡		
	df	F-ratio	*p*-value	df	F-ratio	*p*-value
Chlorophyll content						
PGR rate	2	2.0962	0.1241	5	3.2225	0.0075
Growth stage	3	1.1663	0.3221	3	13.2066	0.0000
PGR X Growth stage	6	1.2627	0.2731	15	0.7589	0.7229
Days to harvest						
PGR rate	2	9.5298	0.0001	5	1.7387	0.1253
Growth stage	3	285.4033	0.0000	3	148.5691	0.0000
PGR X Growth stage	6	1.9226	0.0755	15	0.7792	0.7007
Spikelet number						
PGR rate	2	2.0606	0.1285	5	0.3699	0.8691
Growth stage	3	0.7484	0.5237	3	1.1571	0.3262
PGR X Growth stage	6	1.7512	0.1075	15	0.7027	0.7818
Floret number						
PGR rate	2	0.2762	0.7588	5	1.5922	0.1678
Growth stage	3	0.2411	0.8676	3	10.1867	0.0000
PGR X Growth stage	6	2.1365	0.0480	15	0.9911	0.4641
Spike length						
PGR rate	2	1.0578	0.3480	5	3.1735	0.0083
Growth stage	3	0.6388	0.5903	3	0.1907	0.9027
PGR X Growth stage	6	0.5655	0.7578	15	2.3101	0.0039

† Rates of AVG in 2002 are control, 50 ppm, and 100 ppm.

‡ Rates of AVG in 2003 are control, 100 ppm, 150 ppm, and 200 ppm and of TE are 1.3 g a.i. L^{-1} and 2.5 g a.i. L^{-1}.

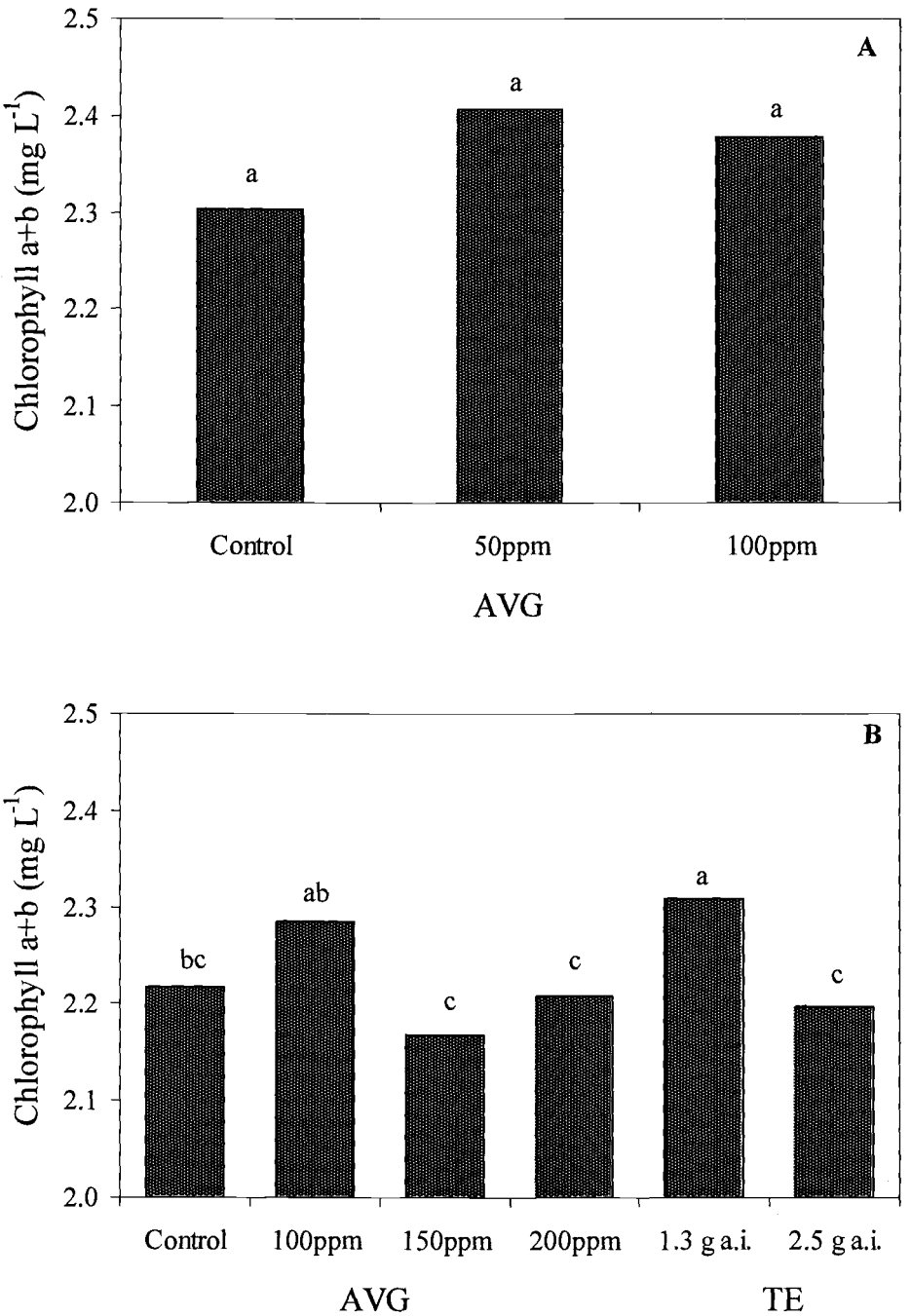

Fig. 1-3. PGR treatment effects on leaf chlorophyll content of Cutter perennial ryegrass in 2002 (A) and 2003 (B). Rates of AVG in 2002 are control, 50 ppm, and 100 ppm. Rates of AVG in 2003 are control, 100 ppm, 150 ppm, and 200 ppm and for TE rates are 1.3 g a.i. L^{-1} and 2.5 g a.i. L^{-1}. Means with the same letter are not significantly different at LSD (p=0.05).

chlorophyll content was consistently, but not significantly increased (Fig. 1-3). No effect of TE on chlorophyll content has been observed by other investigators.

Chlorophyll content was significantly decreased at the anthesis stage ($p \leq 0.01$) in 2003 (Fig. 1-4). Ethylene production increases after the anthesis stage and accelerates at seed filling period because plants accumulate ethylene precursors before anthesis (Yang and Hoffman, 1984). Beltrano et al. (1999) observed that ethylene hastens maturity and senescence at the same time in wheat. Since ethylene hastens maturity of plants, chlorophyll concentrations are lower in the presence of higher ethylene concentrations. In wheat cultivars with higher ethylene production rates, lower chlorophyll content was observed compared to the normal condition (Balota et al., 2004). This was evident in the second trial in 2003 where chlorophyll content was reduced at anthesis, but no effect was apparent in 2002.

Days to harvest maturity

The days to harvest maturity is a measurement of the time interval between PGR treatment and the time of harvest maturity. Like chlorophyll content, the number of days to harvest maturity may be influenced by PGR treatment. There were the expected differences among growth stages for days to harvest maturity and these differences were significant at $p \leq 0.01$ in both 2002 and 2003 (Table 1-1).

AVG treatments tended to promote the early harvest of spikes in 2002 by shortening the days to harvest maturity, especially at 50 ppm (41.6 days) ($p \leq 0.01$), but that tendency was not evident in 2003 ($p=0.13$) (Table 1-2). Likewise, TE treatment had no effect on the number of days to harvest maturity in 2003.

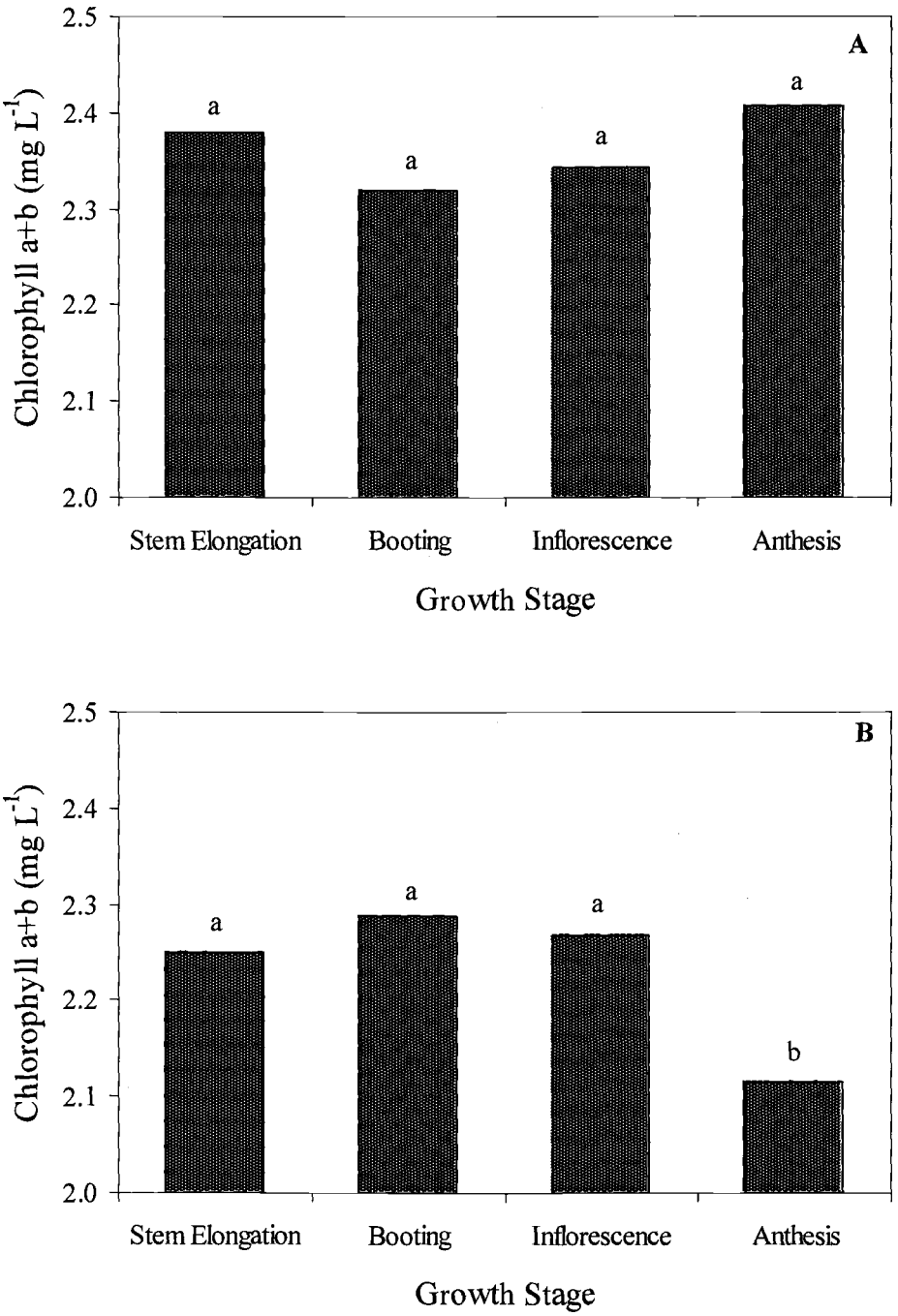

Fig. 1-4. Growth stage effects on chlorophyll content of Cutter perennial ryegrass in 2002 and 2003 (B). Rates of AVG in 2002 are control, 50 ppm, and 100 ppm. Rates of AVG in 2003 are control, 100 ppm, 150 ppm, and 200 ppm and for TE rates are 1.3 g a.i. L^{-1} and 2.5 g a.i. L^{-1}. Means with the same letter are not significantly different at LSD (p=0.05).

Number of Spikelets and Florets

Two of the important seed yield components in perennial ryegrass are the number of spikelets and florets per spike (Hampton and Hebblethwaite, 1985). PGR treatment with AVG and TE did not affect the numbers of spikelets and florets in perennial ryegrass in this study (Table 1-2). However, Silberstein et al. (2002) reported that floret number was increased following the application of TE and another GA inhibiting growth regulator. The application of GA inhibitors and their effect on reducing internode length, have been found to improve the utilization of seed yield potential as a result of better pollination and fertilization with the lessening of crop lodging (Elgersma, 1985). The number of florets per spike at late growth stages was increased at the anthesis stage in comparison with the early growth stages ($p \leq 0.01$) (Table 1-2).

Spike Length

The relative compactness of the inflorescence in grasses may present a physical impediment to seed and floret loss associated with seed shattering. Falcinelli et al. (1984) reported that seed shattering was less in densely packed inflorescences of orchardgrass compared with longer and broader inflorescences. The length of the spike in perennial ryegrass is one measure of inflorescence compactness. The application of AVG had no effect on the length of the spike in either year (Table 1-2). AVG does not seem to be involved in spike length modification. However, TE application decreased spike length about 14~15% compared to the control in 2003 ($p \leq 0.01$) with the application rate of 2.5 g L^{-1}. There was also a tendency, albeit non-significant, for shortened spikes to be produced with the lower rate of TE (1.3 g L^{-1}) compared with the control. Both TE rates

Table 1-2. PGR treatment impacts on days to harvest maturity, number of spikelets and florets per spike, and spike length applied at growth stages in Cutter perennial ryegrass.

Year	Main effects	Days to harvest maturity	Number of		Spike length
			Spikelets	Florets	
2002†					--- cm ---
	PGR				
	Control	48.3 a	19.1	130.6	11.8
	AVG 50ppm	41.6 b	18.8	126.2	11.6
	AVG100ppm	43.4 ab	19.4	127.1	12.0
	LSD (0.05)	3.8	NS	NS	NS
	Growth stage				
	Stem elongation	64.2 a	19.4	129.8	11.8
	Booting	42.1 b	19.3	128.8	11.8
	Inflorescence	33.0 c	19.0	127.1	12.0
	Anthesis	25.1 d	18.8	126.6	11.6
	LSD (0.05)	4.2	NS	NS	NS
2003‡					
	PGR				
	Control	53.6	18.6	136.2	13.1 ab
	AVG100ppm	55.3	19.2	131.3	13.2 a
	AVG150ppm	53.0	19.0	133.9	13.7 a
	AVG200ppm	51.1	18.7	130.3	14.0 a
	TE 1.3 g a.i. L^{-1}	54.3	18.8	129.5	11.5 bc
	TE 2.5 g a.i. L^{-1}	52.2	18.5	125.5	11.4 c
	LSD (0.05)	NS	NS	NS	1.6
	Growth stage				
	Stem elongation	73.3 a	18.8	126.6 bc	12.4
	Booting	54.7 b	19.2	123.3 c	12.9
	Inflorescence	49.8 c	18.8	132.8 b	13.0
	Anthesis	35.1 d	18.4	141.8 a	12.9
	LSD (0.05)	3.4	NS	6.9	NS

† Means with the same letter are not significantly different at LSD (p=0.05).
‡ NS = non-significant.

produced shorter spikes than the three AVG rate treatments in 2003.TE is known to reduce spike length in perennial ryegrass from previous studies (Chastain et al., 2003). There were no differences among growth stages for spike length.

Evaluation of Shattering

Each harvested spike was subjected to a standardized shaking protocol with a modified wrist action shaker to evaluate seed shattering losses. In the 2002 investigation, there were considerable differences among AVG treatments in both shattering losses as spikelets ($p<0.01$) and as florets ($p<0.05$), leading to significant reductions in total shattering losses with AVG application at the 100 ppm rate ($p<0.01$) (Table 1-3 and Fig. 1-5). The 100 ppm AVG rate reduced shattering losses by 8.2 florets per spike when compared to the untreated control and the 50 ppm AVG rate treatment. This reduction in total shattering was attributable to less shattering of both shattering as spikelets and florets per spike.

In 2003, there were no differences among AVG and TE treatments for any of the shattering characteristics measured (Table 1-3). PGR treatments reduced shattering losses compared with the untreated control, but these numerical differences were not statistically significant (Fig. 1-5). Despite the reduction in spike length caused by TE (Table 1-2), this increase in spike compactness had no effect on shattering losses in 2003 (Table 1-4).

There were no differences for shattering characteristics treated between growth stages in 2002; however, there were significant differences ($p\leq0.1$) applied between growth stages in 2003 for shattering of spikelets (Table 1-4).

Table 1-3. ANOVA table for shattering test results of Cutter perennial ryegrass under treatment with AVG only in 2002, AVG and TE in 2003.

Characteristic	2002†			2003‡		
	df	F-ratio	*p*-value	df	F-ratio	*p*-value
Total shattering						
PGR	2	8.5967	0.0003	5	1.3041	0.2645
Growth stage	1	0.2346	0.6288	1	2.9363	0.0885
PGR X Growth stage	2	0.9295	0.3970	5	1.3845	0.2325
Shattering (as spikelets)						
PGR	2	6.6864	0.0017	5	1.4663	0.2034
Growth stage	1	0.1024	0.7495	1	3.0183	0.0842
PGR X Growth stage	2	1.4879	0.2292	5	1.4098	0.2232
Shattering (as florets)						
PGR	2	4.2400	0.0162	5	0.6256	0.6805
Growth stage	1	0.5477	0.4687	1	0.0377	0.8463
PGR X Growth stage	2	1.3162	0.2712	5	0.8969	0.4846

† Rates of AVG in 2002 are control, 50 ppm, and 100 ppm.
‡ Rates of AVG in 2003 are control, 100 ppm, 150 ppm, and 200 ppm and of TE are 1.3 g a.i. L^{-1} and 2.5 g a.i. L^{-1}.

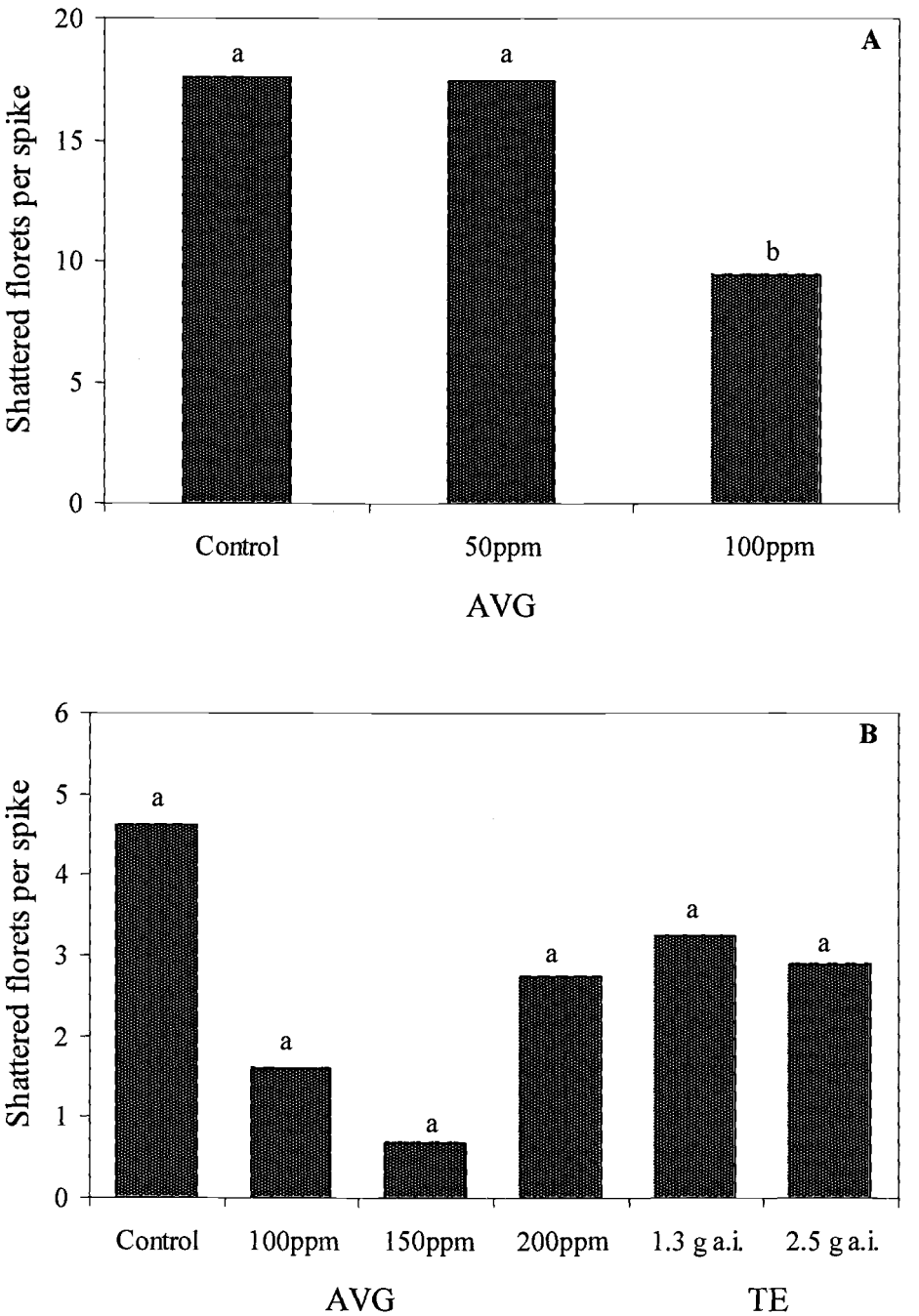

Fig. 1-5. PGR treatment effects on shattered florets of Cutter perennial ryegrass in 2002 (A) and 2003 (B). Rates of AVG in 2002 are control, 50 ppm, and 100 ppm. Rates of AVG in 2003 are control, 100 ppm, 150 ppm, and 200 ppm and for TE rates are 1.3 g a.i. L^{-1} and 2.5 g a.i. L^{-1}. Means with the same letter are not significantly different at LSD (p=0.05).

Table 1-4. PGR treatment impacts on shattering applied in greenhouse trials at inflorescence and anthesis stage in Cutter perennial ryegrass.

Year	Main effects	Shattering per spike	
		Spikelets	Florets
2002			
	PGR		
	Control	14.78 a	2.94 ab
	AVG 50ppm	14.44 a	3.06 a
	AVG100ppm	8.11 b	1.28 b
	LSD (0.05)	5.33*	1.74*
	Growth stage		
	Inflorescence	12.18	2.07
	Anthesis	12.54	2.65
	LSD (0.05)	NS	NS
2003			
	PGR		
	Control	4.60	0.03
	AVG100ppm	1.47	0.13
	AVG150ppm	0.33	0.33
	AVG200ppm	2.43	0.30
	TE 1.3 g a.i. L^{-1}	3.07	0.17
	TE 2.5 g a.i. L^{-1}	2.73	0.18
	LSD (0.05)	NS	NS
	Growth stage		
	Inflorescence	1.59 b	0.20
	Anthesis	3.29 a	0.18
	LSD (0.10)	1.64†	NS

† and * significant at $p \leq 0.1$ and 0.05 levels, respectively.
‡ NS = non-significant.

There was a strong relationship between length of the spike and the number of shattered florets per spike for AVG or TE treatments regardless of the rate of application (Fig. 1-6 and Fig. 1-7). These data show that shorter spikes lost fewer florets due to shattering than longer spikes in perennial ryegrass. This confirms the observations of Falcinelli et al. (1984), that the compact inflorescences (panicles) of orchardgrass have less shattering than open inflorescences. The panicle of orchardgrass is a branched inflorescence with the spikelets and florets borne on the branches while perennial ryegrass has an un-branched inflorescence, the spike. Since spikes have no branches, they can only become more compact by being shorter.

Spikes having greater numbers of florets also had greater numbers of florets lost due to shattering regardless of AVG treatment rate (r^2=0.40) (Fig. 1-6), but the relationship was not as marked when the perennial ryegrass was treated with TE (r^2=0.11) (Fig I-6).

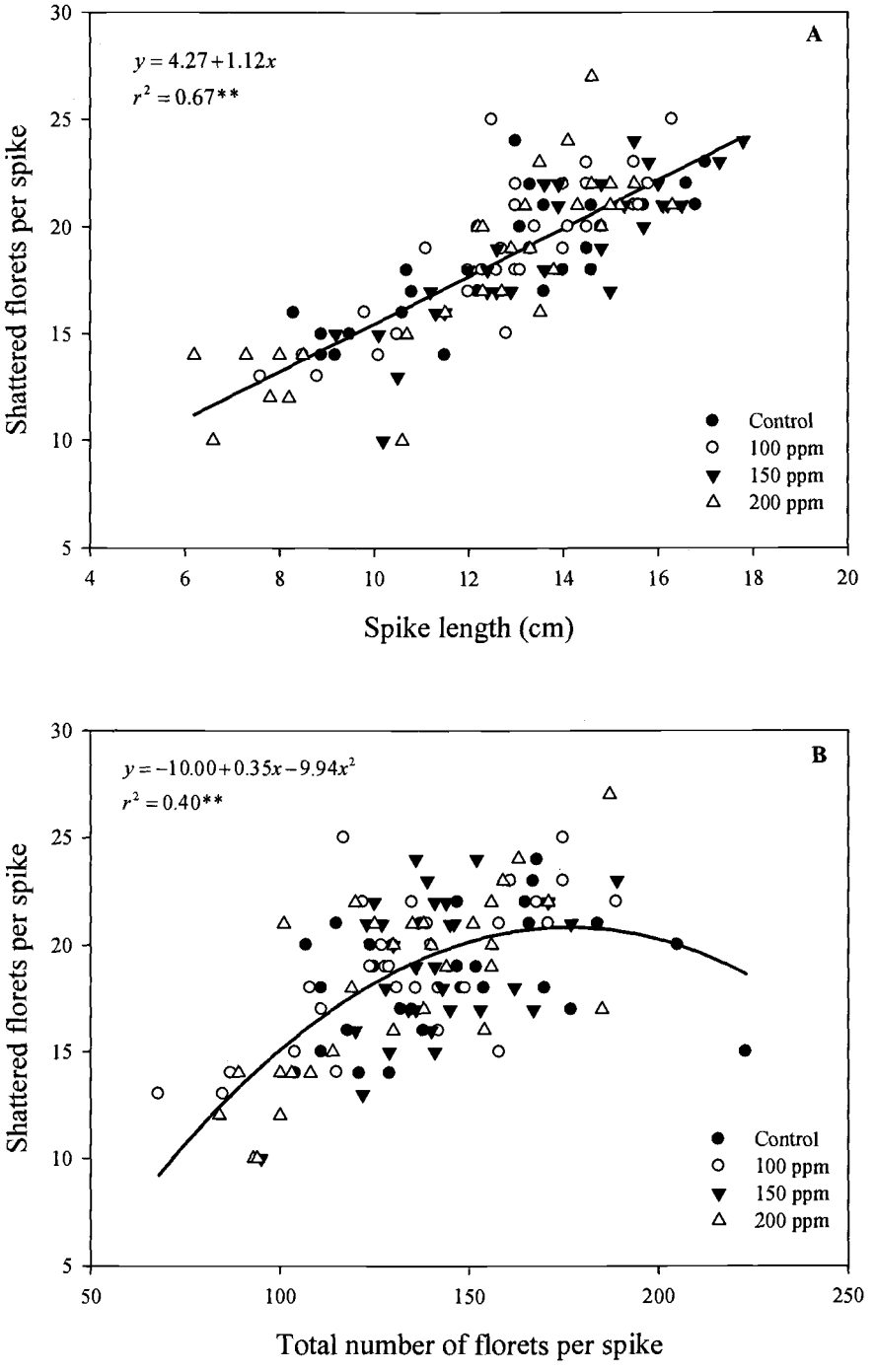

Fig. 1-6. Relationship of spike length (A) and total number of florets (B) to seed shattering in 2003. Values are for AVG-treated plants. Regression coefficients are significant at $p \leq 0.01$ levels.

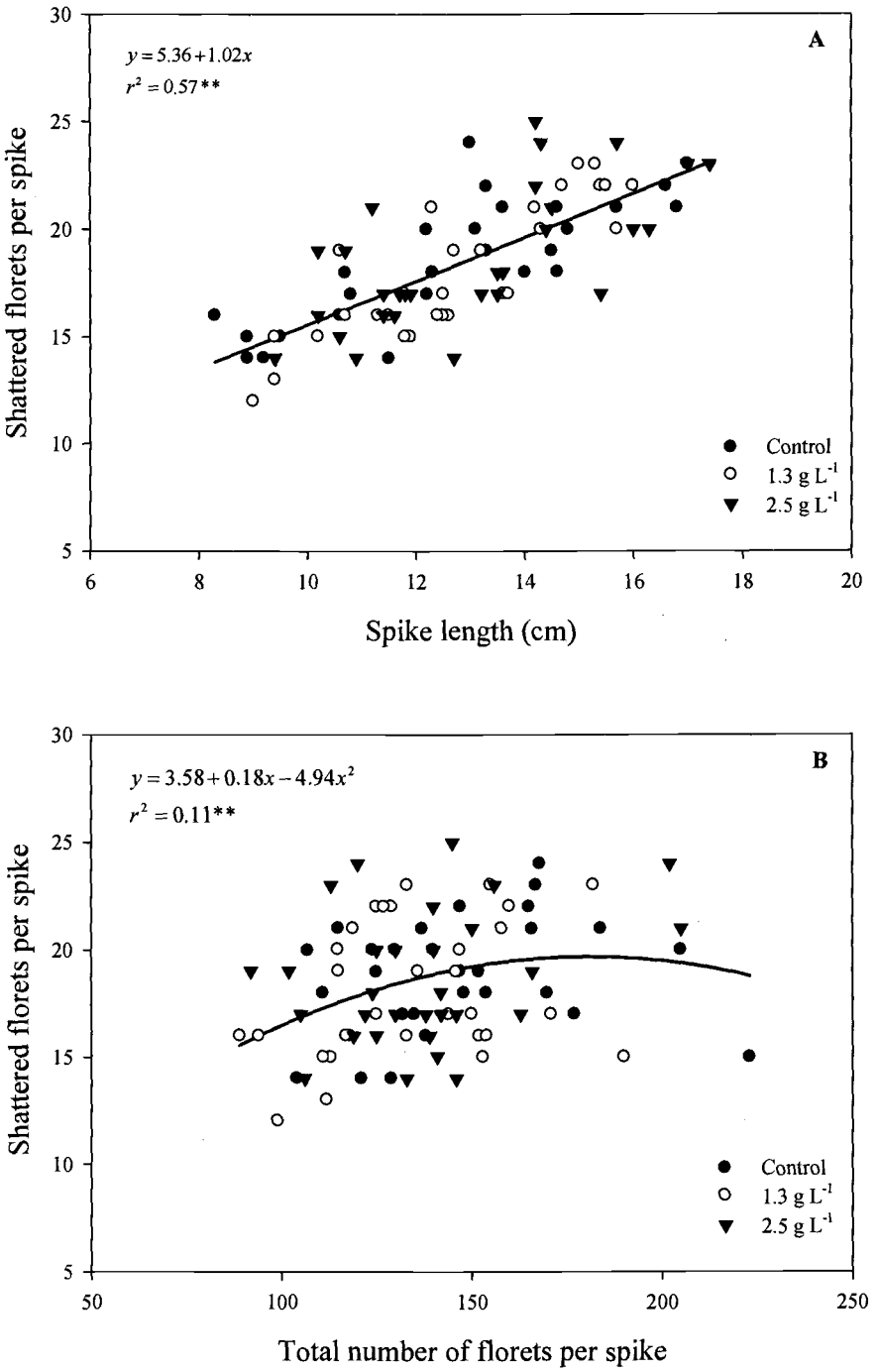

Fig. 1-7. Relationship of spike length (A) and total number of florets (B) to seed shattering in 2003. Values are for TE-treated plants. Regression coefficients are significant at $p \leq 0.01$ levels.

CONCLUSION

Inconsistent effects of both AVG and TE were noted for several physiological and morphological characteristics of perennial ryegrass. Higher rates of both AVG and TE treatments reduced chlorophyll content of the flag leaf. This result was in contrast to earlier reported findings that AVG increased chlorophyll content in the flag leaf of wheat. Lower rates of AVG (50-100 ppm) increased chlorophyll content but it is inability to detect differences using the statistical tests. Variable results were also noted for the number of days to harvest maturity. Neither AVG nor TE application affected the number of spikelets or florets formed in the spike. AVG treatment had no effect on spike length, but TE treatment did reduce spike length. Shorter spikes had lower shattering losses than longer spikes.

The effects of the PGRs, AVG and TE, on shattering in perennial ryegrass were inconsistent and variable under greenhouse conditions. However, there is some evidence of activity of both compounds in reducing seed shattering in perennial ryegrass, thereby warranting further work. Further work might involve tracking the concentrations of SAM, ACC, and final product, ethylene, as well as the activity of ACC synthase and ACC oxidase enzymes after AVG treatment.

TE is currently in widespread use by growers of perennial ryegrass seed in Oregon and significant seed yield increases by use of this compound are typical. Some of that beneficial seed yield increase may be attributable to reduced shattering losses. AVG is registered for use in fruit crops in Oregon to prevent premature loss of fruit prior to harvest, but the compound is not registered for use in grass seed crops.

SOURCES of MATERIALS

Sunshine Mix SB40 potting mix: Canadian Sphagnum Peat Moss (35-45%), Fir bark, Pumice/Cinders, Dolomitic Limestone, Gypsum, Starter Nutrient Charge, Wetting Agent, SunGro Horticulture Inc., 110th Avenue NE, Suite 490, Bellevue, WA 98008

SPAD-502: Ltd. Radiometric Instruments Div., Minolta Camera Co., Osaka, Japan.

Drying Oven: MO1040A1-1, Lindberg/Blue M Inc., 275 Aiken Road Asheville, NC28804, USA

UV-2101PC: Spectrophotometer, Shimadzu, Kyoto, Japan.

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MANUSCRIPT II: THE EFFECT OF PLANT GROWTH REGULATORS ON SHATTERING CONTROL IN COOL-SEASON PERENNIAL GRASSES SEED PRODUCTION: FIELD INVESTIGATION

ABSTRACT

Seed shattering is a major cause of yield losses in cool-season grasses. This study was conducted to determine whether the use of plant growth regulators, aminoethoxyvinylglycine (AVG) and trinexapac-ethyl (TE), can decrease seed shattering and increase harvested seed yield under field conditions in perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.).

Two field experiments were conducted at the Oregon State University Hyslop Research Farm, 10 km north of Corvallis, Oregon. In field experiment 1, AVG was applied at 75 ppm (135 g a.i. ha^{-1}) was applied once (T1) at peak anthesis and again (T2) 14 days after peak anthesis in perennial ryegrass. In field experiment 2, AVG was applied at the following rates: 100 ppm (180 g a.i. ha^{-1}), 200 ppm (360 g a.i. ha^{-1}), and 300 ppm (540 g a.i. ha^{-1}), and TE was applied at 200 g a.i. ha^{-1}, and 400 g a.i. ha^{-1}, on perennial ryegrass and tall fescue.

The application of AVG at T1 and/or T2 increased chlorophyll content of perennial ryegrass flag leave over the control 24 days after peak anthesis. Seed yield in perennial ryegrass was not affected by AVG application in experiment 1, but was reduced by the low rate of AVG (100 ppm) in experiment 2. Seed weight in perennial ryegrass was reduced by AVG in experiment 1, but not in experiment 2. No effect of PGRs on

seed yield or seed weight of tall fescue was measured. PGR treatments had no effects on the level of unutilized florets in perennial ryegrass, but differences among PGR treatments were evident in tall fescue. Tall fescue had much higher levels of unutilized florets than perennial ryegrass regardless of treatment.

For both perennial ryegrass and tall fescue, the total number of seeds lost due to shattering (prior to and after swathing), was significantly reduced by the low rate of TE (200 g a.i. ha^{-1}). There were no significant differences in final germination percentage and mean germination time in perennial ryegrass and tall fescue over various rates of AVG and TE application.

While seed shattering was apparently reduced in the trials, the lack of a positive effect of the late application of TE on seed yield does not warrant an expansion of its use for the purpose of seed shattering control.

Keywords: Shattering, Perennial ryegrass, Tall fescue, Plant growth regulators (PGRs), Aminoethoxyvinylglycine (AVG), Trinexapac-ethyl (TE)

INTRODUCTION

The seed production industry in the Pacific Northwest has developed rapidly since 1940. The Willamette Valley is the center of seed production in the region, with a favorable climate and producers familiar with specialized agricultural practices. The success of the Willamette Valley as a seed production region results from extensive research, and cooperative development of seed production practices. The Willamette Valley's climate is ideally suited for seed production. Mild and wet winters assist the re-growth of grasses in the late fall to early spring. Dry summers allow two-step harvesting, which eliminates the need for drying and reduces the cost of production. Highly dependable yields can be expected every year in a variety of grass seed crops.

Seed shattering plays a critical role in determining yield losses of grass seed crops. Hides et al. (1993) reported that seed shattering reduced seed yield of Italian ryegrass (*Lolium multiflorum* Lam.). Despite high seed yield potential, the actual seed yield harvested in perennial ryegrass (*L. perenne* L.) can be much lower than its potential as a result of unproductive florets, seed shattering prior to and during harvest, and seed losses in cleaning processes (Anslow, 1964; Meijer, 1985). Seed yield and seed shattering were found to be strongly correlated in perennial ryegrass (Elgersma et al., 1988). Shattering in economically valuable grasses leads to harvesting difficulties and results in low yields with poor quality of the seed produced (McWilliam, 1980). Little is known about shattering in tall fescue (*Festuca arundinacea* Schreb.), another important species for Oregon seed producers.

Several special harvest practices have been developed to reduce seed shattering.

Spraying a water-soluble lacquer before seed maturity reduced shattering, but presents problems related to good coverage and additional costs (McWilliam and Schroeder, 1974).

Shattering is caused by abscission, which is the result of ethylene production (Yang and Hoffman, 1984). Inhibition of ethylene synthesis by plant growth regulators (PGRs) may delay abscission of florets, thereby contributing to increased seed yield. Prolonged seed retention on the mother plant may enable greater quantities of photosynthates to be translocated to the seed, resulting in higher seed weight.

Several compounds have been developed to block ethylene synthesis, but only two are commercially available PGRs: AVG (aminoethoxyvinylglycine) and 1-MCP (1-methylcyclopropene) (Sisler and Serek, 1997; Jobling et al., 2003). AVG (EPA Reg. No. 73049-45, Est. No. 33967-NJ-1) is sold by the commercial name ReTain® while 1-MCP (EPA Reg. No. 71297-1-32258, Est. No. 32258-SC-001) is marketed under the trade name EthylBloc®.

GA biosynthesis inhibitor-type PGRs have been utilized for lodging control in grasses. Lodged crops have limited opportunities for pollination and shading reduces photosynthesis in the lodged crop canopy. Trinexapac-ethyl (TE) is a widely used plant growth retardant in cool-season grass seed production (Zapiola et al., 2006). TE can be applied to crop foliage, blocking the activity of 3β-hydroxylase, an important enzyme in GA biosynthesis. Chastain et al. (2001) reported that the application of TE increased seed yield 25% in the 1st year and 41% in the second year over the untreated control with increased floret number and floret conversion to seeds.

Application timing of the PGRs may be critical for reducing shattering in grass

seed production. Ethylene is produced at booting stage whereas abscission zone development begins already at inflorescence stage (van Doorn and Stead, 1997). Seed shattering starts as early as 15 days after anthesis in reed canarygrass (*Phalaris arundinacea* L.) (Baltensperger and Kalton, 1958 and 1959).

The optimum swathing moisture percentage for harvesting seed of tall fescue is approximately 43%, while for perennial ryegrass, the moisture content is 35% (Klein and Harmond, 1971; Singh et al., 2000). This implies that tall fescue is more prone to shattering than perennial ryegrass because it needs to be harvested at higher moisture content. Silberstein et al. (2004) reported that harvesting a few days late doubles seed losses due to shattering compared to harvesting a few days early.

The objective of this study was to determine the efficacy of two PGRs, AVG and TE, on seed shattering and seed yield in experimental fields of perennial ryegrass and tall fescue.

MATERIALS and METHODS

Two field trials on PGRs and their effects on shattering-induced seed losses and crop yield in perennial ryegrass and tall fescue were conducted at the Oregon State University Hyslop Research Farm, located 10 km north of Corvallis Oregon, in 2002 and in 2003.

Field Experiment 1

An AVG application timing experiment was conducted in 2002 in a field of Cutter perennial ryegrass that had been planted in the spring of 1999. AVG was applied to the treated plots at 75 ppm (135 g a.i. ha^{-1}) with a surfactant (SYLGARD 309) at 0.1% (v/v). Applications were made at walking speed by using a 10-foot wide bicycle-type boom sprayer with XR TEEJET 8003VS nozzles arranged at an 18-inch wide spacing. The three treatments consisted of a one time application of AVG at peak anthesis on June 6, 2002 (T1), a repeated application of AVG 14 days after the first application on June 20, 2002 (T2), and a water treatment control. The T2 treatment plots were subjected to an accumulated application of 150 ppm AVG over a 14-day period. Each plot was 1.8 m wide by 9.1 m long.

Field Experiment 2

An AVG and TE rate of application experiment was conducted in 2003 in a field planted to plots of Cutter perennial ryegrass and Velocity tall fescue in the spring of 1999. AVG and TE were applied in the treated plots when the inflorescence had emerged (May

20, 2003). AVG was applied with the surfactant (SYLGARD 309) at 0.1% (v/v) at the following rates: 100 ppm (180 g a.i. ha^{-1}), 200 ppm (360 g a.i. ha^{-1}), and 300 ppm (540 g a.i. ha^{-1}). TE was applied at 200 g a.i. ha^{-1} and 400 g a.i. ha^{-1}. PGR treatments were applied at walking speed using a 10-foot wide bicycle-type boom sprayer with XR TEEJET 8003VS nozzles arranged at an 18-inch wide spacing. A water control was included for comparison to the PGR treatments. Each plot was 3.1 m wide by 12.2 m long.

Chlorophyll Measurement

Chlorophyll content was determined in Field Experiment 1 on 9 flag leaves randomly selected from 9 locations in each treatment plot by using a Minolta chlorophyll meter (SPAD-502). The chlorophyll meter was calibrated in the manner described in Manuscript 1. Measurements were initiated at 2 days after the T2 AVG application (22 June) and repeated on two subsequent dates (28 June and 1 July).

Shattering and Seed Yield Assessment

Seed shattering losses and unutilized florets were determined by collecting random samples of 10 inflorescences from each plot at three times: early bloom, prior to swathing, and in the windrow after swathing. Unutilized florets implicate florets fail to pollination or fertilization. The number of unutilized florets and fertile florets in each inflorescence were counted and recorded. Seed yield was determined by harvesting the crops with a small plot swather, allowing the windrows to dry in the field until 12% seed moisture content was reached. The dried windrows were individually threshed with a

small plot combine equipped with a pickup header attachment. Combine-harvested seed samples were cleaned with an M2-B clipper cleaner for the calculation of clean seed yields and 1000 seed weights were determined on the clean seed.

Seed Germination

Germination tests were performed in Oct. 2004 on 6 seed lots each of perennial ryegrass and tall fescue harvested from Field Experiment 2. Each seed lot represented the field treatments (control, AVG application rates 100, 200, 300 ppm, and TE application rates 200, 400 g a.i. ha^{-1}). Seed samples were selected from each treatment and were subdivided into 4 sub-samples of 100 seeds each for the replications required in the germination test. Germination testing was conducted according to AOSA rules (Association Official Seed Analysts, 2000).

Seeds were germinated on top of filter paper soaked in distilled water in plastic germination boxes. The germination boxes were placed in a germination chamber (SG222, Hoffman Manufacturing Inc., Albany, OR) with cool white fluorescence light for 8 h per day at 25±1°C and darkness for 16 h per day at 15±1°C. Seeds were considered to be germinated when the radicle had protruded more than 5 mm. Germinated seeds were counted once a day. The seeds that did not germinate after 7 days were regarded as dormant seeds.

Experimental Design and Data Analysis

The experimental design of the two trials was a randomized complete block with 3 replications in 2002 and 4 replications in 2003. The first experiment had 3 treatments

and the second experiment had 6 treatments. Analysis of variance was performed on all experimental results. Differences among means were tested and LSD values are reported to indicate the significance of differences at $p<0.1$ and 0.05.

RESULTS and DISCUSSION

Field Experiment 1

The application of AVG at peak anthesis (T1) and again 14 days after peak anthesis (T2) increased chlorophyll content of perennial ryegrass flag leaves over the control on July 1st, 2006 (24 days after peak anthesis) (Fig 2-1). There were no differences in chlorophyll content among treatments on June 22 (p=0.53), sixteen days after the T1 treatment and two days after the T2 treatment was applied. Likewise, there were no differences among treatments when chlorophyll measurements were made on June 28 (p=0.54). These results imply that the natural rate of decline in flag leaf chlorophyll that takes place after anthesis is complete (20 ~ 24 days after peak anthesis) and when seed development is underway, was reduced by the application of AVG. Both AVG treatments had a similar effect on flag leaf chlorophyll content in perennial ryegrass. Wheat flag leaves had higher chlorophyll concentrations during seed development when treated with AVG, presumably as a consequence of reduced ethylene biosynthesis, than the untreated control and ethephon-treated (ethylene) plants (Beltrano et al., 1994).

Ethylene production increases after the anthesis stage and accelerates during the seed filling period because plants accumulate ethylene precursors before anthesis (Yang and Hoffman, 1984). Beltrano et al. (1999) observed that ethylene hastens maturity and senescence at the same time in wheat. Since ethylene hastens maturity of plants, chlorophyll concentrations are lower in the presence of higher ethylene concentrations. In wheat cultivars with higher ethylene production rates, lower chlorophyll content was observed compared to the normal condition (Balota et al., 2004).

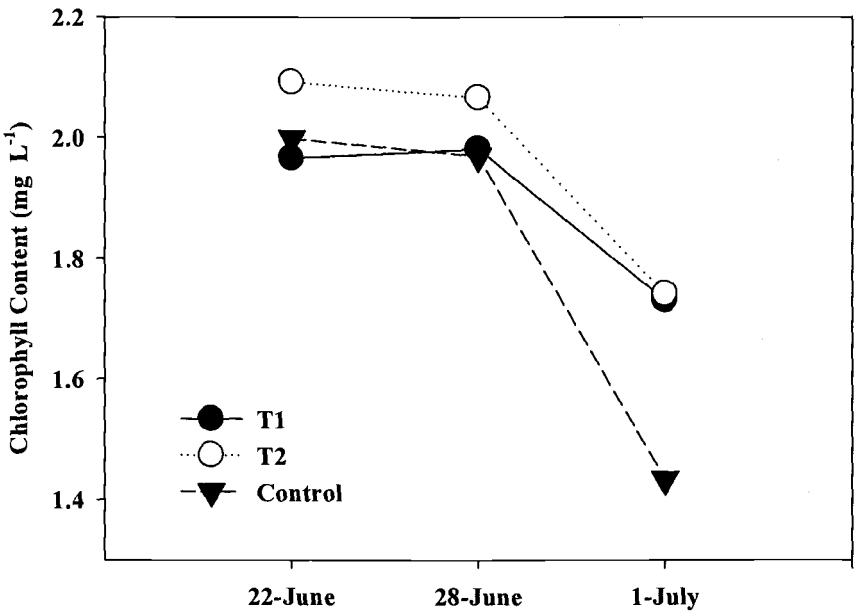

Fig. 2-1. Chlorophyll content after one and two AVG applications at anthesis (T1) and 14 days after anthesis (T2) in Cutter perennial ryegrass in 2002.

Seed yields were not different whether the perennial ryegrass seed crop was treated with AVG application at peak anthesis (T1) or 14 days after peak anthesis (T2) (Table 2-1). Neither treatment yielded differently than the water control treatment.

Seed weight was reduced by both AVG treatments compared with the control, with the average loss in seed weight exceeding 4% (Table 2-1). The reason for the loss in 1000-seed weight as a result of AVG application is unclear and unexpected. AVG application in wheat resulted in increased seed weight when compared to an un-treated control and ethephon treatment (Beltrano et al., 1994).

Field Experiment 2

There was a significant difference (p=0.01) among PGR treatments in seed yield of perennial ryegrass, but not in tall fescue (Table 2-2). Seed yield in perennial ryegrass was reduced by the low rate of AVG (100 ppm) compared to the water control and increased all other treatments except for the intermediate AVG rate (200 ppm) (Table 2-3). The date of TE application in this experiment was made later in the season than recommended for grass seed crops, so the typical increase in seed yield that has been found in other trials (Chastain et al., 2003; Zapiola et al., 2006), and anecdotally by seed growers, was not observed. Unlike in field experiment 1, no impact of AVG on seed weight was observed for either perennial ryegrass or tall fescue (Table 2-3). Likewise, TE had no effect on seed weight in perennial ryegrass or tall fescue.

Table 2-1. AVG treatment effects on seed yield and 1000-seed weight in Cutter perennial ryegrass after one and two AVG application (75ppm, 135 g a.i. ha^{-1}) at anthesis (T1) and 14 days after anthesis (T2) in 2002.

PGR treatment	Yield	1000-Seed weight
	-- kg ha^{-1} --	---- g ----
Control	1412	1.89 a
T1 (75ppm)	1363	1.80 b
T2 (75ppm X 2)	1300	1.82 b
LSD (0.05)	NS	0.06

† Means with the same letter are not significantly different at LSD (p=0.05).
‡ NS = non-significant.

Table 2-2. Summary of analysis of variance for characteristic of yield, shattering, and seed germination for two PGRs (AVG and TE) and two crop species in 2003.

Characteristic	Perennial ryegrass	Tall fescue
	Significance of F-value	
Seed yield	**	NS
1000-seed weight	NS	NS
Unutilized florets	NS	†
Shattered at swathing	*	NS
windrow	NS	NS
total	*	*
Final seed germination	NS	NS
Mean germination time	NS	NS

† , *, and ** significant at $p \leq 0.1$, 0.05, and 0.01 levels, respectively.

Table 2-3. PGR treatment effects on seed yield and 1000-seed weight in Cutter perennial ryegrass and Velocity tall fescue in 2003. PGR treatments were made at the inflorescence stage.

PGR treatment	Perennial ryegrass		Tall fescue	
	Yield	1000-Seed weight	Yield	1000-Seed weight
	-- kg ha^{-1} --	---- g ----	-- kg ha^{-1} --	---- g ----
Control	909 a	1.78	651	2.48
AVG 100 ppm	846 b	1.79	647	2.53
AVG 200 ppm	902 ab	1.78	655	2.52
AVG 300 ppm	985 a	1.76	668	2.50
TE 200 g a.i. ha^{-1}	955 a	1.80	691	2.49
TE 400 g a.i. ha^{-1}	958 a	1.79	753	2.46
LSD (0.05)	128	NS	NS	NS

† Means with the same letter are not significantly different at LSD (p=0.05).
‡ NS = non-significant.

Shattering Assessment

PGR treatments had no significant effects on the level of unutilized florets in perennial ryegrass, but differences among PGR treatments was evident in tall fescue (p=0.05) (Table 2-2 and Table 2-4). Tall fescue showed much higher levels of unutilized florets (control = 136) than perennial ryegrass (control = 43). The low rate of AVG (100 ppm) and the high rate of TE (400 g a.i. ha^{-1}) increased unutilized florets, while the low rate of TE (200 g a.i. ha^{-1}) increased floret number by 42% (Table 2-4).

When assessed after swathing, seed shattering in perennial ryegrass was significantly reduced by the intermediate and high rates of AVG (200 and 300 ppm) and the low rate of TE (200 g a.i. ha^{-1}) when compared to the control (Table 2-4). No reductions in seed shattering were observed in tall fescue when the impact of PGRs was assessed after swathing.

When the assessment of shattering was made during windrow, inflorescences that were taken out of windrows did not show any differences among PGR treatments for seed shattering losses for perennial ryegrass or tall fescue (Table 2-4). Ethylene is a stress response hormone, which is increased in concentration in the plant by stress conditions such as flooding, chilling, drought, and wounding (McKeon et al., 1995). During the process of swathing the crop into windrows, the standing crop is cut by a sickle bar and laid in the field. It is possible that the wounding of the plants caused by the action of swathing might result in the increase of ethylene concentrations in the cut crop. The cutting of the crop might have negated the benefits of an earlier AVG application on seed shattering.

Table 2-4. Effect of PGR application on seed shattering of Cutter perennial ryegrass and Velocity tall fescue in 2003.

Species	Treatment	unutilized florets	Shattered seeds		
			Swathing	Windrow	Total
		--- no. of seeds per inflorescence ---			
Perennial Ryegrass	Control	43	85 a	16	101 a
	AVG 100 ppm	47	60 ab	31	92 ab
	AVG 200 ppm	46	56 b	27	88 ab
	AVG 300 ppm	53	49 b	32	80 ab
	TE 200 g a.i. ha^{-1}	57	45 b	32	77 b
	TE 400 g a.i. ha^{-1}	49	61 ab	36	96 ab
	LSD (0.05)	NS	23	NS	21
Tall fescue	Control	136 b	104	80	184 a
	AVG 100 ppm	161 ab	68	78	146 ab
	AVG 200 ppm	147 b	96	49	145 ab
	AVG 300 ppm	126 b	97	54	150 ab
	TE 200 g a.i. ha^{-1}	194 a	54	56	110 b
	TE 400 g a.i. ha^{-1}	156 ab	68	62	130 ab
	LSD (0.05)	45	NS	NS	57

† Means with the same letter are not significantly different at LSD (p=0.05).

‡ NS = non-significant.

For both perennial ryegrass and tall fescue, the total number of seeds lost due to shattering (prior to and after swathing), was significantly reduced by the low rate of TE (200 g a.i. ha^{-1}) (Table 2-4). The shortening of the inflorescence by TE likely resulted in lessened shattering losses as a result of more compact inflorescence architecture (Falcinelli et al., 1984; Chastain et al., 2003). Other PGR treatments showed consistent activity with lowered total seed shattering, but these differences were not considered to be statistically significant.

Despite the apparent reduction in seed shattering by TE, there were no effects of TE on seed yield in either perennial ryegrass or tall fescue (Table 2-3). This reduction in seed loss by TE was not manifested as an increase in seed yield. It could be possible that the increase in seed retention caused seed weight to decline as a result of spreading a finite supply of photosynthates over a suddenly greater number of seeds, thereby causing seed yield to remain the same. However, seed weight was also not influenced by the TE application (Table 2-3). Therefore, the reason for this discrepancy remains undetermined.

Seed Germination

The germination test results showed that all seed had germination ability or viability over 90% in both perennial ryegrass and tall fescue (Table 2-5). Seed germination in perennial ryegrass ranged from 94.3% to 97.3% and for tall fescue, from 91.5% to 94.5%. Germination was somewhat lower in tall fescue than in perennial ryegrass.

The lowest recorded germination value (89.8%) was for tall fescue treated with TE at the high rate (400 g a.i. ha^{-1}). Nevertheless, there were no significant differences

(p=0.05) in final germination percentage and mean germination time in perennial ryegrass and tall fescue over various rates of AVG and TE application.

Table 2-5. Final germination percentage and mean germination time response to PGR application in Cutter perennial ryegrass and Velocity tall fescue.

Crop species	PGR treatment	Final germination	Mean germination time
		-- % --	-- days --
Perennial ryegrass	Control	97.3	2.24
	AVG 100 ppm	94.3	2.15
	AVG 200 ppm	95.0	2.08
	AVG 300 ppm	97.0	2.13
	TE 200 g a.i. ha^{-1}	95.5	2.07
	TE 400 g a.i. ha^{-1}	96.3	2.13
	LSD (0.05)	NS	NS
Tall fescue	Control	92.8	2.55
	AVG 100 ppm	92.0	2.46
	AVG 200 ppm	93.3	2.61
	AVG 300 ppm	91.5	2.45
	TE 200 g a.i. ha^{-1}	94.5	2.45
	TE 400 g a.i. ha^{-1}	89.8	2.63
	LSD (0.05)	NS	NS

† Means with the same letter are not significantly different at LSD (p=0.05).

‡ Mean values for control samples are based on four replicates of 100-seeds. NS = non-significant.

CONCLUSION

AVG treatment at peak anthesis or with an additional application of AVG 14 days after peak anthesis, resulted in a reduction in the natural deterioration of chlorophyll in the flag leaf of perennial ryegrass. Neither AVG nor TE had a positive effect on seed yield in either field experiment. However, inconsistent effects of AVG on 1000-seed weight were observed. Also, mixed effects of PGRs on unutilized florets in tall fescue were measured. The stress of cutting the crop during swathing might accelerate ethylene biosynthesis, resulting in increased ethylene production and seed shattering. It might be useful to apply AVG after swathing for reducing seed shattering.

Results of this study clearly showed that AVG and TE treatment play a role in chlorophyll content change, seed yield, 1000-seed weight, and seed shattering prior to harvest, but not seed germination in perennial ryegrass and tall fescue. While seed shattering was apparently reduced in the trials, the lack of a positive effect of the late application of TE on seed yield does not warrant an expansion of its use for the purpose of seed shattering control.

SOURCES of MATERIALS

SPAD-502: Ltd. Radiometric Instruments Div., Minolta Camera Co., Osaka, Japan.

Drying Oven: MO1040A1-1, Lindberg/Blue M Inc., 275 Aiken Road Asheville, NC28804, USA.

Germination Chamber: SG222, Hoffman Manufacturing Inc., Albany, OR, USA.

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SUMMARY and CONCLUSIONS

Greenhouse experiment and field experiment were undertaken to determine weather the use of the plant growth regulators (PGRs), aminoethoxyvinylglycine (AVG) and trinexapac-ethyl (TE), might reduce seed shattering in perennial ryegrass and tall fescue.

Inconsistent effects of both AVG and TE were noted for several physiological and morphological characteristics of perennial ryegrass. Higher rates of both AVG and TE treatments reduced chlorophyll content of the flag leaf. This result was in contrast to earlier reported findings that AVG increased chlorophyll content in the flag leaf of wheat. Although chlorophyll content increased in lower rates of AVG (50-100 ppm), it is inability to detect differences using the statistical tests. However, AVG treatment at peak anthesis or with an additional application of AVG 14 days after peak anthesis resulted in a reduction in the natural deterioration of chlorophyll in the flag leaf of perennial ryegrass.

Variable results were also noted for the number of days to harvest maturity. Neither AVG nor TE application affected the number of spikelets or florets formed in the spike. AVG treatment had no effect on spike length, but TE treatment did reduce spike length. Shorter spikes had lower shattering losses than longer spikes.

Neither AVG nor TE had a positive effect on seed yield in either field experiment. However, inconsistent effects of AVG on 1000-seed weight were observed. Also, mixed effects of PGRs on unutilized florets in tall fescue were measured. The stress of cutting

the crop during swathing might accelerate ethylene biosynthesis, resulting in increased ethylene production and seed shattering. It might be useful to apply AVG after swathing for reducing seed shattering.

Further work might involve tracking the concentrations of SAM, ACC, and final product, ethylene, as well as the activity of ACC synthase and ACC oxidase enzymes after AVG treatment. While seed shattering was apparently reduced in the trials, the lack of a positive effect of the late application of TE on seed yield does not warrant an expansion of its use for the purpose of seed shattering control.

On the basis of results obtained in this study, the following conclusions can be drawn.

(1) Higher rates of both AVG and TE treatments reduced chlorophyll content of the flag leaf.

(2) AVG treatment at peak anthesis or with an additional application of AVG 14 days after peak anthesis delayed the natural deterioration of chlorophyll in the flag leaf of perennial ryegrass.

(3) Neither AVG nor TE application changed the number of spikelets or florets formed in the spike.

(4) Shorter spikes had lower shattering losses than longer spikes. AVG treatment had no effect on spike length, but TE treatment did reduce spike length.

(5) Neither AVG nor TE had a positive effect on seed yield and seed weight.

(6) AVG and TE application did positive effect on seed shattering prior to and after swathing.

(7) The stress of cutting the crop during swathing might accelerate ethylene biosynthesis,

resulting in increased ethylene production and seed shattering. It might be useful to apply AVG after swathing for reducing seed shattering.

(8) Seed germination and period were not affected by AVG and TE applications.

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