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My sincerest thanks to the USOA for the support given me throughout my Masters Degree

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Factors Affecting Seed set in *Poa annua* L. Using an Excised Stem Technique

A thesis submitted to the faculty of the graduate school of the University of Minnesota

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Table of Contents

Introduction

Poa annua L. is a fine to coarse textured grass with many ecotypes found worldwide under the most variable of environmental conditions. When managed successfully, as a turfgrass, it often is considered the best putting surface for a golf course green. However, because many types tend to be winter annuals, disease prone and not very heat tolerant, it is often considered a weed by turfgrass managers. *Poa annua* is normally divided into two subspecies based on morphology and growth habit. Annual types (*P. annua* var. *annua*) have bunch type growth, are often coarse in texture and often have the undesirable characteristics mentioned above. Perennial, or weakly perennial forms, (var. *reptans*) are also widespread. Many of these types have desirable turfgrass characteristics, such as dark green color, reduced flowering (both in quantity and time of year), stoloniferous habit, and cold hardiness. Breeding potential for the species is enormous, as seen by its variability and adaptability to many conditions (Hovin, 1957).

As with any species that has not been the object of improvement work, the first task, by necessity, is studying the reproductive biology of the species. In addition, techniques need to be developed to conduct an efficient breeding program. Some of the troublesome characteristics in working with *Poa annua* include 1) the small flower size (1-2 mm), 2) the large number of spikelets (up to 40-50), and tightly arranged florets and 3) the general habit of the plant (Plate 1&2).

Some of these problems have been alleviated by adapting an excised stem technique for use with the species (Ruemmele, 1989). Flowering stems

Plate 1: Typical *Poa annua* inflorescence showing tight arrangement of the flowers. The inflorescence is composed of many spikelets that have from 2 to 6 florets, or flowers, each.

Plate 2: Typical growth habit of some *Poa annua* genotypes, that causes difficulty in working with the flowers for breeding purposes. Note that the large number of flowers that tend to be expressed horizontally rather than vertically.

are removed from a *Poa annua* plant, and inserted into a floral pic, containing a sugar solution. The use of this technique has enabled much greater flexibility in handling the *Poa annua* flowers and completing desired pollinations, as well as improved efficiency in studying reproductive biology. However seed set variation, either due to the technique or the plant itself, often made analysis and interpretation of results difficult (Ruemmele, 1989).

It became apparent that factors affecting seed set, while using the excised stem technique, needed to be addressed. Results of this thesis have identified some variables that must be accounted for or controlled to best use this technique.

Review of the Literature

Improvement of *Poa annua* L., has received little attention, with the recent exception of the University of Minnesota breeding project. As with any new project, there is a need to develop techniques to deal with specific problems. In the case of *Poa annua*,handling the flowers, especially for controlled pollinations, has been a major challenge. Emasculations have proven to be especially difficult, as *Poa annua* flowers do not lend themselves to easy removal of the anthers (Johnson & White, 1990).

In an effort to overcome some of these problems, an excised stem technique was adapted from similar work done on chrysanthemum and *Petunia* (Ruemmele, 1989). Similar techniques have also been described in the literature for some grasses, primarily for seed maturation (Verret, *et al*, 1925; Harlan & Pope, 1926; Pope, 1935; Keller, 1943). Excised flowering stems have also been used to investigate physiological processes occurring in the stems (Donovan & Lee, 1977; 1978; Brocklehurst, et al, 1978; Barlow, et al, 1983; Singh & Jenner, 1983; Johnson & White, 1990). These included studies of nutrient solutions, light, stage of floral development, additives to control detrimental organisms in the solution, and time of seed maturation.

Use in grasses

Through personal communication it is known that excised stems were used with horseradish in the early 1900's. However, the first report found documenting the use of an excised stem technique was by Verret, *et al*, in 1925 with sugarcane. It was noted that seed development was found to be much improved on cut stems if they were kept moist or left in standing water.

Harlan & Pope (1926) described a similar technique and reported comparable results using wheat. Additional studies grew out of this work, where similar methods were used on barley and some forage grasses (Pope, 1935; Keller, 1943). Some effects of the technique were seen in these early investigations, because the seed tended to be lighter in weight (40-83%), and slower to germinate, compared to seed from non-detached culms. However, germination percentages were similar to seed from intact culms. Once germinated, few differences in the plants were observed (Keller, 1943). Excised stem methods have also been described for *Agrostis, Podagrostis, Polypogon* (Carlbom, 1969) and *Festuca* (Wofford, et al, 1986). Carlbom proposed many possible uses of his technique ranging from breeding work to studies into the physiology of flowering.

No improvements of excised stem methods were found in the literature until that by Wofford, et al (1986). The feasibility of the technique was investigated with *Festuca arundinacea* (tall fescue) to evaluate effects of selected variables on the method. Significant sources of variation for seed production were genotype, stage of floral development when excised from the plant, culm length, genotype x floral development, and genotype x culm length. None of the factors were reported to have influenced seed germination.

Potato

Excised stem methods have also been used successfully with potato (McLean & Stevenson, 1952; Peloquin & Hougas, 1958). The methods consisted of placing detached flowering stems in milk bottles, containing tap water or nutrient solutions, located in a greenhouse. The technique was

found to be effective for increasing seed set in difficult matings. Comparisons of decapitated and attached flowers in a wide cross resulted in 10 seeds set from the attached flowers, while 157 seeds were collected from the excised stems. Additional advantages of the technique that Peloquin & Hougas (1959) listed include its adaptability to large scale use and the ability to perform matings under controlled environments.

Chrysanthemum & Petunia

More recently, the chrysanthemum breeding project at the University of Minnesota successfully used an excised stem technique. Excised flower stems were inserted through the bottoms of Styrofoam cups which floated on a solution of 2% by weight sucrose and 200 ppm 8-hydroxy quinoline citrate (8-HQC). As a result, it was named "the floating garden technique". This method allowed for easy emasculation and crossing of the breeding materials (Ascher, 1989). This technique was adapted from similar uses in a *Petunia* breeding program (Flaschenriem, 1974). Flaschenriem investigated the influence of various concentrations of 20-20-20 and 18-24-16 fertilizer as well as tap vs. distilled water. Results showed that tap water, with no added nutrients or other ingredients, produced the greatest seed set. Distilled water and dilute fertilizer solutions followed (Flaschenriem, 1974).

Bactericide

In some of the excised stem literature, compounds have been added to the nutrient solution to control growth of detrimental organisms. As nutrients, such as sugars, were added to the solutions, growth of bacteria and fungi disrupted the water supply to the developing seeds by clogging the

vessels in the stems (Aarts, 1957; Peloquin & Hougas, 1958; 1959; Coorts, et al, 1965; Larsen & Scholes, 1965; 1966; Marousky, 1969; Wilfret, 1971). Sugar cane flowering stalks remained fresh for one month with additions of sulfurous and phosphoric acids, while stems in plain water lasted only 1-2 days (Verret, et al, 1925). In potato cut stems, streptomycin sulfate was commonly used for the control of soft rot infection (Peloquin & Hougas, 1959). It is possible that the sulfates and acids gave a nutrient effect rather than biocidial effects. However, the concentrations are quite low (5-10 ppm streptomycin sulfate; .05% sulfurous acid), therefore, the observed effects are not likely due to the addition of nutrients.

The bactericide 8-hydroxy quinoline citrate (8-HQC) has commonly been used in cut flower preservation. In addition to its biocidial properties, 8-HQC has also been found to reduce vascular blockage and increase uptake and retention of water in carnations (Larsen & Scholes, 1965), roses (Marousky, 1969), and gladiolus (Wilfret, 1971). Similar literature was also found for other species.

In addition to its bactericidal properties, 8-HQC was observed to reduce vascular blockage in roses (Marousky, 1969). It is theorized that 8-HQC acts as a chelating agent, disabling the enzyme responsible for producing callose, which is often involved in blocking of the vascular system (Coorts, *et al*, 1965).

Carbohydrate

The amount of carbohydrate available to a developing seed has been the topic of research for many investigations. When flowering stems have been removed from their source plant, carbohydrate supply has the potential

of being interrupted. Therefore exogenous sources of carbohydrate may be needed for effective use of the excised stem method.

A significant correlation (r^2=.847) was reported between the concentration of sucrose (from 0-3% by weight) in an excised stem solution and starch synthesis in wheat grains. Concentrations in the 3-8% range resulted in no further increase in seed set or seed weight (Jenner, 1970). The flow of sucrose into the developing seed appears tightly controlled. At higher concentrations (3-8%), the extra available sugars were directed to other parts of the flower, rather than to the seed. The reasons for this pattern of sucrose uptake is unknown, however some suggest a mechanical control, relating to a lack of plasmodesmatal connections between maternal and embryonic tissue (Felker, 1980; Thorne, 1981). In various crop seeds, solutes must pass from the phloem symplastically, through maternal seed coat layers before reaching the developing seed. It is suggested that concentrations above 3% sucrose, saturate this transport system, such that excess sugar is diverted elsewhere (Jenner, 1970; Barlow, 1983).

In most of the excised stem techniques described in the literature, sucrose is the carbohydrate used. While sucrose is a common form of sugar found in plants, fructose or fructosans, are the primary forms in most of the temperate region grasses (DeCugnac, 1931; Okajima & Smith, 1964; Smith & Grotelueshcen, 1966). Therefore fructose might be used more efficiently by *Poa annua* in an excised stem technique.

Light effects

It is clear from the literature, that in an excised stem technique, supplying a carbon source can be beneficial to the developing seed. However,

it has been proposed that flowering stems of wheat are self-sufficient in supplying photosynthate to the developing seed by the photosynthetic contribution of the floral parts (Bremner & Ingham, 1960).

To investigate the contribution of photosynthesis within wheat stems, to the developing seeds, shading treatments were imposed on detached and attached flowering stems (Puckridge, 1968; Willey & Holliday, 1971; Brocklehurst, 1978; Martinez-Carrasco, 1979). Seed weight of detached wheat stems was affected little by shading treatments when ample sucrose (4-6% by weight) was available in the solution (Singh & Jenner, 1983). However, light intensity did influence seed weight when low concentrations of sucrose (0-1%) were available. It is apparent that light has an influence on dry weight accumulation in the seed.

With inadequate light, results suggest that undetached wheat stems are unable to fully support themselves in terms of seed set. When shading was imposed on flowering plants, seed number was reduced. This indicated a limited supply of, and competition for, carbohydrate. The shade was imposed after floral initiation, so the number of flowers was not affected by the treatments. Similar shading experiments on barley did not affect seed weight. This difference might be explained by lower photosynthetic levels in wheat ears (Willey & Holliday, 1971; Singh & Jenner, 1983; Brocklehurst, et al, 1978; Martinez-Carrasco, 1979). Due to the difference in responses to light between species, generalizations cannot be made to cover light effects with *Poa annua,* especially since *Poa annua* appears to be relatively shade tolerant.

Poa annua

In preliminary excised stem work with *Poa annua,* a comparison of 0 and 2% sucrose was made, with 2% resulting in greater seed set (Ruemmele, 1989). In addition, 8-HQC was investigated in preliminary studies for preservative effects. A concentration of 200 ppm of 8-HQC gave the best seed set, when compared with concentrations of up to 1000 ppm.

Preliminary experiments were also done to investigate the photosynthetic contribution of the culm to the developing seed. Rather than shading treatments, as used in wheat studies, the photosynthetic area of each stem was altered. All the leaves, or none of the leaves on the culm were removed, but no effects were observed (Ruemmele, 1989). The leaves apparently had little effect on seed development. In light of the fact that flower parts and the stem themselves, may contribute photosynthate, shading treatments applied to them as well are needed to effectively test the influence of light.

Nitrogen

In addition to carbon source, nitrogen was also considered as a nutrient supplement for excised stem work with wheat. Mixtures of amino acids or ammonium nitrate were used as sources (Donovan & Lee, 1977, 1978; Singh & Jenner, 1983; Barlow, et al, 1983). In the initial work, it was reported that amino acids were needed by the developing seed on the basis of the nitrogen uptake (Donovan & Lee, 1977). However it was later determined that nitrogen did not play a major role in dry weight accumulation. Variation of nitrogen levels with consistent sucrose levels showed no effect on starch

accumulation. This was similar to the effect of nitrogen fertilization after heading of wheat: where nitrogen content of the grain increased, but yield was not affected (Donovan & Lee, 1978).

Excised stem techniques have enabled the study of individual nutrients and their impacts to developing seeds and various plant parts. Amounts of carbohydrate, nitrogen, and other elements, could be controlled independently by altering concentrations in the nutrient solution. Such work had been very difficult in attached flowers (Donovan & Lee, 1977;1978; Barlow, Donovan & Lee, 1983; Singh & Jenner, 1983).

Summary

Excised stem techniques have been used in breeding programs of various crops for many years. The technique has proven useful for producing seed as well as studying some physiological phenomena. However, where the number of seed become important, variation in seed numbers can present problems. Ruemmele (1989) encountered large amounts of variation in seed set data with *Poa annua*. In order to improve the ability to complete genetic studies of *Poa annua*, the seed set variation encountered while using the excised stem technique must be considered.

General Materials & Methods

Plant material and growing conditions

The experimental units for all studies were *Poa annua* flowering stems, cut from cloned stock plants, maintained uniformly in the University of Minnesota Horticulture greenhouses. The stock plants were grown in 4" standard pots and repotted every 2-3 months. Regular repotting was practiced to prevent the plants from becoming too crowded and root bound. This practice also reduced disease problems. Temperatures in the greenhouse were maintained at 18-21°C day and 13°C night in winter, with slightly higher day temperatures in summer. During periods of hot weather, temperatures occasionally reached 32-35°C. Cooling was supplied by fans and evaporative pads.

For experiments conducted after June, 1990, stock plants were maintained out of doors, in a cold frame, next to the greenhouse. This was to avoid the extremely high temperatures encountered in the greenhouse during the warmest days of summer. High temperatures appeared to negatively affect seed setting which could have affected experimental results.

General Excised Stem Technique

Individual culms were cut near the crown of the source plant to obtain the longest stem possible. This improved the handling of the culms and ensured sufficient immersion into the solutions. No parts of the crown or roots were included.

Culms were inserted through rubber caps into plastic floral pics, 10 cm in length and 1 cm in diameter (Plate 3), containing the appropriate solution

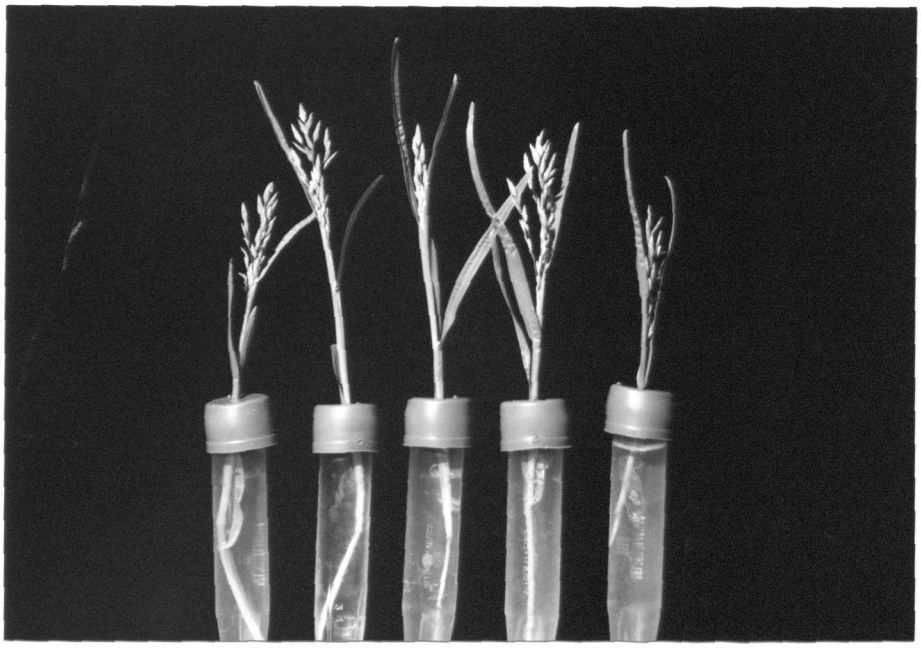

Plate 3: Floral pic unit, filled with a sugar solution, and culm inserted.

Plate 4: One of the three light racks that were used in all the experiments.

treatment. Solutes were diluted in distilled and deionized water. Appendix 1 shows water test results. These pics were then placed on a light rack (Plate 4) with illumination provided by cool white florescent lights giving 70-110 μE $m^{-1}s^{-1}$ intensity at inflorescence level. Intensities were highest in the center of the rack, decreasing slightly towards the edges. To prevent any uncontrolled effects due to differing light intensities within a rack, culms were placed in locations of similar intensities, usually near the edges of a shelf. Room temperature was maintained at 22±2°C with relative humidities ranging between 30-85%, depending on the time of day and outdoor weather.

Pollinations

To ensure adequate pollination, culms were grouped at the time of anthesis for coincidental pollen shed. Anthesis on the culms did not all occur on the same day, so grouping at anthesis, rather than at the time of collection, ensured simultaneous pollen shed between flowering stems. To enhance pollen exchange, culms were brushed together providing a mixture of pollen from the inflorescences. This was repeated each day until pollen shed ceased, usually after 3-4 days. These methods are similar to those described by Ruemmele (1989).

Culms remained in the solution for 15-20 days, unless otherwise noted. Inflorescences were then cut from the stem and placed in coin envelopes for drying under room conditions. When air dry (usually 2-3 days), seed was counted and weighed. For the majority of the experiments, only the top most 10 spikelets were used in seed counting (Appendix #2). Seed weight was calculated by dividing the total weight of the seeds by the number of seeds. Most work in the literature measured seed weight by using a constant

number of seeds. However, because of low seed counts in some treatments (ranging from 0-50 seeds), a constant number of seed could not be used.

Statistics

Statistical analysis for each study was performed using MacAnova 2.21, a statistics application for the Macintosh. Seed set and seed weight data were transformed, to partially control non-constant variance, using $\sqrt{sds+1}$ and $\sqrt{sd.wt.+.0001}$ respectively. Tukey's Honest Significant Difference (HSD), at p=.01 and p=.05 levels, was used for mean separation (Lertner & Bishop, 1986). To ensure validity of the conclusions, given the sometimes substantial variation, the conservative HSD test was used. Variation in the data was compared by using the coefficient of variation (CV).

Chapter 1: Time of Seed Maturation on Excised Stems

In the literature and in practical applications, the optimum time for seed harvest is usually determined by one or more of three measurements: 1) dry weight, 2) seed number, and 3) germination percentage of the seed (Grabe, 1956). All three measurements were observed for up to 18 days after anthesis in *Poa annua*, using an excised stem technique, in a randomized complete block design.

Materials & Methods:

Flowering culms of *Poa annua* genotypes #709 and #1667, were excised on each of five dates (7/4, 7/10, 7/20, 8/2, 8/28/89), and inserted into floral pics containing a 2% by weight sucrose solution. Two percent sucrose was selected because of its previous use in an excised stem solution (Ruemmele, 1989). The inflorescences were collected approximately one day before anthesis. A randomized complete block design (blocking on date of collection) was used to account for possible variation due to the different collection dates. The four pairing combinations of the two genotypes (709x709, 709x1667, 1667x709, 1667x1667) were used as one factor of the analysis. The second factor was length of maturation in the pic. At three day intervals from the average day of anthesis, two inflorescences were randomly selected from the group of inflorescences (collecting one of each of the four pairing combinations). This occurred at 3, 6, 9, 12, 15, and 18 days. The inflorescence was cut from the remainder of the stem, and put in a coin envelope. The 'zero' day was omitted because all the florets on any one inflorescence do not open on the first day of anthesis. However by the third day, all the flowers on the top ten

spikelets usually have been open for at least one day. The inflorescence was cut from the stem and air dried in an envelope for approximately one week before seed counting and weighing. The five blocks and two replications per block, gave a total of 10 replications per treatment.

Germination Tests

Germination tests were performed approximately two to three months after seed collections were made. Seeds were placed on moist blotter paper in a aluminum box under similar light conditions as described for the excised stem technique. Counts of germinated seeds were taken 14-21 days later. No dormancy of the seed was observed in these tests.

Results & Discussion:

The number of days that an excised *Poa annua* stem remained in the solution for maturation was found to have a significant effect ($p<.001$) on seed set and seed weight (Fig. 1&2). Main effects and interactions involving genotype pairing were not observed for either seed set or seed weight measurements. Seed was found on inflorescences only three days after anthesis, but the three and six day treatments resulted in much lower seed set and weight than longer maturation times. Seed set and weight reached a plateau at 12-15 days (15-18 days for seed weight). Coefficient of variation decreased as treatment means increased, up to 12 days, after which it remained consistent (Fig. 1&2). The decrease in seed set for the 18 day treatment was somewhat puzzling, and may be explained by experimental error. It did not appear to be due to shattering of the seed. Unlike in the field, where shattering of mature seed is a problem, shattering rarely occurs with

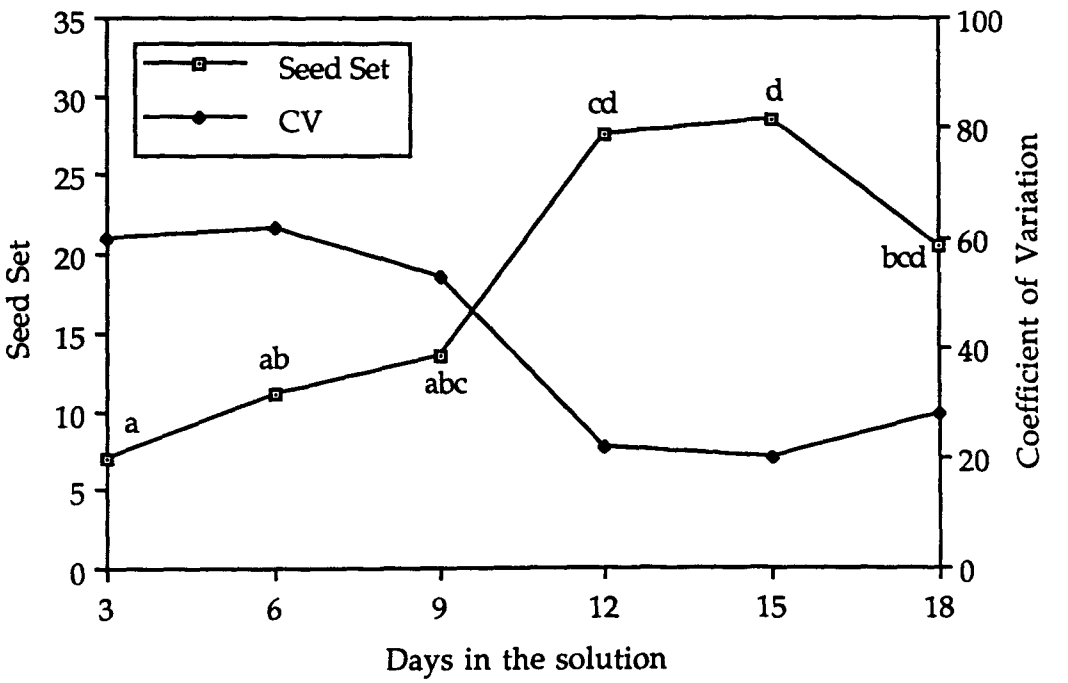

Fig. 1. Time of seed maturation on excised stems using seed set data. Data is not transformed. Values with different letters are significantly different at HSD(.01). Seed set reached maturity at 12 days in terms of maximum seed set, with a slight drop after 18 days. Coefficient of Variation exhibited an inverse relationship to that of sed set.

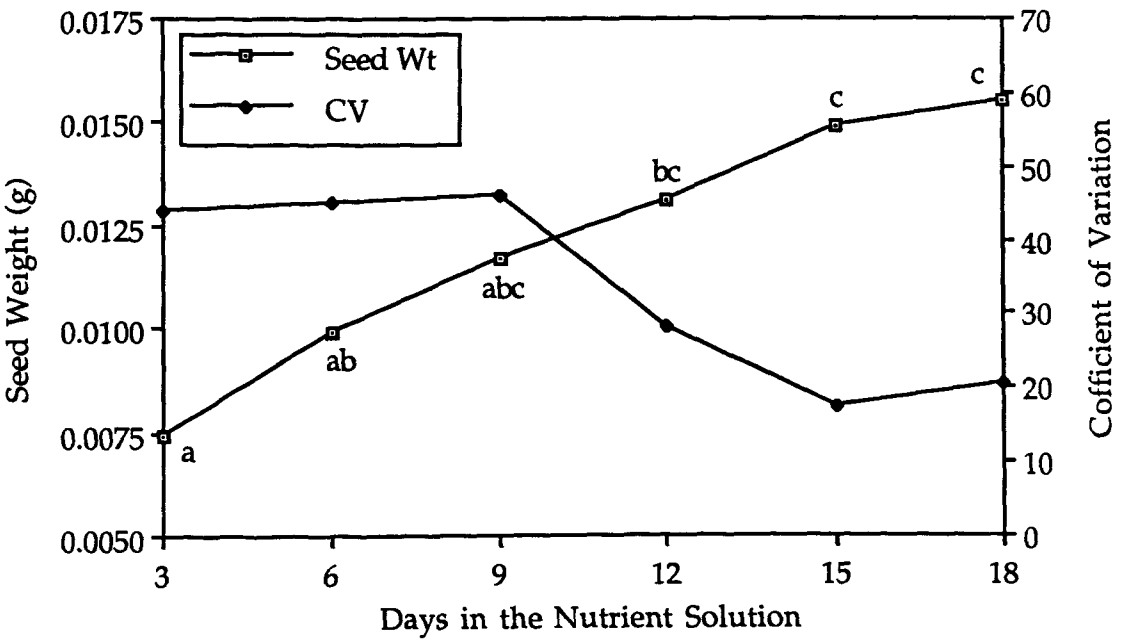

Fig. 2. Time of seed maturation on excised stems using seed set data (not transformed). Values with different letters are significantly different at HSD(.01). Seed set reached maturity at 12-18 days in terms of maximum seed weight. Coefficient of variation exhibited a nearly inverse relationship to that of seed weight.

excised *Poa annua* stems. In fact, when harvested, seeds often needed to be coaxed out with a needle. Reasons for this are unknown, but might involve hormonal relations and merit further research.

The seed germination data was not statistically evaluated due to large amounts of missing data. This was especially a problem in treatments having low seed set, resulting in a very unbalanced design, which was difficult to interpret. However germination mean trends were comparable to the seed set and seed weight measurements (Fig. 3).

Relationships between seed maturity and seed set or seed weight have been published for several different species, including some grasses. Work with bromegrass presented nearly identical results, for all three measurements, as shown in this experiment (Grabe, 1956). Similar results were also found in soybean and fescues, except the time of maturation for those species was longer than in *Poa annua* (Burris, 1973; Anderson & Anderson, 1980).

The reduction in seed set and seed weight due to early removal from the solution might be due to insufficient seed development. Literature discussed later in chapter 3, outlines processes in seed development, including endosperm cell division (production) and cell expansion (Brocklehurst, 1978; Martinez-Carrasco, 1979). Early harvest of the inflorescence may result in the loss of access to assimilates, as well as moisture, interfering with grain filling processes.

However, these results do not coincide with common grass seed production methods. Grass seed stalks are often cut and windrowed soon after flowering to prevent excessive shattering of the seed. The remainder of seed development then occurs while on the cut stalk, with little loss in quality and

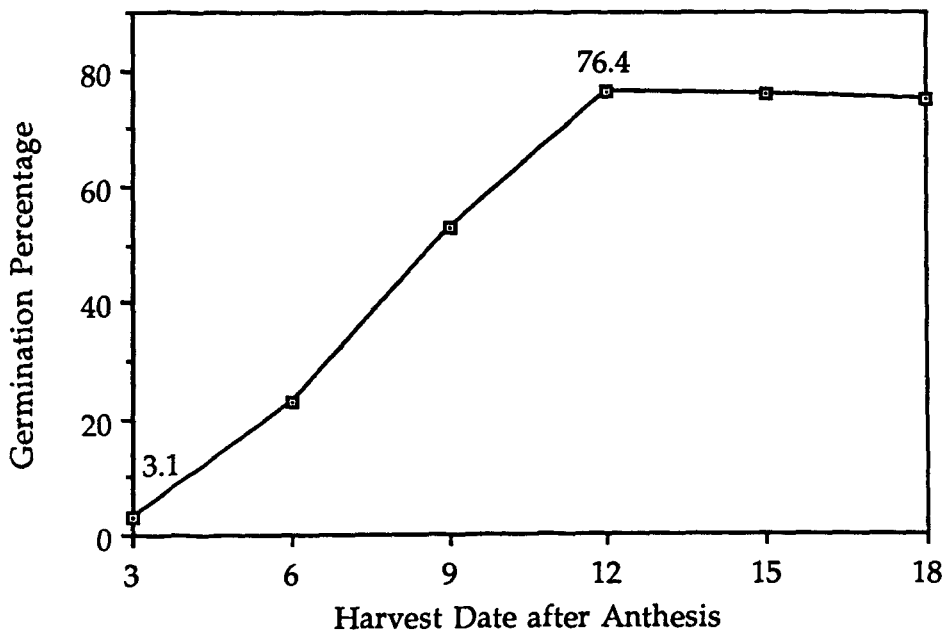

Fig. 3. Time of seed maturation on excised stem seed germination data. This data was not statistically tested, however a clear pattern exists with a plateau in germination percentage beginning at 12 days.

viability of the seed (Cowen, 1969). In fact *Poa annua* has been reported to set viable seed only one to two days after anthesis (Beard, 1973). Some seed was collected in the three day treatment, however in this excised stem work, at least 12 days with external nutrients was shown to be optimum for seed set and seed weight.

Stored assimilates and water in the stalk could serve as a reservoir, with a longer and more vigorous stalk supplying more nutrients. The literature of various species does not agree whether or not the culm length affects eventual seed set. Work has been reported with several species where the effects of the culm length on seed set and seed weight were significant (van Wijk, 1985; Wofford, 1986; Ensign, 1989). In work with *Poa annua*, culm length did not appear to effect seed set (Ruemmele, 1989). Similarly, tall cultivars of wheat were shown to be no more able to draw on stem reserves for grain filling than short culmed types (Rawson & Evans, 1971). The experiments in chapter 4 may indicate that the *Poa annua* culm is probably not a storage reservoir, but may rather be merely a vehicle for transport of water, solutes and carbohydrate. Nutrient movement in excised stems and attached stems could be observed to determine possible differences in seed maturation requirements.

Waiting longer than the optimum number of days on the culm would most likely not reduce seed yield, since shattering is not a real problem in the excised stem technique. However, for greatest efficiency, a seed maturation period of 15-18 days is recommended for *Poa annua* excised stems.

Chapter 2: Effects of 8-HQC in the Excised Stem Solution

Additives that reduce growth of fungal and bacterial organisms, have been shown with some species to increase the life of an excised stem while in a nutrient solution. Bacteria, or other organisms, can plug vascular tissues of the stem, disrupting the water and nutrient supply to the flowers and developing seeds. The additive 8-hydroxy quinoline citrate (8-HQC), a common additive in cut flower preservation, was evaluated for beneficial effects in terms of seed set for excised *Poa annua* stems.

Materials & Methods:

Experiment 1:

A completely randomized design experiment was used to evaluate three concentrations of 8-HQC (0, 100, and 300 ppm) in the excised stem solution and the four pairing combinations of two *Poa annua* genotypes (#709 and #1667 with two selfs and two crosses). A 2% concentration of sucrose was used in all the solutions in addition to the 8-HQC (Ruemmele, 1989). Stems were excised and handled as described in the general materials and methods section with five replications of each treatment.

Experiment 2:

A completely randomized design was used to evaluate four concentrations of sucrose (0, 2, 4, and 6% by weight) and five concentrations of 8-HQC (0, 50, 100, 200, and 300 ppm) as components in the excised stem solution. One genotype (#709) was selfed to obtain the seed. Ten replications of each treatment were used.

Results & Discussion:

Both experiments investigating 8-HQC concentrations, showed no significant effect on seed set. In the second study (Experiment 2), seed weight was not evaluated, but might be expected to be similar to the seed weight results of experiment 1. Significant main and interaction effects involving genotype pairing treatments were not observed.

The second study was designed as a factorial to investigate differential effects of the 8-HQC as sucrose concentration changed. However, no interaction was observed. Therefore, effects of sucrose concentration will be addressed in chapter 3, as part of a more comprehensive discussion of the carbohydrate needs of the excised stem. Results of both experiment 1 and experiment 2 are summarized in Tables 1 and 2.

The benefits of 8-HQC in the solution have been demonstrated for cut flower preservation. Larson and Scholes (1965, 1966) and Wilfret (1971), attributed an increase in water uptake and longevity of the flowers to the bactericidal properties of 8-HQC. They suggested that reduction in stomatal opening, resulted in greater water retention and fresh weight. Odom (1954) reported that cut flowers treated with 8-HQS (sulfate), a related compound, did not wilt.

Investigations studying 8-HQC and sucrose together in the excised stem solutions revealed an interaction in terms of water uptake. In gladiolus, sucrose alone reduced water absorption by the stem, while 8-HQC counteracted some of the decreased uptake by decreasing vascular blockages (Marousky, 1968). Both sucrose and 8-HQC reduced stomatal opening, but the combination reduced stomatal opening further than each of them singly, further increasing longevity of the gladiolus flowers.

Table 1. Experiment 1, Seed set and seed weight data for three 8-HQC concentrations

Concentration of 8-HQC	seeds	CV	seed wt.	CV
ppm			g	
0	18.86	39.04	.00012	29.61
100	15.53	44.21	.00012	43.34
300	16.53	39.60	.00009	29.67

No differences at HSD (.05)

Table 2. Experiment 2, Sucrose and 8-HQC treatment means

Treatment		Sds/spk	CV
8-HQC:	0ppm	0.73	31.93
	50ppm	0.71	31.77
	100ppm	0.75	32.09
	200ppm	0.74	29.91
	300ppm	0.62	33.25

No differences at $HSD_{(.05)}$

Sucrose:	0%	0.09	15.63
	1%	0.57	25.79
	2%	1.17	23.58
	4%	1.21	25.46

$HSD_{(.01)}$=0.1589 (Transformed data)

In contrast to the literature of other species, no beneficial effects of the 8-HQC, in terms of seeds set or seed weight, were observed in these current experiments with *Poa annua*. The excised stem system used with *Poa annua* is relatively sterile, since only the culm, may introduce organisms to the sugar solution. The stem is cleaned of soil by removal of some lower leaves. Also, not all organisms are capable of inducing vascular blockages (van Doorn, 1986). It was observed that when culms remained in solution for several days after full maturation (>25 days after anthesis), a slime began to form on the surface of the solution. Such a growth was rarely observed during the essential seed maturation period of 12-15 days, indicating too short a time for a detrimental level of growth to build up. Sucrose alone may not be a very good media for organism growth.

The seed, acting as a strong sink for assimilates, may also be a factor in the minimal ability of the organisms to grow in the sugar solution. Many species have nutrient and hormonal flow down the stem, as well as up to the seed. Such downward flow into the solution may create a favorable environment for organism growth. The investigations concerning assimilate reallocation from the stem to the seed (chapter 3) indicated the seed as a very strong sink, which may result in little if any downward flow of nutrients in *Poa annua*.

No benefit in terms of seed set or seed weight was observed for *Poa annua* due to the use of 8-HQC in the excised stem solution.

Chapter 3: Carbon Source and Light Effects on *Poa annua* seed set

The concentration of carbohydrate or carbon source available in solution to an excised wheat stem significantly affected seed set and seed weight (Jenner, 1970; Barlow, 1983). Light intensity and the interactions of light with the amount of carbohydrate available have also been implicated. However, the significance of these factors is not consistent between species (Willey & Holiday, 1971). Factors influencing the carbohydrate availability to the developing seed of *Poa annua* were investigated by comparing light intensity in relationship to type and concentration of two sugars in the solution.

Materials & Methods:

Experiment 1: Carbon source and Light comparisons

A split plot design was used with the whole plot treatments as three light intensities: 0, 55, and 110 $\mu E\ m^{-1}s^{-1}$. A completely randomized design, using two sugars (sucrose and fructose) each at four concentrations (0, 2, 4, and 6% by weight) in the solution, made up the split plot. Two replications of each treatment were made. Five of these split plot designs were used as blocks for an overall RCB design. Blocking was done to account for variation due to different dates of collection of the flowering culms (7/20, 7/24, 7/30, 8/11, and 8/16/90). This resulted in a total of 10 treatment replications. One *Poa annua* genotype, breeding project #709, was selfed to produce the seed.

Light intensities were accomplished with 50% shade cloth over the flowering stems for the 55 $\mu E\ m^{-1}s^{-1}$ group. Black plastic was used for the 0 $\mu E\ m^{-1}s^{-1}$ light intensity treatment. The reduced light intensities were confirmed

with a light meter, to within 5 percentage points. The air temperature in all the light treatments were maintained between 20 and 25°C., with the higher temperatures during the day and the lower at night.

Experiment 2: High sucrose concentrations

Five concentrations of sucrose (10, 15, 20, 25, and 30% by weight) were investigated using eight replications of each treatment at full light intensity. Again, *Poa annua* genotype 709 was selfed to produce seed. A one-way ANOVA was used to compare seed set and seed weight at sugar concentrations higher than in part I.

Results & Discussion:

Carbon source and Light interactions:

Part I of this experiment focused on the interactions between the sugars and light intensity. The analyses indicated significant interactions of light x concentration and light x carbon source x concentration in seed weight measurements (Table 3), and marginal significance (.06) of light x concentration in seed set measurements (Table 4). Higher light intensity increased seed set and seed weight for culms in the lower sugar concentrations, but had less effect for those in the higher sugar concentrations (Table 5). The significant three way interaction appears to be the result of sucrose influencing the light x concentration interaction greater than fructose (Fig. 4). As shown in Fig. 4, the 6% sucrose, 0 $\mu E\ m^{-1}s^{-1}$ treatment appeared to be negatively affected compared to the 4% sucrose treatment. The cause of this decrease was unknown, but seems likely to be random variation. These light x sugar concentration interactions indicate that developing seeds are able to use exogenous carbohydrate as needed for seed development and filling,

Table 3. ANOVA table for Seed weight data (transformed): Carbon source and Light Experiment

Source of Variation	DF	SS	MS	p
CONSTANT	1	8.759e-006	8.759e-006	
block	4	4.288e-007	1.072e-007	
light	2	3.617e-007	1.809e-007	0.02
ERROR1	8	2.226e-007	2.783e-008	
csource	1	1.26e-009	1.26e-009	0.73
conc	3	1.186e-006	3.955e-007	<0.001
csource.conc	3	5.984e-008	1.995e-008	0.14
light.csource	2	3.275e-008	1.638e-008	0.22
light.conc	6	1.587e-007	2.645e-008	0.03
light.csource.conc	6	2.011e-007	3.352e-008	0.01
ERROR2	204	2.195e-006	1.076e-008	

Table 4. ANOVA table for Seed set data (transformed): Carbon source x Light Experiment

Source of Variation	DF	SS	MS	p
CONSTANT	1	2781.400	2781.400	
block	4	46.733	11.683	
light	2	130.100	65.048	<0.01
ERROR1	8	52.887	6.611	
csource	1	20.352	20.352	<0.001
conc	3	233.090	77.697	<0.001
csource.conc	3	4.138	1.379	
light.csource	2	6.927	3.464	0.10
light.conc	6	17.789	2.965	0.06
light.csource.conc	6	6.177	1.030	
ERROR2	204	296.440	1.453	

Table 5. Interaction between sugar concentration and light intensity.

Light Intensity	Seed weight*	No. seeds*
$\mu E\ m^{-1}s^{-1}$	g	
0	.00021**	2.75**
55	.00023**	2.83**
110	.00011	1.47

*Measurement differences between 0% and 6% sugar concentration at the indicated light intensity (transformed data).

**Significance between concentrations within an intensity level at HSD(.01). Seed set and seed weight was most affected by sugar concentrations in the excised stem solutions, while at low light intensities.

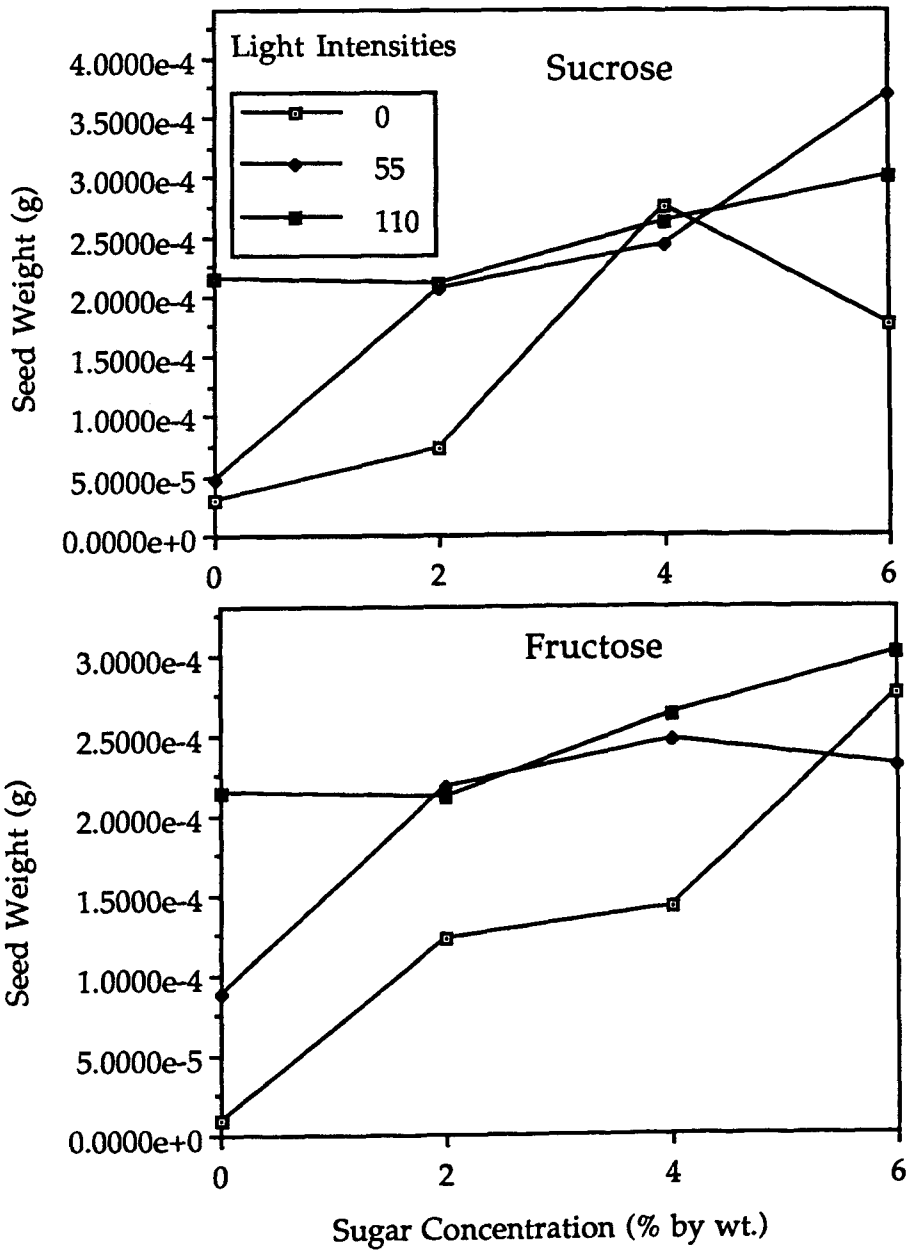

Fig. 4. Three-way interaction graphs representing three light intensities and four sugar concentrations of sucrose and fructose (transformed data). Fructose appeared to affect the light x concentration interaction less than sucrose. Flowering culms exposed to the maximum light intensity (110μE $m^{-1}s^{-1}$) were least affected by sugar concentrations.

when under less than optimum light conditions for photosynthesis. It also indicates that sugars and other carbohydrates are being produced, in the culm itself, for use by the developing seed.

Photosynthetic area of the excised stem could have a similar effect as that observed with light intensity. In other words a greater leaf area could supply additional photosynthate to a developing seed, much like higher light intensity did. However, Rawson & Evans (1971) as well as Ruemmele (1989) reported in wheat and *Poa annua,* respectively, that little or no effect was seen as a result of altering leaf photosynthetic area on a flowering stem. It appears in *Poa annua* that, although photosynthesis plays an active role in seed filling, the assimilates from photosynthesis in the leaves may not be partitioned to the seed. Rather, the flower itself may be self-sufficient, or nearly so, in photosynthetic ability. This was the finding for wheat ears (Rawson & Evans, 1971).

Type of Carbon Source:

Previous investigations of sucrose and fructose as the sugar in the excised stem solution indicated no differences in terms of *Poa annua* seed set (Ruemmele, 1989). However this experiment resulted in fructose giving significantly higher seed set than sucrose (Table 3 & 6). Seed weight measurements, however, showed no such effect (Table 4 & 6). The coefficient of variation for both measurements tended to be lower in the fructose treatment (Table 6).

Preliminary work had not shown significant differences in seed set measurements, but did consistently find lower variation due to the fructose treatments. Increased power of the statistical test due to greater replication is a

Table 6. Seed set and seed weight means and CV for sucrose and fructose.

	No. Seeds		Seed Wt.	
Sugar	Mean	CV	Mean	CV
			g	
Sucrose	9.17a	59.19	0.00018a	86.12
Fructose	13.19b	49.00	0.00018a	61.88

Values with different letters within each column are significantly different at HSD(.05). The fructose treatments result in significantly greater seed set, but not differences in seed weight measurements. Less variation is observed for both seed set and seed weight measurements

possible reason for detecting significant differences in this experiment using this genotype. The differences in average seed set measurements may be rather small, and may vary among genotypes (Ruemmele, 1989), however the reduced variation in the fructose treatments merit its use in future work. It may also indicate that proper type of carbon source may be an important factor for excised stem work with other species as well.

Carbon source concentration:

The results of experiment 1 show a significant effect of carbon source concentration on seed set (Table 4) and seed weight data (Table 3) due to sugar concentration in the excised stem solution. Treatment means increased between 0 and 4% sugar, followed with no significant difference between the 4 and 6% levels. Fig. 5 shows seed set data along with coefficient of variation for each mean. Seed weight data followed a very similar pattern, both in mean and CV and, therefore, are not presented. In experiment 2, effects of sugar concentrations in the excised stem solution (10-30% sucrose) were investigated, and resulted in a significant drop in both seed set and seed weight at the 25% and 30% levels (Fig. 5). Sugars available in the nutrient solution over 4% do not appear to increase seed capacity (weight) or number until concentration exceeds 20-25%, when seed set decreases.

The results of experiment 1 are comparable to those from carbohydrate concentration experiments in wheat (Jenner, 1970; Barlow, et al, 1983). The mechanism for this control of carbohydrate entering the seed is not known, but *Poa annua* may be similar to wheat in such controls.

Sucrose concentrations greater than 10% were not found in any literature researched. Therefore, the results of experiment 2 (high

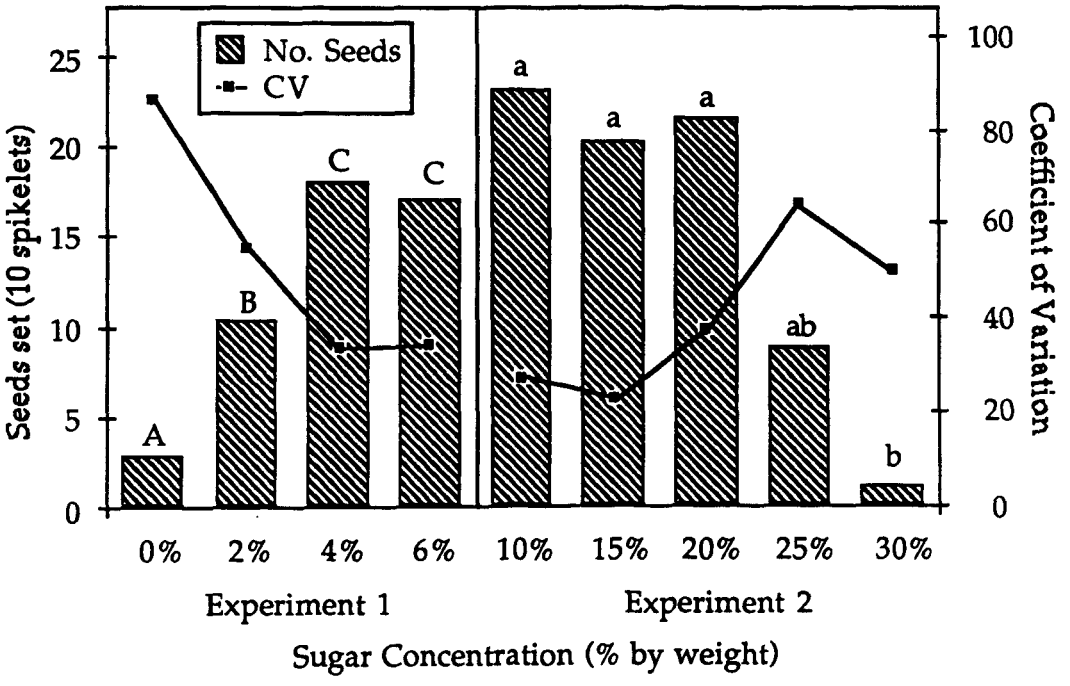

Fig. 5. Sugar concentration means and CV values are presented for experiment 1 and 2, with means having different letters being significantly different at HSD(.05). Data are not transformed. This graph represents two experiments (0-6% and 10-30% sugar); points between the experiments cannot be compared directly. Seed set reaches a plateau in the 4-20% range. Coefficient of variation has an inverse relationship with seed set means with the lowest amount of variation present between 4-15%.

concentration levels) could not be compared to other species at these levels. Osmotic imbalance due to the extreme solute concentration is a probable cause for the observed reduction in seed set and seed weight. Decreased water uptake by detached culms of wheat and cut flowers (gladiolus and rose) were observed as sucrose concentrations increased, even at relatively low concentrations (0-2%) (Aarts, 1957; Barlow, et al, 1983). The very high levels used in the current study may reduce water uptake too severely, decreasing seed set.

Coefficient of variation of seed set and seed weight data indicated an inverse relationship with the means (Fig. 5). Part of the reduction in CV is due to greater seed set means of the better performing treatments. However, variance often remained constant or actually decreased as the treatment means increased.

Light Intensity Main Effects:

The level of light intensity under which the excised stems were kept, was also shown to be a significant factor in determining the seed set (Table 4) and seed weight (Table 3) of *Poa annua*. Increases in light intensity led to increases in seed set and seed weight, and coincidentally, a decrease in coefficient of variation (Table 7). The upward trend, that still occurred at the 110 $\mu E\ m^{-1}s^{-1}$ intensity level, may indicate that greater intensities might further increase seed set. However, excised *Poa annua* stems located in a greenhouse under sunny, summer conditions (much higher light intensities), gave very similar seed set when compared to that of the 110 $\mu E\ m^{-1}s^{-1}$ illumination treatment. Although the greenhouse study results cannot be directly compared to treatments in the main study, it seems to

Table 7. Seed set, seed weight, and CV for three light intensities

	No. Seeds		Seed Wt.	
Light intensity	Mean	CV	Mean	CV
μE m-1 s-1			g	
0	5.36a	73.16	0.00013a	95.37
55	12.53b	47.64	0.00020ab	76.62
110	17.06b	38.37	0.00022b	52.68
Green Hse.	14.17	n.a.	0.00015	n.a.

Values with different letters within each column are significantly different at HSD(.05). Higher light intensties result in significantly greater seed set and seed weight. Variation decreases with increasing intensities. Separate greenhouse treatments, although not in the same study, are similar to that of the artificial light conditions.

indicate little further benefit in terms of seed set, with higher light intensities beyond the level obtained with the artificial lights. An experiment directly comparing these much higher intensities needs to be conducted to make definitive statements.

Mechanisms for the seed set and seed weight effects reported here may be similar to those discussed in shading experiments of wheat. Seed filling was divided into two stages in these reports. The first was termed cell production or division phase (of the endosperm), and the second, the cell expansion phase. 50% shading during the cell division phase, decreased the number of endosperm cells (Brocklehurst, 1978). The later cell expansion phase reduced the dry weight of seeds by 11%. Similar shading effects were reported in other wheat investigations as well (Thorne, 1965; Willey & Dent, 1969; Willey & Holliday, 1971).

Since higher light intensity often correlates with higher temperature, the observed effects to seed set or seed weight might be explained as a result of temperature differences, rather than the light intensity itself. Wheat ears, provided with a 5% sucrose solution and a temperature of 30°C, produced more than 4 times the starch found in ears with the same solution at 15.5°C (Jenner, 1968). Large temperature differences, like that reported by Jenner, were not present in this study, as temperatures were consistently between 20 and 25°C. Therefore, seed set and seed weight effects do not appear to be the result of temperature effects.

The data presented, show reductions in seed number or seed dry weight not equal in quantity to the reduction in light intensity, both in the previous wheat data as well as those presented for *Poa annua* in this study. For example in the *Poa annua* data, a 50% reduction in light intensity (55 μE

$m^{-1}s^{-1}$) resulted in a 26% decrease in seeds set and a 10% decrease in seed weight. Brocklehurst attributes similar less than expected differences, to light saturation in the full illumination (field) conditions, as well as reallocation of assimilates within the stem. In the experiments with *Poa annua*, however it is highly unlikely that light saturation could occur at the relatively low light intensities (110 $\mu E\ m^{-1}s^{-1}$). Therefore reallocation of assimilates may play a role in seed development under less than optimum light conditions. These results may also reflect the shade tolerance of some *Poa annua* genotypes. *P. annua* may be more efficient in responding to low light intensities as compared to wheat or other non-shade tolerant plants.

Evidence of reallocation of assimilates within a stem included investigations where shading was found to reduce the amount of dry matter in the stem and chaff of wheat (Martinez- Carrasco, 1979; Rawson & Evans, 1971). A reduction in stem dry weight may not be a result of carbohydrate translocation to the flower. Instead, lower than optimum light conditions may reduce the carbohydrate diverted there from photosynthesis in the attached leaves. As discussed previously, excess sugars were found in the non-seed parts of the flower. Photosynthesis, during seed development, may be adequate to supply carbohydrate, both to the developing seed and the stem, unless low light conditions are encountered, upon which more assimilates may be sent to the seed at the expense of the stem (Wardlaw, 1970). To answer this question more thoroughly, carbohydrate partitioning experiments are needed to determine where assimilates are going, during seed and floral development.

Another possible explanation for the lower than expected effects due to shading may be a non-linear relationship between light intensity and seeds set, endosperm cell number, or other measurement.

If assimilates are being translocated up the stem to the developing seed, the sugar solution supplied to the excised stem should have some effect, as was shown in the carbon source concentration discussion. Analysis of the interactions between the sugar concentration in the solution and light intensity indicated differential effects depending on light intensity and the form of sugar used. Because beneficial effects of supplied sugars, in terms of seed set and weight, were observed even at the 100% light intensity level, the sugar solution is an important component needed for good seed set results with *Poa annua* in an excised stem technique.

For use of this excised stem technique in *Poa annua*, concentrations of 4-6% fructose together with maximum artificial light, resulted in the best seed set and seed weight yield. Fig. 5 shows a plateau of high seed set values between 4 and 20% carbon source. In experiment 2 of chapter 2, where sucrose concentrations (0, 2, 4, 6%) were tested with levels of 8-HQC, the 2% and 4% treatments were not significantly different in terms of seed set results. The reason for this discrepancy is unknown, but could be due to random variation or uncontrolled factors. It may be an indication of the small practical differences present between the 2 and 4% treatments since Ruemmele (1989) found 2% satisfactory for her work. However, for optimum seed set and lowest variation, 4-6% is recommended based on the genotypes studied here. The lower concentrations are easier to work with, and it is unlikely that additional seed set would be gained, by using sugar concentrations greater than 10% by weight.

Chapter 4: Some factors influencing inflorescence seed capacity

Influences on *Poa annua* source plants used for excised stems may have significant effects on the seed set of a particular inflorescence. Similar to wheat, where shading and inadequate nutrition are known to reduce the number of viable flowers (Willey & Dent, 1969; Willey & Holliday, 1971; Martinez-Carrasco, 1979), a reduction in flower number is reflected in a reduction in potential seed set. When assimilates are limiting, the most mature flowers become stronger sinks, leading to fewer viable flowers to produce seed (Puckridge, 1968).

In this chapter, genotype and stage of floral development, as well as the shoot density of the source plants were investigated for effects on the seed set in *Poa annua* L. Culms length was also observed for effects on seed set.

Materials & Methods:

Experiment 1: A RCB design was used with dates of stem collection (3/17, 3/20, 3/26, 5/2, and 5/7/90) as the five blocks. Within each block a split plot was used with five genotypes (493, 42, 1824, 1538 and 1798) as whole plot treatments and three developmental stages, defined by 1) 0-1 spikelets emerged from the boot, 2) 5-7 spikelets emerged and 3) all spikelets emerged. These stages of development were used because they represented inflorescences approximately three, two and one days before anthesis. The first stage was the earliest that culms could be collected and reliably produce seed. Two replications of each treatment were used, giving a total of ten treatment replications for the experiment. Details on the excised stem method are described in the general materials and methods section.

Culm length measurements were made from the top of the first spikelet to the excision point when the inflorescence was fully exserted from the boot just prior to anthesis. The average number of florets in the top 10 spikelets of each inflorescence were counted one day prior to anthesis. Seed set, seed weight, floret number and culm length were then analyzed to test for differences due to genotype and stage of development. Correlations between seed set, floret number, and culm length were also calculated to evaluate the strength of any relationships. Due to the loss of some culms through various means, a small number of data values (six) were estimated using MacAnova's estimation techniques (Oehlert, 1989).

Experiment 2: A split plot was used to evaluate effects of the number of florets per spikelet of a *Poa annua* inflorescence. Six genotypes (234, 709, 948, 1476, 1538, 1667) were the whole plot treatments, each at two population densities resulting in the split plot treatment. Five to seven inflorescences were removed from a plant and each counted for the average number of florets/spikelet in the top ten spikelets.

Plant density was divided into a high and low group. The high plant density conditions consisted of root bound four inch pots located in a greenhouse, having very high shoot density. The low density group consisted of clones grown under field conditions in the 1989 spaced planting at the University of Minnesota *Poa annua* and *Poa supina* breeding project. Plants were spaced 20 cm. apart within rows spaced 76 cm. apart. This environment allowed ample space for growth and spread. The experiment was conducted during July 1989.

Results & Discussion:

Genotypic Effects

No significant differences in seed set or seed weight due to genotype were observed in experiment 1 (Table 8). In both experiments, florets per spikelet differed significantly (Table 9) among genotype. It would be expected that a difference in flower number would correlate with a difference in seeds set. However, such a correlation does not exist, possibly due to the unpredictable nature of seed yield in grasses (van Wijk, 1985).

Some differences in seed set due to genotype were noted in preliminary experiments, as well as differences in potential seed production as measured by florets per spikelet. Therefore, comparisons of seed set alone between genotypes, for measures of fertility, may be difficult. Accounting for the differing seed capacities may be possible by factoring in the number of florets per spikelet in seed set measurement. The number of florets per spikelet in some genotypes were observed to be as large as six or as small as two. "Seed potential" was calculated by dividing measured seed set by the potential seed set. Potential seed set was determined by multiplying florets per spikelet and the number of spikelets used in counting. The result was a proportion of observed to potential seed that was set. In theory this statistic bases seed set on the number of florets, or actual possible seeds, rather than on the number of spikelets which can differ in flower number. However attempts at accounting for seed set variation in this manner have been unsuccessful. The use of this measurement for seed set did not reduce the variation, possibly because of the large amounts of variation in general, mentioned earlier in grass seed yield (van Wijk, 1985). In work with *Poa pratensis*, correlation coefficients for floret number and seed set or seed weight were only .1776 and .1422 respectively,

Table 8. Experiment 1, Seed set and seed weight means of five genotypes.

Genotype	Seed set	Seed wt.
		g
493	5.55a	.00009a
42	5.59a	.00009a
1824	9.27a	.00010a
1538	5.25a	.00010a
1798	4.69a	.00012a

Values with different letters within each column are significantly different at HSD(.01). No significant differences were seen in seed set or seed weight between these five genotypes

Table 9. Experiment 1, Number of florets per spikelet in five genotypes

Genotype	Flor/spk	Culm Length
		cm
1538	3.34a	8.31c
1824	3.73b	4.84a
1798	3.81b	6.40b
493	4.19c	5.35ab
42	4.32c	5.75ab

Values with different letters within each column are significantly different at HSD(.01). Both florets per spikelet and culm length are significantly different among the five genotypes used in this experiment.

along with low repeatability of measurements (Ensign, et al, 1989). These data from the literature further support the great amounts of variation evident in *Poa annua.*

Stage of Development

The stage of floral development of an inflorescence at the time of excision was a significant factor in seed set ($p<.01$) and seed weight measurements ($p<.01$). Lesser developed inflorescences produced fewer, lighter, and more variable seed set than the more mature inflorescences (Table 10). All the genotypes were equally affected by the stage of development as indicated by the lack of genotype x stage interaction. A similar experiment in tall fescue, however, reported such an interaction (Wofford, 1986). Competition between the spikelets for available assimilates needed for floral development may be involved in the reductions in seed set. In wheat, the most developed spikelets are more competitive than those less developed, if nutrients are limiting (Puckridge, 1968). When a *Poa annua* inflorescence is excised too early, or in an immature stage, the available assimilates may go only to the top most florets of the top most spikelets in the inflorescence. These are the most developed parts of the flowers (Evans & Gordon, 1940). Therefore the remainder of the florets remain under-developed and not available for seed production.

Culm length

Culm length has also been investigated for its effects on seed set in *Poa annua,* however Ruemmele (1989) reported no such effect, unlike similar studies in *Poa pratensis* and *Festuca arundinacea* (Ensign, 1989; van Wijk,

Table 10. Stage of development means and CV for seed set and seed weight.

	No. seeds		Seed wt.	
Stage*	mean	CV	mean	CV
			g	
0-1 spk.	2.57a	73.31	.00007a	68.27
5-7spk.	4.41a	64.66	.00010ab	60.44
All spk.	12.97b	47.79	.00015b	39.25

Values with different letters within each column are significantly different at HSD(.01)

* Stage of floral development as shown by the number of spikelets emerged from the boot

1985; Wofford, 1986), where seed setting was significantly affected by culm length. Ruemmele attributed her results to lower amounts of variation in culm length of *Poa annua* as compared to the other species. In light of these conflicting results, culm length was again investigated, by taking a slightly different approach. Rather than collecting inflorescences, and cutting the culms to a specific length, culms were cut normally (as described in the general materials and methods) and measured, then the data was analyzed using correlations. It was observed that a longer culm was normally accompanied by a larger inflorescence compared to a short culm. This seems to occur even within a genotype. Therefore, cutting to the same length on a long and short culm may not be equal treatment, especially with the relatively short culms of *Poa annua.*

Culm length was significantly different between genotypes (Table 9), however correlations of culm length with seed set and seed weight were not (.116 and .123 respectively). If translocation of assimilates, as described in chapter 3, from the excised stem to the developing seed did occur, stem length seems to have no effect. One might reason that a larger culm would have greater reserves available to be used by the seed. However tall cultivars of wheat were also no more dependent than short ones on stem reserves, and no more able to draw on reserves for grain filling (Rawson &Evans, 1971). This evidence may indicate that stem reserves are not a source of carbohydrate for seeds. But, if reallocation does occur, sucrose present in the excised stem solution may have masked any effects of culm length. A study investigating culm length and differing carbon source levels in the nutrient solution might be able to test the effect of culm length more definitively.

Seed Capacity of an Inflorescence: Floret number variation

In addition to lower seed set, the less developed inflorescences had significantly fewer florets per spikelet (Table 11). In wheat, the florets are formed very early in the ear development (Bonnet, 1936). Since *Poa annua* floret initiation occurred well before the earliest stage collected in this experiment, what caused the decrease in floret number? The decreases are most likely due to not counting those less developed florets. Counts were made by observation, thus the small, undeveloped florets were not seen due to their small size. The less developed florets likely are not viable, contributing to the decreased seed set of the less developed inflorescences. The literature appears to be lacking in references outlining developmental processes occurring between floral initiation and anthesis. The majority of the literature on floral development deals with initiation and sex determination, both of which have occurred well before the earliest collection stage of this experiment. However, conditions during development of the flower are vital in determining the eventual seed capacity of an inflorescence (Willey & Dent, 1969; Willey & Holliday, 1971; Martinez-Carrasco, 1979).

Lower numbers of florets per spikelet and seed set due to nutrient competition are supported by experiment 2, where nutrients were possibly limited due to interplant competition. It was observed that some *Poa annua* genotypes were affected more due to plant density than others as indicated by the significant genotype x plant density interaction (Table 12&13). This interaction is related to morphology when the larger (& longer) stemmed genotypes (1667, 709) were affected more by the high plant density than the finer texture genotypes (1476). The long stemmed types tend to have a more open habit naturally, with low shoot density, while fine types are very dense,

Table11. Florets per spikelet means at three stages of development.

Stage*	Flor/spk.
0-1 spk	3.723a
5-7 spk	3.787a
All spk	4.123b

Values with different letters within each column are significantly different at HSD(.01)

* Stage of floral development as shown by the number of spikelets emerged from the boot

Table 12: ANOVA table for florets per spikelet data, Genotype and Location

Source of Variation	df	SS	MS
genotype	5	7.35	1.469*
error	24	2.02	.084
location	1	10.50	10.500*
loc. w/in genotype	5	3.18	.636*
error	24	1.66	.069

* <.001 prob.

Table 13. Interaction of two plant densities and six genotypes for florets/spk.

Genotype	mean (field) - mean (pt. bd.)†
1667	1.60*
709	.85*
948	.81*
1538	.74*
234	.70*
1476	.04

* significant at HSD(.01).

† Number of florets per spikelet in field grown conditions minus florets per spikelet in pot bound grown conditions.

even with ample growing space. These effects are not due to early collection or less developed inflorescences, since all culms were counted just one day prior to anthesis. The question arises, what then is the cause of this reduction in floret number?

Yield in wheat is determined by two factors, the capacity of the ear and carbohydrate supply. Competition occurs for the available carbohydrate as the flowers are forming. When carbohydrates were limiting to some degree, the ear capacity was reduced (Willey & Dent, 1969; Martinez-Carrasco, 1979).

In addition, competition for light may be involved. At high plant densities, many culms may be shaded, reducing the photosynthetic capability, similar to the shading experiments of chapter 3. When shading effects due to high plant density occurred during floral development in wheat, ear capacity was reduced. (Martinez-Carrasco, 1979). Some genotypes are affected more, due to plant density and possibly nutrient competition than others, as indicated by the significant genotype x plant density interaction.

For the best and least variable seed set of excised*Poa annua*, inflorescences they need to be collected as late as possible. However, if early collection is necessary, additional nutrients may be experimented with in the nutrient solution. Hoagland's solution or similar media may help satisfy the needs not supplied by the sugar solution.

Although one of the goals of this work is to reduce the seed set variation of excised stems, the natural amount of variation in the plant appears to limit this objective to some extent. Further work investigating seed set under a variety of conditions is needed to learn more about this natural variation.

Thesis Summary & Conclusions

Factors influencing seed set on excised *Poa annua* stems, particularly carbohydrate availability, have been discussed. These include:

1) Carbohydrate type; fructose over sucrose
2) Concentration of sugar in the solution; 4-6% by weight
3) Light intensity for the excised stems; ≥110 $\mu E\ m^{-1}s^{-1}$
4) Carbon source concentration and light intensity interaction; light intensity is most important at low sugar levels and vice versa. The interaction is less affected by fructose than by sucrose.
5) Optimum time in the solution; 15 days
6) Genotypes differ in seed capacity
7) Optimum stage of development at the time of excision; just prior to anthesis (1 day)
8) Plant density of source plants; conditions allowing open, relatively unrestricted growth, favor greater floret number and possible seed capacity.
9) Plant density and Genotype interaction; Some genotypes are more affected by population density than others in terms of seed capacity (number of florets per spikelet)

Factors that did not appear to affect seed set of *Poa annua* include:

1) Additives (8-HQC) to control bacterial or fungal growth as well as vascular blockage in the excised stem solution

2) Number of florets per spikelet on the inflorescence of some genotypes (however was involved in significant effects in population density experiments

3) Length of the excised stem

Interactions between carbon source concentration and light intensities, along with floret/spikelet and culm length data, presented evidence for some patterns of carbohydrate partitioning within the excised stem. It is apparent that excised *Poa annua* stems were able to use sugars supplied in the solution if needed. Stems also maintained the capability to produce carbohydrate by photosynthesis. However, photosynthetic activity alone was not adequate for optimum seed set and seed weight. There appears to be some evidence that the photoassimilates partitioned to the seeds were the result of photosynthetic activity in the floral parts of the inflorescence.

The literature contains evidence for reallocation of photosynthate within the stem, favoring the seed, at the expense of the stem. However, such reallocation did not appear to be occurring in this study, but further work is necessary to make more definite conclusions. Since these experiments were not designed to explicitly address such nutrient partitioning and source-sink questions, only speculations can be presented. The need for continued research into the carbohydrate partitioning within the excised *Poa annua* stem is indicated.

Even with the decreases in seed set variation described in this thesis, substantial variation still exists. Further research into identifying sources of this seed yield variation would be useful.

Protocol Summary

For the best and least variable seed set results with excised *Poa annua,* stems, flowering culms need to be excised from the source plants, (under uniform growing conditions) as close to anthesis as possible and placed in the sugar solution containing 4-6% by weight fructose. A reasonable artificial light intensity (110 $\mu E\ m^{-1}s^{-1}$), without increasing temperature (>5°C) over the non-illuminated conditions, offers the best results for seed set and seed weight measurements. These protocols work well for seed set and seed weight comparisons within a genotype, and in qualitative comparisons between genotypes.

Appendix 1: Water Test Results of Distilled-Deionized Water

These measurements are the average of four samples taken throughout Alderman Hall

Element	mg/l	Element	mg/l
P	0.150	Zn	<0.007
K	<0.756	Cu	<0.028
Ca	0.241	B	<0.025
Mg	<0.203	Pb	<0.090
Na	<0.193	Ni	<0.024
Al	<0.010	Cr	<0.015
Fe	<0.022	Cd	<0.006
Mn	<0.006		

Tests were performed October, 1989 by:

Research Analytical Laboratory
Department of Soil Science
University of Minnesota
1903 Hendon Avenue
St. Paul, Minnesota, 55108

Appendix 2: Use of all spikelets vs the top 10 for seed set measurements

The number of spikelets that open on an excised *Poa annua* inflorescence may vary from 8 to 50, but, often only the top half of the spikelets commonly open. In addition, the number of florets per spikelet tends to decrease in the lower parts of the inflorescence, ranging from 2 to 6 at the proximal end, to often only 1 to 2 florets/spk on the distal end. This variation appears to be caused by competition for assimilates within an inflorescence (Willey & Dent, 1969; chapter 3 discussion). Growing conditions and stresses, caused by population density can also cause the number of florets per spikelet to decrease in the progression from the top to the bottom of the stem (chapter 4).

All of these characteristics mentioned have the potential of biasing or influencing seed set numbers. Attempts to avoid this variation in seed counting could help in making results more meaningful.

One such method is the use of the top ten spikelets only on an inflorescence for seed counting purposes. These first ten spikelets almost always open on inflorescences in the excised stem technique. Also, these top spikelets have much more uniform numbers of florets, than the inflorescence as a whole, reducing the overall variation in seed capacity. Finally, counting seeds in the first ten spikelets makes the work much more efficient than using all the spikelets. Counting seeds can be a very slow process and allow more chance for error.

To examine the effectiveness of this counting method, seed set in two experiments were counted in two ways: 1) using seeds from all open spikelets and seeds per spikelet as the seed set measurement, and 2) using only the

number of seed collected from the top ten spikelets. Statistical analyses used both counts for comparison. No differences in tests of significance were observed between the two methods, but the variation in the measurements tended to be lower in the counts using the top 10 spikelets. This lower variation in the measurement may be the result of using the more uniform spikelets near the top of the inflorescence for seed counting. The data in Table 1 compared sucrose and fructose carbon sources, and is representative of this decrease in variation, as expressed by the coefficient of variation.

Use of the top ten spikelets in seed counting can be very useful when applied in the pic technique where seed counts are used as a fertility measure. Variation of the measurements may be reduced and efficiency increased.

Table 1. Comparison of means and CV of the two counting methods.

	All spk		Top 10 spk	
Treatment	mean	CV	mean	CV
	sds/spk		sds/spk	
fructose	2.23	34.4	2.74	32.4
sucrose	1.99	48.0	2.50	45.6

Tests of significance were not different between the two counting methods, but CV was slightly reduced in the top 10 spikelets.

Appendix 3: Origin of Genotypes Used

42 (PA3A): 2nd generation of a plant collected at Nassau CC, New York

234 (PA21): Collected from a green at the Les Bolstad GC, University of Minnesota

493 (NY12): 3rd generation (see 1824)

709 (AZ17-A6): Open pollinated progeny from plants collected from Oro Valley CC, Tucson, Arizona

948 (OH8407-4): Open pollinated progeny of a plant collected at Meadowview GC, Kent State University, Kent, Ohio

1476 (Interlachen Gr. 8): From Interlachen CC, Edina, Minnesota

1538 (HK15 #17): From Club de Nimes, France

1667 (CS-2B): Open pollinated progeny of a plant collected in Canada

1824 (NY11-6): 3rd generation open or self pollinated seed from plants collected at Galloping Hills CC, New York

1978 (MN88 26): Minnesota collection

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