AN ABSTRACT OF THE DISSERTATION OF

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Title: Seed Development in Orchardgrass (*Dactylis glomerata* L.) in Relation to Plant Growth Regulators and Spring Nitrogen.

Abstract approved: _____
Thomas G. Chastain

Orchardgrass (*Dactylis glomerata* L.) is an important seed crop, but unlike other cool-season perennial grass seed crops such as perennial ryegrass (*Lolium perenne* L.) and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.], seed yields have not increased over time so there is considerable room for improvement. Research suggests that plant growth regulators (PGRs) such as trinexapac-ethyl (TE), chlormequat chloride (CCC), and spring nitrogen (N) application have been found to increase seed yield in orchardgrass through its effect on increasing seed number. Field trials were conducted at Hyslop Experimental Farm near Corvallis, OR in three crop years (2016-2017, 2017-2018, and 2018-2019) to examine the effects of spring N and PGRs on seed production characteristics in orchardgrass. Spring N was applied at four rates: 0, 112, 157, and 202 kg N ha^{-1} and PGR applications were timed using the BBCH [Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie] scale. The four PGR treatments consisted of : 0 g ai ha^{-1}, 210 g ai ha^{-1} TE applied at BBCH 32, 210 g ai ha^{-1} TE applied at BBCH

51, and a combination of 105 g ai ha^{-1} TE + 1500 g ai ha^{-1} CCC applied at BBCH 32. Four treatments were used to examine seed development, and carbon and nitrogen deposition in orchardgrass: control, TE (210 g ai ha^{-1}), N (spring N applied at 112 kg ha^{-1}), and TE + N. Regression analyses were used to aid in understanding the development of seed in three spikelet seed positions and the effects of treatments. An interaction of spring N and PGR resulted in increased seed yields in two of the three years while spring N and PGR worked independently in the other year to increase seed yield. The combination of TE and CCC PGRs did not increase seed yield over TE alone. Spring N effects on the observed increases in seed yield were largely due to a corresponding increase in seed number m^{-2} evident with spring N application. Increases in orchardgrass seed yield attributable to TE PGR were the result of increased seed number m^{-2} and improved harvest index (HI), but not seed weight.

In 2018, seed weight increased over GDD in bi-phasic segmented pattern in seed from distal and central spikelets but increases were linear in seed from proximal spikelets. In 2019, seed weight increase in proximal spikelets followed a bi-phasic segmented function, while seed weight increase in central spikelets segmented except for TE treatment. Seed growth rate (SGR) varied among spikelet positions and ranged from 0.22 to 0.34 mg GDD^{-1} per 100 seed. The SGR varied among treatments and ranged from 0.31 to 0.47 mg GDD^{-1} per 100 seed. Seed filling duration (SFD) varied among spikelet positions and treatment. Seed moisture content declined over seed development in a linear manner in 2018, and the decrease in seed moisture followed mixed linear and segmented models in 2019. The TE + N treatment had both the shortest SFD and one of the shortest SGR values among treatments, and as a result tended to have low seed

weight. The TE + N treatment produced high seed yields and seed number so either seed abortion or seed shattering loss was reduced, or both. The content of C and N in seed increased over growing degree days ($P \leq 0.001$) and followed a segmented model except for N content in the N treatment. Both C and N content of seed were increased by spring N application. The N treatment reached peak C deposition earliest and had the shortest duration of C filling in seed among the four treatments. The peak deposition of C and N preceded physiological maturity of the seed. During seed development, flag leaf chlorophyll declined in treatments with no N and either declined or were constant with N application. There were no consistent effects of TE PGR on the deposition of C or N in the seed. This study suggests that the combination of spring N applied at 112 kg N ha^{-1}, and TE PGR application at 210 g ai ha^{-1} would be the best practice to increase seed yield in orchardgrass.

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Seed Development in Orchardgrass (*Dactylis glomerata* L.) in Relation to Plant Growth Regulators and Spring Nitrogen

by
Mohammed M. Morad

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Mohammed M. Morad, Author

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CONTRIBUTION OF AUTHORS

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DEDICATION

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Chapter 1. Introduction and Review of Literature

1.1 Introduction

Oregon is a world leader in seed production. The state is the nation's leading producer of cool-season turf and forage grass seed and these crops rank among Oregon's top five agricultural products each year. Seed production is one of the most important revenue resources for Oregon where domestic and foreign seed production exports are valued at $922 million (USDA, 2019).

One of these important cool-season perennial seed crops produced in the state is orchardgrass (*Dactylis glomerata* L.). The earliest U.S. seed production of orchardgrass was mostly in Virginia and Kentucky (Wheeler and Hill, 1957). Orchardgrass seed production began in Oregon in 1934 and by 1962, orchardgrass seed crop acreage had grown to 2,500 acres (Rampton and Warren, 1963). Today, orchardgrass seed production is mainly focused in Oregon where 98% of the national seed production takes place. About 10 million pounds of orchardgrass seed were harvested in Oregon in 2017.

1.2. What is Orchardgrass?

Orchardgrass is a cool-season perennial grass species that is native to western and central Europe where it is known as "cock's foot" (Myers, 1962). The plant is a bunch-type grass that produces many tillers and the flowering culm can grow up to more than 1.2 m in height at peak anthesis. The leaves are sharp-pointed, long, wide, v-shaped in cross section, and the leaf blades are flat, and folded in the bud. Orchardgrass does not form a complete sod since the plant produces no stolons (Wheeler and Hill, 1957). Flowering in orchardgrass is promoted by low winter temperatures through the process of vernalization (Gardner and Loomis, 1953), thus the seed crop must be produced in an

environment that has sufficient cold in winter for successful seed production. The crop's primary use is for forage and hay.

The inflorescence in orchardgrass is a panicle consisting of multiple spikelets deployed in a complex pattern of branching that is not common among cool-season grasses grown for seed. The panicle exhibits an unusual pattern of dorsiventrality in development and bilateral symmetry of panicle apices (Fraser and Kokko, 1993). The spikelets are arranged in clusters borne in an irregular manner at the apex of lateral branches within the panicle and at the apex of the rachis. Lateral branches in the lower third of the panicle tend to produce greater numbers of spikelet clusters than branches at the base and tip of the panicle. The development of the spikelets and the florets they contain is asynchronous in nature, meaning that both spikelets and florets in various stages of development can be found in the inflorescence at any given time. Each spikelet has two glumes and may contain up to six florets (flowers) developed in alternate positions along the rachilla.

The florets of orchardgrass are cross-fertilized by windborne pollen, with self-fertilization being rare. The lemma, palea, awn (if present), and rachilla are parts of the floret and are present prior to seed development. Within the floret is the caryopsis – a single-seeded dry fruit characteristic of the grasses that does not split at maturity to release the seed.

1.3. Seed Production Practices

Seed yields of perennial ryegrass (*Lolium perenne* L.) and tall fescue [*Schedonorus arundinaceus* (Schreb.)] seed crops in the state have more than doubled in the last 40 years. Reasons for the seed yield increases include better agronomic and pest

control practices, and modernization of harvest equipment. Orchardgrass seed crops have not experienced similar advancements in seed yield over that time, so there is considerable room for improvement. Some of this lack of seed yield improvement in orchardgrass is likely due to the propensity of the crop for seed shattering losses and to the widespread incidence of choke disease. The review of literature reveals that very little research has been conducted to date on seed production of this valuable crop.

The orchardgrass seed crop is planted in spring in wide row spacings (up to 0.9 m) (Rampton and Warren, 1963). Narrow row spacings (0.3 m) produce lower seed yields than wide rows. No seed crop is harvested in the year of establishment because of juvenility (Niemeliäinen, 1990). Orchardgrass seed crops typically have been established without the use of a cereal companion crop because of poor first-year seed yield of this perennial crop, but profitable establishment with wheat might be possible (Chastain and Grabe, 1989). The seed crop is best harvested by cutting into windrows and field drying before combining because seed dried in artificial dryers had much lower seed germination (Rampton and Lee, 1969). Seed yield in orchardgrass was not improved by burning of post-harvest residues (Rampton and Jackson, 1969; and Chastain, unpublished).

1.4. Seed Development and the Relationship to Seed Yield

Seed development begins in the floret following double fertilization and ends with the mature seed. Seed development in cool-season perennial grasses has a two-phase pattern: seed growth and seed maturation (Grabe, 1956; Anslow, 1964). During the seed growth phase, seed weight increases as a result of starch reserve accumulation in the endosperm and growth of the embryo. Seed moisture content declines slightly. When

the seed reaches maximum dry weight, it has attained the state of development known as physiological maturity. During seed maturation, seed moisture content declines rapidly but seed weight does not change appreciably.

The seed development process affects seed yield of cool-season perennial grasses. Seed yield is primarily influenced by two components: seed number and seed weight (Chastain and Young, 1998). The relationship of seed yield to these components of yield can be expressed in the following manner:

Seed yield = Seed number x Seed weight

The seed number component is determined by establishment of yield potential (manifested at anthesis) and by the realization of that potential at the time of seed harvest (Chastain and Young, 1998; Abel et al., 2017). Seed yield potential is an expression of seed yield components including panicles m^{-2}, spikelets $panicle^{-1}$, and florets $spikelet^{-1}$ found in the crop at anthesis. This expression of seed yield potential is affected by the production practices and cumulative effect of the production environment leading up to that point in crop development. Seed yield in orchardgrass was found to increase in proportion to an increasing number of panicles m^{-2} (Chastain and Grabe, 1989). However, the effects of spikelets $panicle^{-1}$ and florets $spikelet^{-1}$ seed yield components on harvested seed yield is not known in orchardgrass.

Only a small portion of the seed yield potential leading to seed number in cool-season grass seed crops is captured at the time of seed harvest (Chastain and Young, 1998; Abel et al., 2017). Much of this loss in potential seed yield is attributable to failures in pollination, as well as reduction in seed set and abortion of seed during the

seed growth phase of seed development. The environment during seed development has an impact on seed yield as precipitation during this period can reduce seed set, and as a result, seed number is adversely affected (Abel and Boelt, 2018). Seed set can also be affected by seed position within the spikelet as seed set was lower when florets were located in the distal portion of the spikelet than when positioned in the central or proximal locations (Chastain et al., 2014b). Abortion of developing seeds is common in cool-season perennial grasses and has often been attributed to lack of sufficient photoassimilates during the seed growth phase of development (Elgersma, 1990).

Seed shattering is the loss of harvestable seed where seed falls to the ground before full harvest maturity. Seed shattering in cool-season grasses starts at physiological maturity and increases from that point forward in seed development (Piccirilli and Falcinelli, 1989; Andrade et al., 1994). Andrade et al. (1994) compared moisture content loss and seed shattering between different tall fescue cultivars and season years. Maximum yields were higher when the crop was windrowed at 350 to 410 g kg^{-1} seed moisture content. Subsequently, seed shattering losses further reduces seed yield during the seed maturation phase of seed development. This is a well-known, but unresolved problem in orchardgrass despite some efforts to breed for resistance to seed shattering losses (Piccirilli and Falcinelli, 1989). Like other grass seed crops, Piccirilli and Falcinelli (1989) ascertained that seed shattering in orchardgrass is determined by the presence of abscission layers. The abscission process is divided into chemical and mechanical categories (Fahn and Werker, 1972). Two cellular layers across the pedicel and the rachilla were distinguished during boot stage (Piccirilli and Falcinelli, 1989). The primary layer under the floret causes the detachment of a single seed, while the secondary

layer under the pedicel causes the whole spikelet to fall. The formation of the primary abscission layer was recorded earlier in orchardgrass cultivar Hallmark compared to Marta. The two abscission processes were observed in the primary abscission layer where cell lyses occurs, while the disarticulation of the caryopsis by the secondary layer is rarely observed. To minimize seed yield losses from shattering, orchardgrass seed crops are harvested by windrower at 420 to 460 g kg^{-1} seed moisture content rather than at low seed moisture content (Silberstein et al., 2010).

Seed weight in cool-season grasses is affected by the length of seed growth period and by the rate of growth over that period. Seed position within the spikelet has a major impact on seed weight in cool-season grasses (Anslow, 1964; Warringa et al., 1998; Chastain et al., 2014b). The seed growth period has been found to be shorter for distal seed than for central or proximal seed within the spikelet. Seed weight was incrementally increased acropetally from the proximal to the distal locations of the spikelet (Warringa et al., 1998; Chastain et al., 2014b). Seed weight was consistently greater in the medial region of the spike and was lower in the apical and basal regions. No work has been done on the relationship of seed position and seed weight or seed set in the cool-season grasses in species such as orchardgrass that has panicles rather than spikes.

To date, no studies have investigated the seed development process in orchardgrass seed crops. Moreover, there is no information available on the effects of management practices such as plant growth regulators and nitrogen on seed development and the manifestation of these practices on seed yield in orchardgrass.

1.5. Plant Growth Regulators

Under conditions of high soil water and N availability – common in the Willamette Valley – the culm (reproductive stem) cannot support the increasing weight of the panicle and developing seed. As a result, the culm together with the panicle that it supports lodges or falls to the ground under its own weight. Lodging reduces seed yield in cool-season grasses (Hebblethwaite et al., 1978).

Stem elongation in grasses that leads to lodging is promoted by gibberellic acid (GA), but this elongation can be counteracted by the application of specific plant growth regulators (Rademacher, 2015). Plant growth regulators (PGRs) are chemical compounds that affect metabolic processes in plants. True PGRs alter the balance of plant hormones by directly inhibiting the biosynthesis and translocation of plant hormones. Atypical PGRs are phytotoxins that inhibit hormonal systems indirectly. GAs are plant hormones involved in fruit development, elongation, bolting in long-day plants, and induction of seed germination seed.

There are three different groups of GA-inhibiting PGRs (Rademacher, 2015). The first group of GA-inhibiting PGRs such as chlormequat chloride and mepiquat chloride block GA metabolism by inhibiting cyclase activities in the early stages of GA biosynthesis. The second group inhibit cytochrome P450-dependent monoxygenases which serve as a catalyst of ent-kaurene oxidation into GA12-aldehyde. Triazoles, such as paclobutrazol and uniconazole-P, and pyrimidines, such as ancymidol and flurprimidol, are examples of the second group. The third group inhibits dioxygenase

reaction by blocking 2-oxoglutaric acid which includes daminozide, prohexadione-calcium, and trinexapac-ethyl. Trinexapac-ethyl [4-(cyclopropyl- α-hydroxymethylene)-3, 5-dioxo-cyclohexanecarboxylic acid ethylester] is a lodging control agent that inhibits GA biosynthesis because of the structural similarity to 2-oxoglutaric acid, a co-substrate in dioxygenase catalysis in the late stages of GA biosynthesis (King et al., 2004). Trinexapac-ethyl (TE) has been widely adopted for use as a plant growth regulator (PGR) for lodging control and seed yield-enhancing tool in cool-season forage and turf grass seed production. (Zapiola et al., 2006; Borm and van den Berg, 2008; Chastain et al., 2014a; Chastain et al., 2015).

Studies from around the globe have reported large seed yield increases from the use of TE in grass seed crops. Ryegrass (*Lolium* spp.) and tall fescue seed yield increased by 50% and 67% on average with TE applications at BBCH 32 (stem elongation) due to increase of floret site utilizations (Chastain et al. 2003; Rolston et al., 2004). Chynoweth et al. (2010) found that higher seed yields in perennial ryegrass were achieved with a TE rate of 400 g a.i. ha^{-1} and followed by a decline in yield with increased rate. More studies show that seed yield has been increased up to 38% in strong creeping red fescue (*Festuca rubra* L. subsp. *rubra*) (Zapiola et al., 2006; Zapiola et al. 2014), up to 40% in tall fescue (Chastain et al., 2014a; Chastain et al., 2015) and up to 45% in perennial ryegrass (Borm and van den Berg, 2008, Rolston et al., 2010; Chastain et al., 2014b). These studies have found that these high and consistent increases in seed yield resulting from TE applications have for the most part resulted from an increase in seed number with little or no effect on seed weight. Chynoweth and Moot (2017) reported that seed growth in perennial ryegrass over the seed development period followed a sigmoidal pattern over

three rates of TE PGR. Seed yield increased up to 28% with 200 g a.i. TE ha^{-1}. TE application at 400 g a.i. ha^{-1} increased seed growth rate with a mean slope in the linear phase of 0.450 mg per $^{\circ}C$ day which is faster than the control and the 200 g a.i. ha^{-1} TE application. No other work on the effects of TE PGR on seed development have been reported.

One previous study has examined the effects of TE PGR on seed yield in orchardgrass (Rolston et al., 2014). This work conducted in New Zealand indicates that applications of TE PGR increased seed yield in orchardgrass by up to 37% with TE application rate between 100 to 300 g a.i. ha^{-1}. This study did not provide insights into the underlying mechanism for the TE-induced seed yield increases in orchardgrass.

1.6 Spring Nitrogen

Rampton and Jackson (1969) reported that the best results for nitrogen applications in terms of seed yield were observed when the nitrogen was placed on the field in spring (March) over fall applications (October) or split between fall and spring. As a result, application of spring nitrogen is one of the most important management practices for production of orchardgrass seed crops (Doerge et al., 2000). The purpose of the spring N application is to support the nutritional needs that arise during rapid growth in biomass of the crop starting with stem elongation through flowering and seed development. This in turn, is essential for producing higher seed yields in orchardgrass.

In a five-year field trial with spring N applications, Rampton and Jackson (1969) showed that orchardgrass seed yield was increased incrementally from no N to 90 kg N ha^{-1}, but was not further improved with N applications beyond that rate up to 179 kg N

ha^{-1}. By contrast, Young et al. (1999) found that spring applications of 120 kg N ha^{-1} produced optimum results for seed yield in orchardgrass. Higher rates of spring N application did not increase seed yield over the 120 kg N ha^{-1} rate, but 90 kg N ha^{-1} produced lower seed yields.

Orchardgrass seed yields were increased with spring N because of increases in seed weight according to Rampton and Jackson (1969). Conversely, Young et al. (1999) indicated that the primary reasons for increased seed yield with spring N in orchardgrass were greater seed number and harvest index at 120 kg N ha^{-1} than at 90 kg N ha^{-1}. Another reason was increased numbers of fertile tillers with spring N application at double ridge stage (BBCH 29) (Young et al., 1999). Seed yield was higher when spring N was applied at the double ridge stage of development (about 600 GDD, 5°C base) than later during spikelet initiation. The underlying mechanisms for the seed yield increase effect of spring N in orchardgrass is not clear.

Chapter 2. Seed Development in Field-grown Orchardgrass (*Dactylis glomerata* L.)

Abstract

Orchardgrass (*Dactylis glomerata* L.) is an important grass seed crop, but little is known about the seed development process and its contribution to the most important seed yield components: seed number and seed weight. Trinexpac-ethyl (TE) plant growth regulator (PGR) and spring nitrogen (N) application have been found to increase seed yield in orchardgrass through its effect on increasing seed number. Field trials were conducted in 2018 and 2019 at Hyslop Experimental Farm, Corvallis, OR to investigate seed development in orchardgrass and the effects of four treatments on this process: control, TE (210 g ai ha^{-1}), N (spring N applied at 112 kg ha^{-1}), and TE + N. Regression analyses were used to aid in understanding the development of seed in three spikelet seed positions and the effects of treatments. In 2018, seed weight increased over GDD in bi-phasic segmented pattern in seed from distal and central spikelets but increases were linear in seed from proximal spikelets. In 2019, seed weight increase in proximal spikelets followed a bi-phasic segmented function, while seed weight increase in central spikelets segmented except for TE treatment. Seed growth rate (SGR) varied among spikelet positions and ranged from 0.22 to 0.34 mg GDD^{-1} per 100 seed. The SGR varied among treatments and ranged from 0.31 to 0.47 mg GDD^{-1} per 100 seed. Seed filling duration (SFD) varied among spikelet positions and treatment. Seed moisture content declined over seed development in a linear manner in 2018, and the decrease in seed moisture followed mixed linear and segmented models in 2019. The TE + N treatment had both the shortest SFD and one of the smallest SGR values among treatments, and as a result tended to have low seed weight. The TE + N treatment produced high seed yields and seed number so either seed abortion or seed shattering loss was reduced, or both.

2.1 Introduction

Orchardgrass (*Dactylis glomerata* L.) is one of the most important seed crops in Oregon. Orchardgrass seed production is focused in Oregon where 98% of the national seed production takes place. Seed yields of orchardgrass have not increased appreciably over time unlike other cool-season grasses and to date, no studies have investigated the seed development process in orchardgrass seed crops. Moreover, there is no information available on the effects of management practices such as plant growth regulators (PGR) and spring N on seed development and the manifestation of these practices on seed yield in orchardgrass.

Seed development begins in the floret at double fertilization and ends with the mature seed. Seed development in cool-season perennial grasses has a two-phase pattern: seed growth and seed maturation (Grabe, 1956; Anslow, 1964). During the seed growth phase, seed weight increases as a result of starch reserve accumulation in the endosperm and growth of the embryo. Seed moisture content declines slightly. When the seed reaches maximum dry weight, it has attained the state of development known as physiological maturity. During seed maturation, seed moisture content declines rapidly but seed weight does not change appreciably.

The seed development process affects seed yield of cool-season perennial grasses. Seed yield is primarily influenced by two components: seed number and seed weight (Chastain and Young, 1998). The relationship of seed yield to these components of yield can be expressed in the following manner:

Seed yield = Seed number x Seed weight

The seed number component is determined by establishment of yield potential (manifested at anthesis) and by the realization of that potential at the time of seed harvest (Chastain and Young, 1998; Abel et al., 2017). Seed yield potential is an expression of seed yield components including panicles m^{-2}, spikelets $panicle^{-1}$, and florets $spikelet^{-1}$ found in the crop at anthesis. This expression of seed yield potential is affected by the production practices and cumulative effect of the production environment leading up to that point in crop development. Seed yield in orchardgrass was found to increase in proportion to an increasing number of panicles m^{-2} (Chastain and Grabe, 1989). However, the effects of spikelets $panicle^{-1}$ and florets $spikelet^{-1}$ seed yield components on harvested seed yield is not known in orchardgrass.

Only a small portion of the seed yield potential leading to seed number in cool-season grass seed crops is captured at the time of seed harvest (Chastain and Young, 1998; Abel et al., 2017). Much of this loss in potential seed yield is attributable to failures in pollination, as well as reduction in seed set, seed shattering, and abortion of seed during the seed growth phase of seed development. The environment during seed development has an impact on seed yield as precipitation during this period can reduce seed set, and as a result, seed number is adversely affected (Abel and Boelt, 2018). Seed set can also be affected by seed position within the spikelet as seed set was lower when florets were located in the distal portion of the spikelet than when positioned in the central or proximal locations (Chastain et al., 2014b). Abortion of developing seeds is common in cool-season perennial grasses and has often been attributed to lack of sufficient photoassimilates during the seed growth phase of development (Elgersma, 1990).

Seed weight is affected by the length of the seed growth period (seed filling duration = SFD) and by the seed growth rate (SGR) over that period (Egli, 1998). Seed position within the spikelet has a major impact on seed weight in cool-season grasses (Anslow, 1964; Warringa et al., 1998; Chastain et al., 2014b). The seed growth period has been found to be shorter for distal seed than for central or proximal seed within the spikelet. Seed weight was incrementally increased acropetally from the proximal to the distal locations of the spikelet (Warringa et al., 1998; Chastain et al., 2014b). Seed weight was consistently greater in the medial region of the spike and was lower in the apical and basal regions. No work has been done to date on the relationship of seed position and seed weight or seed set in the cool-season grasses in species such as orchardgrass that has panicles rather than spikes as inflorescences.

Relationships of the growth in seed weight over time (or GDD) during seed development in cool-season grasses have either not been fitted to models (Grabe, 1956; Anslow, 1964), or have been fitted to Gompertz (Warringa et al., 1998) or logistic models (Chynoweth and Moot, 2017). Examination of this published data suggests that piecewise (segmented) regression models might more realistically capture the relationship of growth in seed weight over the course of seed development. Similarly, the relationship of seed moisture content (ordinate) over the seed development process can be fitted to piecewise regression models against time (Warringa et al., 1998) or GDD (Andrade et al., 1994) on the abscissa.

The objectives of this study were to investigate seed development process in orchardgrass under field conditions and ascertain the effects of spring N application and

PGR treatments on floret and seed weight, seed growth (SGR and SFD), seed moisture content, and progress toward physiological maturity in orchardgrass.

2.2 Materials and Methods

2.2.1 Experimental Design

Field trials were inducted in two crop seasons (2018 and 2019) at Oregon State University's Hyslop Experimental Farm (44° 40' N, 123° 11' 36'' W) near Corvallis, Oregon. The soil type at the research site is a Woodburn silt loam (fine-silty, mixed, superactive, mesic, Aquultic Argixeroll). 'Persist' orchardgrass was planted on 7 October 2015 at a seeding rate of 8 kg ha^{-1} in a 60-cm row spacing. The late fall planting meant that the first seed harvest of the crop was delayed to 2017. Development stages of orchardgrass in relation to management practices, experimental treatments, and the timing of sampling were characterized by using the BBCH scale (Lancashire et al., 1991). Growing degree days (GDD) were used to mark the progress of the crop toward seed development and maturity and were calculated from 1 September of each crop year and were based on air temperatures observed at Hyslop Farm. The base temperature used in the GDD calculations was 5°C.

The experimental design was a split-plot, randomized block design with three replications (Chapter 3). Main plots (11.6 m x 13.4 m) were spring-applied N and subplots (3.4 m x 11.6 m) were PGR treatments. Four treatments were chosen for the seed development study from the treatments listed in Chapter 3. These treatments were: control (no N, no TE), TE (210 g ai ha^{-1} TE), N (spring N at 112 kg N ha^{-1}), and TE + N (both TE and N). Fall N was applied to all plots in October of each year as dry ammonium phosphate-sulfate at 45 kg N ha^{-1} (Doerge et al., 2000). Spring N treatments consisted of dry granular urea (46-0-0), applied prior to stem elongation (BBCH 29; mean = 791 GDD). A tractor-mounted orbit-air spreader system was used to make spring

N applications while untreated N control plots were driven on by the tractor and spreader system without application of N.

Applications of trinexpac-ethyl PGR were made at the 2-node stage of early stem elongation (BBCH 32; mean = 858 GDD. The PGR was applied at walking speed by using a bicycle-type boom sprayer operated at 138 kPa with XR Teejet 8003VS nozzles. The spray volume used in the PGR application was 194 L ha^{-1}. All other management of the experimental plots were based on common practices for commercial orchardgrass seed production in the Willamette Valley of Oregon (Doerge et al., 2000).

2.2.2 Sampling and Measurement of Floret and Seed Characteristics

Five panicles were collected periodically from random locations within each plot starting at BBCH 65 (peak anthesis) and continuing until BBCH 85 (seed harvest) in two growing seasons – 2018 and 2019. The panicle samples were immediately transported to the lab from the field for determination of floret number, seed number, floret weight, seed weight, and seed moisture content. Seed and florets were obtained from distal, central, and proximal positions within spikelets located in the apical, medial, and basal region of the panicle in 2018 and from only the medial position in 2019. The number of spikelets in the panicle were counted and recorded.

Seeds were differentiated from unfertilized florets or those ovules that aborted development shortly after fertilization by microscopic examination (Chastain et al., 2014b). The number of florets and seeds present in each spikelet sample was counted and recorded, and the percentage of florets setting seed was expressed as seed set. In 2018, 90 seed samples were collected from each of the three spikelet positions (distal,

central, and proximal) and each of the three different panicle positions (apical, medial, and basal). One treatment was sampled on each sampling day. In 2019, 120 seed samples were collected on each sampling day from each of the three spikelet positions (distal, central, and proximal) from each of the four treatments.

The dry weight of florets and seeds were determined after oven drying. Seed and florets were placed in a metal sample container and weighed prior to oven-drying. The sample containers were placed in a laboratory air-oven and dried at 130°C for 2 hours. After drying was complete, the cover was placed on each sample container. The container was cooled to room temperature prior to weighing in a desiccant-containing chamber. Seed moisture content was calculated from the weights measured prior to and after drying and expressed as a percentage (on a wet weight basis). The seed-filling duration (SFD) was determined by subtracting GDD at pollination (start point of seed filling) from GDD at maximum weight.

2.2.3 Caryopsis Weight

The caryopsis is the single-seeded indehiscent fruit characteristic of the grasses. The “seed” of grasses is composed of the caryopsis and the parts of the floret including the lemma, palea, and rachilla. The weight of the caryopsis was determined by subtracting the average floret weight from the maximum seed weight. Maximum seed weight values were obtained from the estimated plateau from the segmented model using PROC NLIN (SAS 9.4, SAS institute). Caryopsis weight was determined in 2019 for the three spikelet positions.

2.2.4 Data Analysis

To describe seed development processes in orchardgrass, regression analysis was conducted to ascertain the nature of relationships of seed weight and seed moisture content over the course of seed development. The relationship of seed growth (weight) throughout the seed development process is often graphically represented with seed weight plotted on the ordinate and time plotted on the abscissa. Instead of time, the more biologically relevant GDD is a better replacement on the abscissa (Chynoweth and Moot, 2017). Thus, GDD (base 5°C) was used in curve-fitting as the abscissa for both seed weight and seed moisture content relationships.

Regression analysis was conducted to describe spring N and TE effects on seed growth and development using PROC GLM and PROC NLIN in SAS (SAS 9.4, SAS institute). A segmented model was chosen if data fit a linear or a quadratic function as a first segment and then a plateau as a second segment (Appendix A, B). When using PROC GLM, the data were fitted to linear and quadratic models and compared for goodness of fit. If the quadratic term was significant, then data were fitted into a segmented model where a quadratic function was the first segment and a plateau is a second segment. If the quadratic term was not significant, the data were fitted into a segmented model where a linear function was the first segment and a plateau would be the second segment. Initial parameters used in the segmented model were chosen from the quadratic functions found in PROC GLM results. The data analyses were conducted for each year and not combined over years.

Regression analysis was performed on the compiled seed samples from all treatments and panicle positions and seed moisture content from the 2018 season and seed growth was compared among spikelet positions. The analysis was done as preliminary regression to examine the nature of the relationship of seed growth rate and moisture content against GDD. For 2019, the four treatments were compared using regression analysis for differences in maximum seed weights, GDD date of reaching maximum weight, and seed growth rate. Seed moisture content decrease rate was determined using the same regression analysis method for the three different spikelet positions and four treatments.

Seed weight and seed moisture content relationships with GDD over the course of seed development were fit with logistic, Gompertz, segmented piecewise, linear, and curvilinear models. These procedures were used to test relationships of seed weight and seed moisture content with GDD over the course of seed development. The goodness of fit of models with observed data were determined using the coefficient of determination and the best model were used.

2.3 Results

2.3.1 Seed Growth Rate

The environmental conditions during the period of seed development (May and June) in 2018 and 2019 were outlined in Chapter 3. The weather was warmer than the long-term averages in each of the two years at the field site so the accumulation of GDD were accelerated over the seed development period. Precipitation was below average in May 2018 during the early part of seed development but was otherwise at or above the average for the site.

Function parameters for SGRs in the 2018 growing season were estimated and functions were described using PROC GLM and PROC NLIN (Table 2.1; Figure 2.1). The weight of seed in proximal spikelet positions increased ($P < 0.0001$) over the accumulation of GDD and followed a linear function due to lack of convergence with PROC NLIN. The SGR in proximal positions of the spikelet was 0.21 mg GDD^{-1} per 100 seed (2.1 μg GDD^{-1}) ($P < 0.0001$). In contrast, the seed weight in central spikelets increased ($P < 0.0001$) but development of the seed followed a segmented model. The SGR for seed in the central spikelet positions was 0.23 mg GDD^{-1} per 100 seed (2.3 μg GDD^{-1}) where the seed growth ends at 1401 GDD ($P < 0.0001$). The peak seed weight for seed in central spikelets was 0.77 mg per seed ($P < 0.0001$). Seed growth in distal spikelet positions was not statistically significant ($P > 0.05$) over GDD; however, the data was fitted to a segmented model. The SGR for seed in distal positions was 0.34 mg GDD^{-1} per 100 seed (3.4 μg GDD^{-1}) with a peak seed weight at 0.72 mg per seed after 1417 GDD.

In 2019, there were insufficient numbers of seed that were found in the distal spikelet positions, thus only seed from central and proximal spikelet positions were examined and included in the data analysis. Seed weight from proximal positions increased ($P < 0.0001$) and followed a segmented model for all four treatments tested ($R^2 > 0.90$) (Figure 2.3). The SGR and maximum seed weights for seeds in proximal spikelet positions can be found in Table 2.3 and Table 2.7. With the control treatment, SGR was 0.36 mg GDD^{-1} per 100 seed (3.6 μg GDD^{-1}) in proximal spikelet positions. Seed filling ended at 1483 GDD in the control treatment with a maximum weight of 1.22 mg per seed in proximal spikelet positions. The seed filling period with the control treatment started earlier at 1225 GDD when compared to other treatments where seed-filling started at 1246 GDD. Maximum seed weights for seed in proximal spikelet positions were recorded at 1493 and 1498 GDD for the N and the TE treatments, respectively (Figure 2.3). The SGR was higher with seed in the TE treatment (0.36 mg GDD^{-1} per 100 seed) (3.6 μg GDD^{-1}) than with the N treatment (0.34 mg GDD^{-1} per 100 seed) (3.4 μg GDD^{-1}). With combined application of TE + N, SGR was 0.31 mg GDD^{-1} per 100 seed (3.1 μg GDD^{-1}) and the maximum seed weight was reached at 1453 GDD.

The weight of seed developing in central spikelet positions increased ($P < 0.01$) for all treatments, following a segmented model with the exception for seed with the TE treatment where linear growth in seed weight was observed (Table 2.5; Figure 2.5). The SGR for seed in the central spikelet positions was 0.47 mg GDD^{-1} per 100 seed (4.7 μg GDD^{-1}) with the control treatment. In the control treatment, maximum seed weight for central spikelet seed was 0.99 mg at 1420 GDD. Unlike other treatments, seed growth of the TE treatment followed a linear function without a plateau and a maximum seed

weight in the central spikelet positions. The SGR for seed in the central spikelets for the TE treatment was 0.32 mg GDD^{-1} per 100 seed (3.2 μg GDD^{-1}). The N treatment had a SGR of 0.33 mg GDD^{-1} per 100 seed (3.3 μg GDD^{-1}) in the central spikelet positions. The maximum seed weight for the N treatment was 1.08 mg and was observed at 1471 GDD in the central spikelet positions. The combination of TE + N produced a SGR of 0.31 mg GDD^{-1} per 100 seed (3.1 μg GDD^{-1}) with a maximum seed weight of 0.98 mg at 1460 GDD.

2.3.2 Seed Moisture Content

Seed moisture content decreased in all spikelet positions ($P < 0.05$) and followed a linear function in 2018 (Table 2.2; Figure 2.2). The seed moisture content of seed in proximal spikelet positions decreased at the rate of -0.75 mg g^{-1} GDD^{-1} per 100 seed (-7.5 μg g^{-1} GDD^{-1}). In central spikelet positions, seed moisture content decreased by -0.88 mg g^{-1} GDD^{-1} per 100 seed (-8.5 μg g^{-1} GDD^{-1}). The greatest rate of decline in seed moisture content was observed in distal seed positions at -2.22 mg g^{-1} GDD^{-1} per 100 seed (-22.2 μg g^{-1} GDD^{-1}).

In 2019, the treatments differed in their effects on seed moisture content of seed in proximal positions within the spikelet over the course of seed development (Table 2.4; Figure 2.4). There was no significant relationship of seed moisture content and GDD with the TE treatment in seeds sampled from the proximal position. The numerical loss in seed moisture content over the course of seed development with the TE treatment -0.28 mg g^{-1} GDD^{-1} per 100 seed (-2.8 μg g^{-1} GDD^{-1}) ($P = 0.13$). The other three treatments followed a segmented model ($P < 0.05$) where seed moisture content remained constant

initially in the early portion of seed development but then declined without further interruption once a certain GDD value was reached (Table 2.4; Figure 2.4) . With the control treatment, seed moisture content in proximal spikelet positions decreased -1.48 mg g^{-1} GDD^{-1} per 100 seed (-14.8 μg g^{-1} GDD^{-1}) after 1397 GDD. Seed moisture content in the N treatment decreased -1.19 mg g^{-1} GDD^{-1} per 100 seed (-11.9 μg g^{-1} GDD^{-1}) starting at 1371 GDD. The TE + N treatment caused seed moisture content to decrease in proximal spikelet positions starting at 1315 GDD at a rate of -0.64 mg g^{-1} GDD^{-1} per 100 seed (-6.4 μg g^{-1} GDD^{-1}).

The relationship of seed moisture content of seed in central spikelet positions and GDD over seed development was significant in 2019 for all treatments (Table 2.6; Figure 2.6). All treatments followed a segmented model ($P < 0.01$) except for the TE + N treatment where a linear model was followed. The seed moisture content in the TE + N treatment decreased -0.64 mg g^{-1} GDD^{-1} per 100 seed (-6.4 μg g^{-1} GDD^{-1}). With the control treatment, seed moisture content declined in central positions starting at 1397 GDD at a rate of -0.90 mg g^{-1} GDD^{-1} per 100 seed (-9.0 μg g^{-1} GDD^{-1}). Seed moisture content in the N treatment decreased at -0.78 mg g^{-1} GDD^{-1} per 100 seed (-7.8 μg g^{-1} GDD^{-1}) starting at 1353 GDD. Seed moisture content in the TE treatment decreased starting at 1352 GDD at a rate of -0.58 mg g^{-1} GDD^{-1} per 100 seed (-5.8 μg g^{-1} GDD^{-1}) in the central spikelet positions. The initial seed moisture content values (plateau) ranged from 468 to 448 mg g^{-1} except for TE + N treated plots where seed moisture content started at 501 mg g^{-1} and experienced linear reductions in seed moisture thereafter.

2.3.3 Seed Filling Duration

Seed filling duration (SFD) is the period that begins with growth of the seed and ends at physiological maturity. In 2018, SFD differed among seed based on their position within the spikelet (Figure 2.1). The SFD for seed growing in distal and central spikelet positions was 139 and 122 GDD, respectively. No SFD was determined for seed in the proximal positon since seed did not reach maximum weight during the sampling period.

Seed filling duration was different between spikelet positions and among the four treatments in 2019 (Figure 2.3). Overall, the SFD was greater in proximal seed (Table 2.7) than in central seed (Table 2.8). The SFD for distal seed was not determined. For seed in the proximal spikelet position, SFD was the position-longest 259 GDD for the control treatment. This was followed by similar SFD values of 253 GDD for the TE treatment and 248 GDD for the N treatment. The combined spring N and TE treatment had the shortest SFD at 207 GDD among the four treatments in the study.

For seed in the central spikelet position, the variation in SFD among treatments was smaller than in the proximal position. No SFD was determined for the TE treatment in the central position seed. Among the other three treatments, SFD was the highest at 185 GDD with the spring N treatment, while SFD was 173 GDD with the combined application of TE and spring N.

2.3.4 Seed Set and Caryopsis Weight

The percentage of seed set under field conditions in orchardgrass after anthesis and over the course of the seed development period in 2019 is shown in Figure 2.7. The first visible indication of seed forming was evident at 1173 GDD in the control and N

treatments. The initial seed set in TE or TE + N treatments was not observed until 1245 GDD. The maximum seed set for all treatments took place between 1335 and 1466 GDD. The seed set was observed to be somewhat lower for all treatments after this maximum period.

The weight of the caryopsis was calculated for seeds from central and proximal spikelet positions in 2019 (Table 2.7; Table 2.8). To do this, maximum seed weight values were obtained from the plateau in the segmented model function except for the central spikelet position seed with the TE treatment where the final seed weight measurement was used. Seed weights from TE + N and control treatments tended to be lower than TE or N treatments. Floret weight was lowest in the control treatment. Due to higher floret weights in the TE + N treatment, caryopsis weights were similar between TE, N, and control treatments. Caryopsis, floret weight, and seed weight were lower with the TE + N treatment.

2.4 Discussion

The results from 2018 showed lower model fitness (R^2 <0.80) compared to 2019 because the 2018 data were pooled over the four different treatments and four panicle positions. The results show that the three spikelet positions in 2018 fit a segmented model where there are two phases, linear growth and a plateau, except for the proximal seed. Low production of seed in distal positions precluded examination of data for that spikelet position in 2019. However, seed growth in all treatments but one followed a segmented model for the both proximal and distal spikelet positions in 2019. The variation in response could have been due to the study being conducted in the field unlike the greenhouse-grown seed with clonal materials used in the Warringa et al. (1998) study. Moreover, the high natural variability of orchardgrass might be a factor in some of the inconsistent results in the study (Stratton and Ohm, 1989).

Warringa et al. (1998) fitted seed growth in perennial ryegrass (*Lolium perenne* L.) seed from three spikelet positions to a Gompertz model despite having no data to support an initial lag, followed by linear increase as expected in this model. Chynoweth and Moot (2017) found that "seed growth" in three cultivars of field-grown perennial ryegrass fit a logistic model. This result is likely confounded by seed number as "seed growth" in their study was defined as the change in total seed mass over GDD on a per stem basis. In other words, seed growth in this case was not the change in mass of a fixed number of seeds over GDD since the treatments that they employed could have and likely did increase the number of seeds per spike (Chastain et al., 2014b). The segmented pattern of growth in seed weight over GDD observed in this study for orchardgrass was similar to that found in diverse genotypes in maize (*Zea mays* L.) (Borras et al., 2009).

Yang et al. (2010) also fitted sorghum [*Sorghum bicolor* (L.) Moench] seed growth to a segmented piecewise model but used a four-phase model rather than a two-phase model that was employed in this study for orchardgrass.

Chynoweth and Moot (2017) reported that growth in overall seed mass produced on a stem basis in perennial ryegrass was increased consistently by 400 g TE ha^{-1} but in only two of three cultivars with the 200 g TE ha^{-1} rate. The latter rate is close to the 210 g TE ha^{-1} rate used in this study. Since the number of seeds or individual seed weight was not reported there is no way to know whether these TE applications affected individual seed weight, seed number, or both in increasing seed mass (yield per stem) over GDD through seed development.

The SGR for the field-grown orchardgrass seed in this study varied with year, position in the spikelet, and treatment (Table 2.1; Table 2.3; Table 2.5). The SGR ranged from a low of 0.21 mg GDD^{-1} per 100 seed to 0.47 mg GDD^{-1} per 100 seed. To compare with the published values of SGR and SFD by Warringa et al. (1998) in greenhouse-grown perennial ryegrass seed, the values from that study were converted to GDD (Base 5 C°) from days. The SGR values for orchardgrass seed were similar to the converted SGR values for perennial ryegrass seed and ranged from 0.16 mg GDD^{-1} per 100 seed to 0.31 mg GDD^{-1} per 100 seed. The SFD in orchardgrass seed also varied among years, spikelet position, and treatment with the low SFD value at 122 GDD and the high end of the range at 259 GDD. By contrast, the SFD in perennial ryegrass seed is much greater than in orchardgrass seed with SFD values ranging from 308 GDD to 434 GDD. Perennial ryegrass seed are heavier than orchardgrass seed so it is not surprising to find that perennial ryegrass has much longer SFD since the SGR values were similar. Both

species appear to fill seed at the same rate (SGR) but the filling period (SFD) is longer in perennial ryegrass to reach the heavier final seed weight. There are no published values available for SGR or SFD in orchardgrass seed.

The SFD differed among seed of the three spikelet positions in 2018 (Figure 2.1), where proximal seed had the longest growth duration and shorter SFD for central and distal positions. Similar results were reported in perennial ryegrass where the shortest SFD was in distal seed and longest SFD was in proximal seed (Warringa et al., 1998). However, the SFD for orchardgrass seed in distal positions were longer than the SFD for central seed. This observation in 2018 may have been the result of the highly variable and small number of collected distal seed. Nevertheless, maximum seed weights are higher in proximal and lowest in distal seed. In 2019, the SFD for proximal seed was greater than for central seed (Table 2.7; Table 2.8).

In 2019, SGR and SFD varied among the four treatments (Table 2.3; Table 2.5). The control treatment had one of the highest SGRs in both spikelet positions, but this was most evident in the central spikelet position. Both TE and N treatments produced low SGR values in the central spikelet positions. The SGR was consistently the lowest in TE + N treatments in both spikelet positions. Since stronger effects of the TE, N, and TE + N treatments on SGR were notable in the central spikelets this might mean that the seeds in this position are more sensitive to these treatments. The shortest SFDs were observed in the TE + N treatments in both spikelet positions. Egli (1998) reported that SGR and SFD were not generally affected by manipulation of N supply in many species including the cereals. Thus, it is more likely that any effects on SGR and SFD in orchardgrass were

the result of TE-induced effects rather than N. No other study has examined the effects of TE PGR on SGR or SFD in the grass family.

The treatments had no consistent effect on the attainment of physiological maturity in orchardgrass seed in either spikelet position examined (Figure 2.3; Figure 2.5). For proximal spikelets, the attainment of physiological maturity ranged from 1453 to 1498 GDD, and for central spikelets, the range was from 1420 GDD to 1471 GDD. Thus, none of the treatments employed in this study accelerated or delayed seed maturity in orchardgrass as determined by physiological maturity.

The decreases in seed moisture content of orchardgrass seed during seed development was linear in 2018 for all spikelet positions (Figure 2.2). The decrease in seed moisture followed mixed linear and segmented models depending on treatment in 2019 for proximal and central spikelets (Figure 2.4; Figure 2.6). Seed moisture content loss during seed development was fitted to a two-phase segmented model in tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] (Andrade et al., 1994), and to a three-phase segmented model in perennial ryegrass (Warringa et al., 1998). The loss in seed moisture content varied in an inconsistent manner among the four treatments and two spikelet positions (Table 2.4; Table 2.6). In general, seed moisture content loss was greatest in the control treatment for both spikelet positions with N tending to have the next greatest loss in seed moisture among treatments. Treatments with TE tended to have the least loss in seed moisture content. This is important to note since seed moisture content is an important indicator of harvest maturity in orchardgrass and other grass seed crops (Silberstein et al., 2010). No other studies have examined the effects of N or TE PGR on seed moisture content loss during seed maturity.

The maximum seed set values in orchardgrass were found to range from 69% to 92% but no differences were evident among treatments (Figure 2.8). Chastain et al. (2014b) reported that seed set was increased in perennial ryegrass from 69% to 74% with application of TE. Abel and Boelt (2017) found that seed set in perennial ryegrass ranged from 25% to 73% depending on position within the spikelet, and Warringa et al. (1998) reported similar results for perennial ryegrass but the range of seed set was from 53% to 73%. Seed set was observed to be delayed with treatments containing TE until 1245 GDD whereas treatments without TE had seed set start at 1173 GDD. No previous work has reported seed set values for orchardgrass or the effects of TE on seed set in any species.

The weight of the caryopsis was consistently the lowest for both proximal and central seed with the TE + N treatment (Table 2.7; Table 2.8). But the weight of the caryopsis was similar among the other treatments. The late start for seed set with TE treatments and accompanying delay in the beginning of seed filling might have contributed to the lower caryopsis weight with the TE + N treatment. Rampton and Jackson (1969) reported that overall seed weight in orchardgrass was increased by N application as a result of increases in the weight of the caryopsis. They found that the weight of the caryopsis without N was (0.64 mg) and with N was (0.79 mg). The range of caryopsis weights observed in this study were also in this range (0.59 to 0.75 mg).

Interactions of spring N and TE PGR or independent effects of spring N and TE PGR increased seed yield in orchardgrass (Chapter 3). This increased seed yield was most likely attributable to increased seed number. But why was seed number increased by spring N and TE PGR? Much of the failure of cool-season grasses to attain their seed

yield potential has been ascribed to high rates of abortion of set seed (Burbidge, 1978; Elgersma, 1990). Is it possible that the spring N and TE PGR treatments create seed development conditions where many fewer seeds are aborted resulting in increased seed number at the time of seed harvest? The numbers of seed set were high and showed no difference among the four treatments (Figure 2.7). Thus, this increase in seed number must be manifested at some point after the initial setting of seed and prior to the time of seed harvest. The small reduction in seed set values after the maximum seed set was attained might be an indication of early seed abortions taking place.

The SFD for the TE + N treatment was shortest among the four treatments (Table 2.7; Table 2.8). In addition, the SGR for the TE + N treatment was among the lowest observed in the study (Table 2.3; Table 2.5). With a combined low SFD and SGR, it was not surprising that the seed weight tended to be low with TE + N. While seed number was increased by the TE + N treatment (Chapter 3), it is possible that the available photoassimilate source materials to fill those additional 10×10^3 seeds m^{-2} to 30×10^3 seeds m^{-2} that were induced by the TE + N treatment was not concomitantly increased. This potential competition for source materials among this increased numbers of seeds was evident even in the seed formed in proximal spikelets (closest to source materials) where seed weight was low with the TE + N treatment. Egli (1999) found that SGR was reduced when the photoassimilate source was limited by shading in soybean [*Glycine max* (L.) Merr.] when compared to unshaded plants.

On the other hand, the increased number of seeds might not be due to a reduction in abortion of set seed alone. One cannot rule out that the effect of N + TE on increasing seed number is the result of the treatment causing increased seed retention in the panicle,

thereby reducing seed shattering losses. Orchardgrass is especially prone to high losses of seed due to shattering (Piccirilli and Falcinelli, 1989). One possibility is that the TE + N treatment combination increased seed retention by changing the morphology of the spikelet thereby reducing seed shattering. Seed shattering losses were observed to be less with panicles that were treated with TE in this study. There are known effects of TE on the morphology of the grass inflorescence making it more compact (Chastain et al., 2014b) and possibly lessening the potential for shattering losses. Another possibility is that TE affects the abscission zone, causing seed shattering to be reduced in these treatments. Weiser et al. (1979) reported that GA either promoted or had no effect on the abscission zone of spikelets. Since the primary effect of TE is to inhibit the activity of GA (Rademacher, 2015) then it is possible that the increased seed number in orchardgrass at harvest might be at least partly attributable to reduction in shattering losses. The TE PGR appears to increase seed yield in orchardgrass by its effect on increasing seed number but there is more than one possible explanation for this increased seed number and this matter cannot be definitively resolved in this study.

Table 2.1 Function parameters estimated from PROC NLIN to describe seed growth (2018) over growing degree days (x = GDD-1000), where y is the estimate mean for 100-seed weight (mg), B_1 is the rate of 100-seed filling rate (milligram per growing degree day per 100 seed), and j is the breakpoint where maximum seed weight is reached.

	Fitted Curve: If x < j then y = B_o + B_1 x if x ≥ j then y = B_o + B_1 j						
Treatment	**Fitted values and standard errors**						
Spikelet Position	B_o	s.e.	B_1	s.e.	j	s.e.	R^2
Distal	-68.7485	84.8746	0.3366	0.2566	417.2	88.0748	ns
Central	-16.0300	23.4797	0.2323	0.0709	400.6	26.9740	0.329
Proximal	0.6904	6.1652	0.21507	0.01552	-	-	0.750

Table 2.2 Function parameters estimated from PROC NLIN to describe seed moisture content (2018) over growing degree days (x = GDD-1000), where y is the estimate mean for moisture content (mg g^{-1}), B_1 is the rate of seed moisture loss (milligram per gram per growing degree day).

Treatment	**Fitted Curve: $y = B_\circ + B_1 x$**				
	Fitted values and standard errors				
Spikelet Position	B_o	s.e.	B_1	s.e.	R^2
Distal	1322.1	293.4	-2.2195	0.7497	0.687
Central	758.9	48.2671	-0.8750	0.1215	0.448
Proximal	683.5	39.3438	-0.7533	0.0990	0.475

Table 2.3 Function parameters estimated from PROC NLIN to describe proximal seed growth (2019) over growing degree days (x = GDD-1000), where y is the estimate mean for proximal 100-seed weight (mg), B_1 is the rate of 100-seed filling rate (milligram per growing degree day per 100 seed), and j is the breakpoint where maximum seed weight is reached.

		Fitted Curve: If x < j then y = $B_\circ$ + B_1 x if x ≥ j then y = $B_\circ$ + B_1 j						
Treatment		**Fitted values and standard errors**						
TE (g a.i. ha^{-1})	N (kg ha^{-1})	B_o	s.e.	B_1	s.e.	j	s.e.	R^2
0	0	-49.36	16.22	0.3553	0.0497	483.2	27.40	0.97
210	0	-54.77	18.75	0.3597	0.0406	498.4	33.32	0.91
0	112	-37.49	15.09	0.3374	0.0532	493.4	28.25	0.93
210	112	-23.59	17.78	0.3078	0.0505	452.7	27.74	0.91

Table 2.4 Function parameters estimated from PROC NLIN to describe proximal seed moisture content (2019) over growing degree days (x = GDD-1000), where y is the estimate mean for seed moisture content (mg g^{-1}), B_1 is the rate of seed moisture loss (milligram per gram per growing degree day), and j is the breakpoint where seed moisture content starts decreasing.

		Fitted Curve: If x > j then y = B_o + B_1 x if x ≤ j then y = B_o + B_1 j						
Treatment		**Fitted values and standard errors**						
TE (g a.i. ha^{-1})	N (kg ha^{-1})	B_o	s.e.	B_1	s.e.	j	s.e.	R^2
0	0	1050.8	257.5	-1.4794	0.5175	397.2	39.7408	0.847
210	0	518.853	68.652	-0.2772	0.1643	-	-	ns
0	112	909.4	239.2	-1.1902	0.5037	370.9	54.6001	0.642
210	112	675.4	23.1640	-0.6431	0.0519	314.8	16.4896	0.972

Table 2.5 Function parameters estimated from PROC NLIN to describe central seed growth (2019) over growing degree days (x = GDD-1000), where y is the estimate mean for central 100-seed weight (100*mg), B_1 is the rate of 100-seed filling rate (100*milligram per growing degree day), and j is the breakpoint where maximum seed weight is reached.

		Fitted Curve: If $x \leq j$ then $y = B_\circ + B_1 x$ if $x > j$ then $y = B_\circ + B_1 j$						
Treatment		**Fitted values and standard errors**						
TE (g a.i. ha^{-1})	N (kg ha^{-1})	B_o	s.e.	B_1	s.e.	j	s.e.	R^2
0	0	-98.0128	53.8119	0.4692	0.1575	419.9	31.4563	0.867
210	0	-52.5964	18.1534	0.3167	0.0421	593.2		0.890
0	112	-49.0418	23.9267	0.3325	0.0629	471.3	26.4743	0.897
210	112	-44.7303	18.6562	0.3110	0.0517	460.0	21.3056	0.937

Table 2.6 Function parameters estimated from PROC NLIN to describe central seed moisture content (2019) over growing degree days (x = GDD-1000), where y is the estimate mean for seed moisture content (mg g^{-1}), B_1 is the rate of seed moisture loss (milligram per gram per growing degree day), and j is the breakpoint where seed moisture content starts decreasing.

		Fitted Curve: If $x \geq j$ then $y = B_\circ + B_1 x$ if $x < j$ then $y = B_\circ + B_1 j$						
Treatment		**Fitted values and standard errors**						
TE (g a.i. ha^{-1})	N (kg ha^{-1})	B_o	s.e.	B_1	s.e.	j	s.e.	R^2
0	0	808.3	139.3	-0.9011	0.2799	397.2	35.77	0.886
210	0	652.7	27.8984	-0.5817	0.0588	352.0	16.3196	0.977
0	112	743.2	76.2138	-0.7780	0.1714	353.3	32.0211	0.866
210	112	677.9	25.8142	-0.6390	0.0598	277.5	-	0.942

Table 2.7. Caryopsis weight from proximal orchardgrass seed in 2019. Floret weight was averaged. Seed weight values were obtained from the plateau point from PROC NLIN in SAS. Seed-filling duration was calculated by subtracting the pollination date from the date where maximum seed weight was recorded.

Treatment		**Seed Weight**	**Floret Weight**	**Caryopsis Weight**	**Seed Filling Duration**
TE (g a.i. ha^{-1})	N (kg ha^{-1})	(mg)	(mg)	(mg)	(GDD)
0	0	1.223259	0.488622	0.734636	258.57
210	0	1.245038	0.566417	0.678621	252.51
0	112	1.289841	0.580023	0.709818	247.51
210	112	1.157559	0.548333	0.609225	206.81

Table 2.8 Caryopsis weight from central orchardgrass seed in 2019. Floret weight was averaged. Seed weight was taken from the Plateau point from PROC NLIN in SAS. Seed-filling duration was calculated by subtracting the pollination date from the date where maximum seed weight was recorded.

Treatment		**Seed Weight**	**Floret Weight**	**Caryopsis Weight**	**Seed Filling Duration**
TE (g a.i. ha^{-1})	N (kg ha^{-1})	(mg)	(mg)	(mg)	(GDD)
0	0	0.99	0.3640123	0.6259877	179.64
210	0	1.1915951*	0.4376323	0.7539628	N/A
0	112	1.077	0.4068254	0.6701746	184.63
210	112	0.983333	0.3979167	0.5854163	173.33

* Seed maximum weight was not observed and predicted seed weight was calculated from the linear function at the last independent variable reading (last GDD reading) and used.

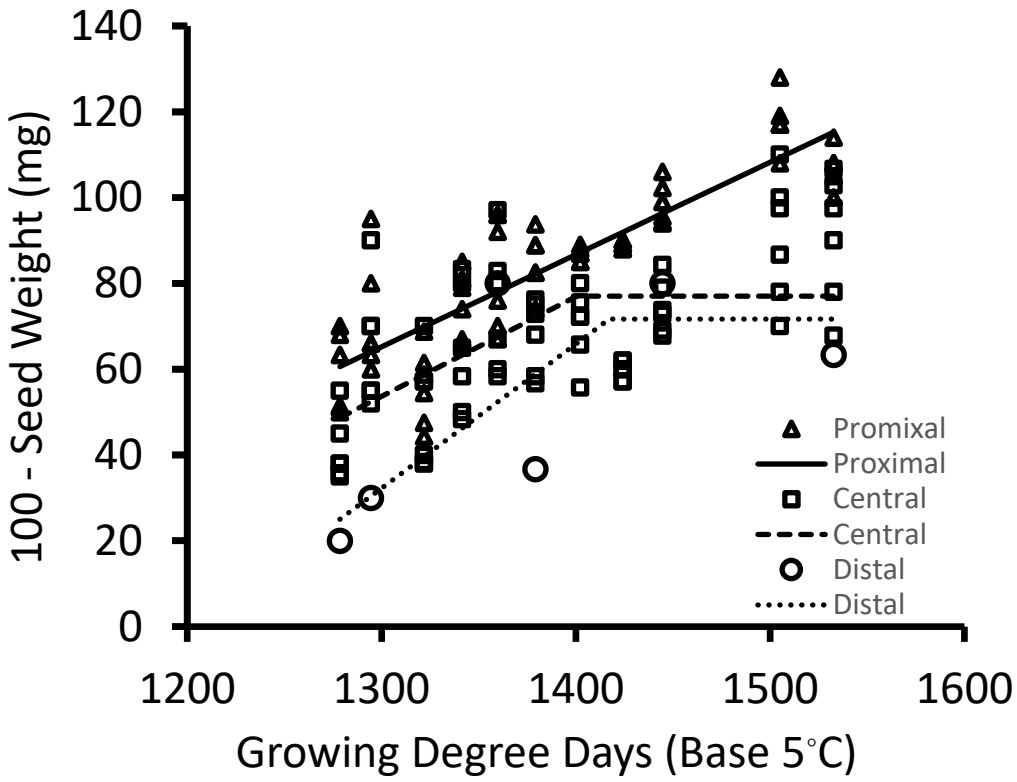

Figure 2.1. Relationship of growing degree days and seed weight over the course of seed development in orchardgrass in 2018. Seed weight is expressed in three spikelet positions (proximal, central, and distal).

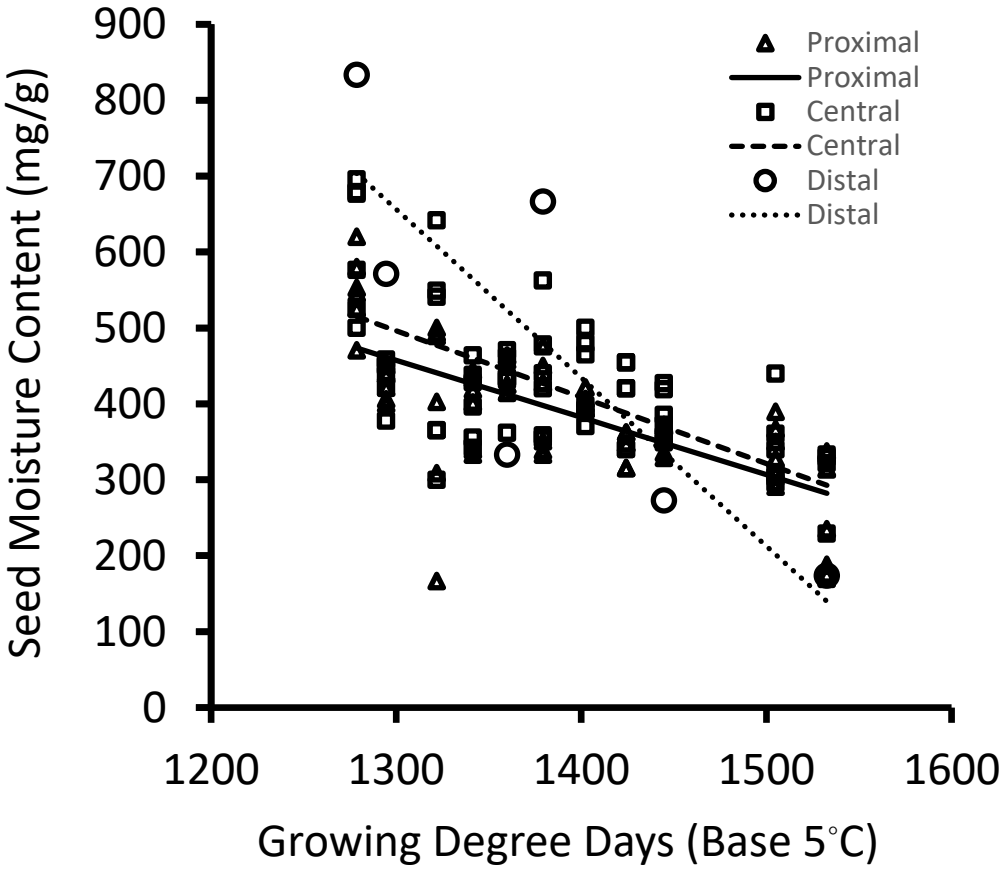

Figure 2.2. Relationship of growing degree days and seed moisture content in orchardgrass seed over seed development in three spikelet positions (proximal, central, and distal) in 2018.

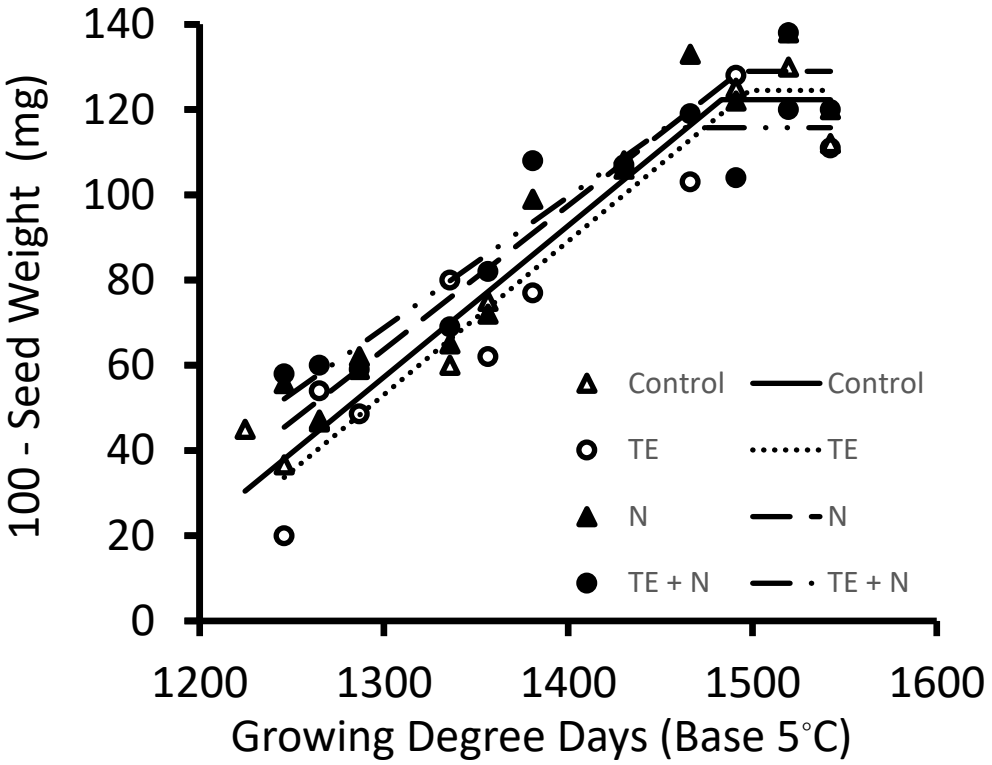

Figure 2.3. Relationship of growing degree days and seed weight in orchardgrass over seed development in proximal spikelet positions in 2019. Orchardgrass was grown under control, spring N, TE PGR, and spring N + TE PGR treatments. Lines represent regression functions for each treatment.

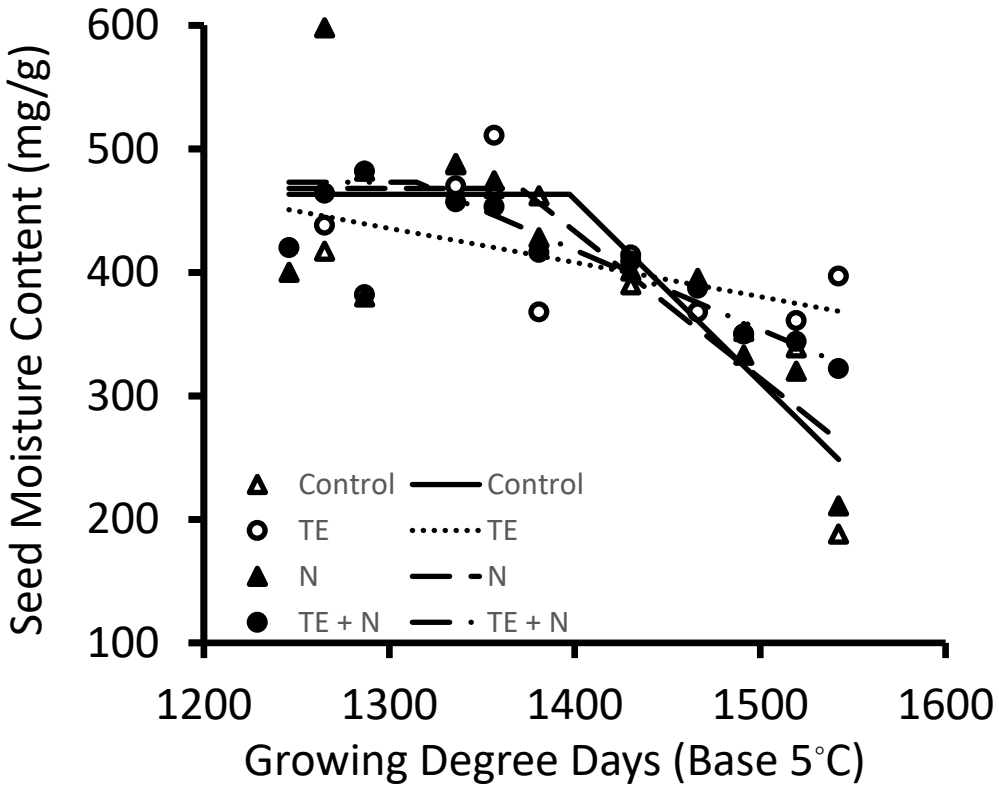

Figure 2.4. Relationship of growing degree days and seed moisture content over seed development in proximal spikelet positions in 2019. Orchardgrass was grown under control, spring N, TE PGR, and spring N + TE PGR treatments. Lines represent regression functions for each treatment.

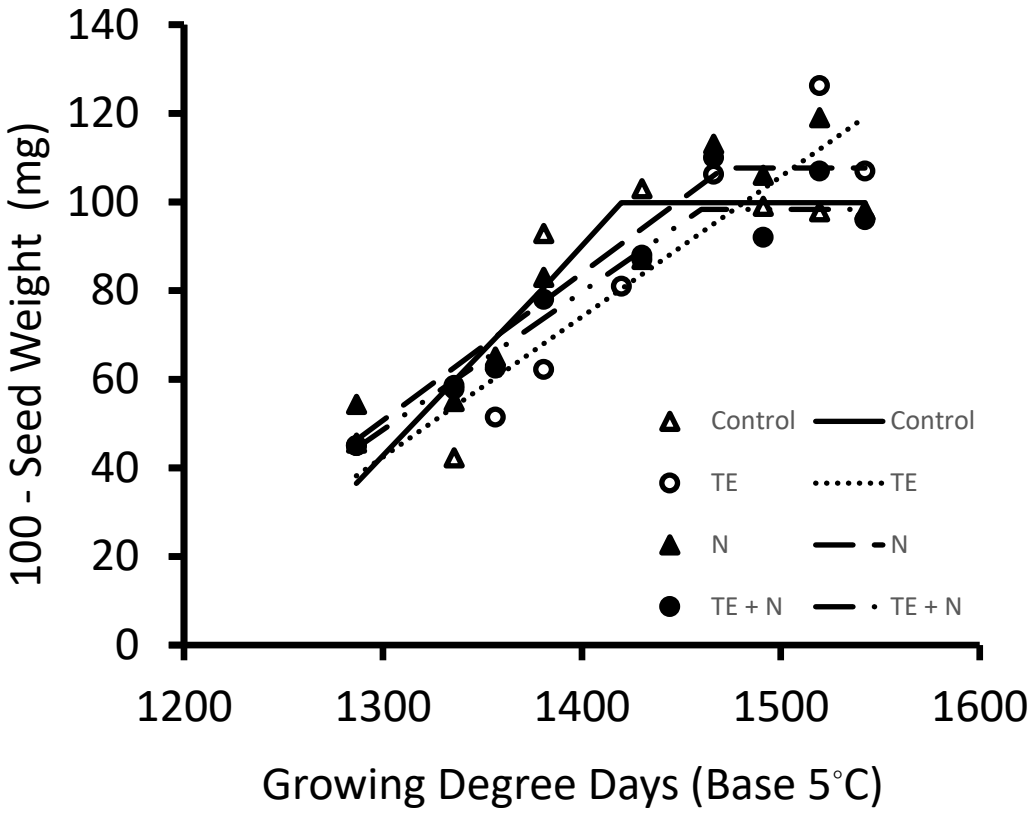

Figure 2.5 Relationship of growing degree days and seed weight in orchardgrass over seed development in central spikelet positions in 2019. Orchardgrass was grown under control, spring N, TE PGR, and spring N + TE PGR treatments. Lines represent regression functions for each treatment.

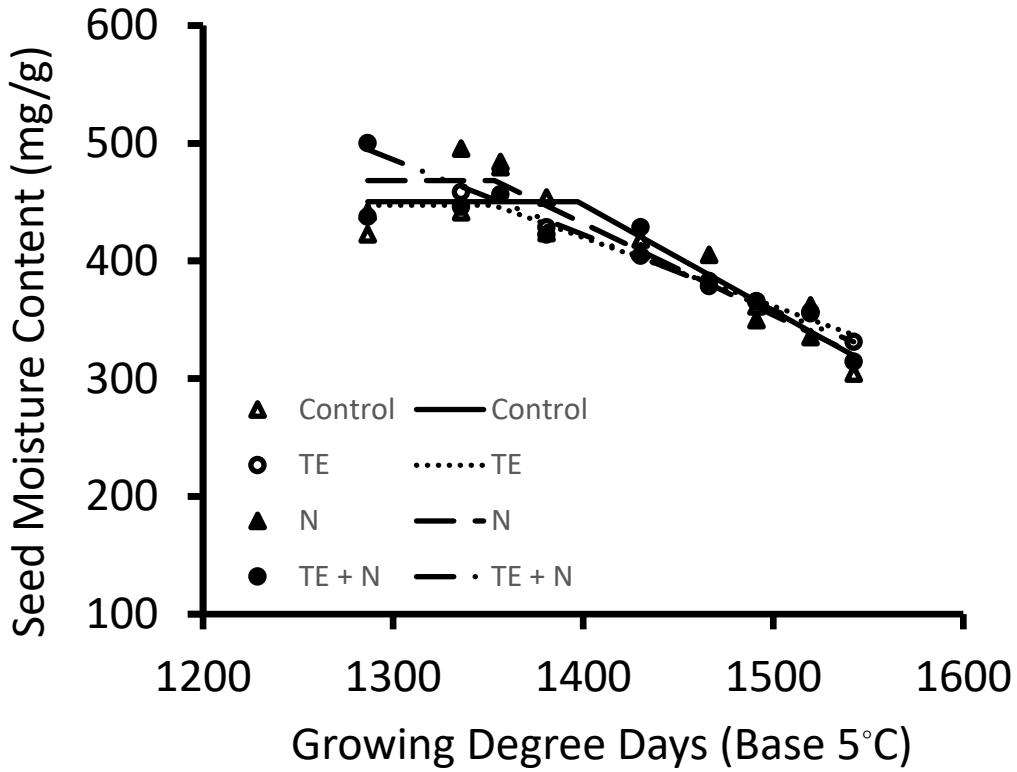

Figure 2.6 Relationship of growing degree days and seed moisture content over seed development in central spikelet positions in 2019. Orchardgrass was grown with control, spring N, TE PGR, and spring N + TE PGR treatments. Lines represent regression functions for each treatment.

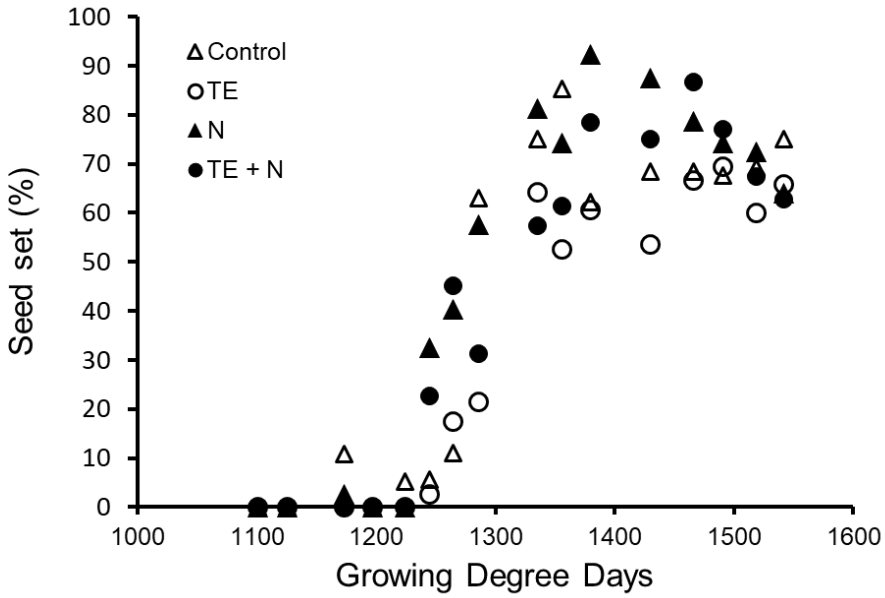

Figure 2.7 Relationship of growing degree days and seed set in Orchardgrass in 2019. Orchardgrass was grown with control, spring N, TE PGR, and spring N + TE PGR treatments. Seed set is expressed as the number of seed present divided by the number of florets.

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Chapter 3. Spring Nitrogen and Plant Growth Regulator Effects on Seed Yield in Orchardgrass (*Dactylis glomerata* L.)

Abstract

Orchardgrass (*Dactylis glomerata L.*) is an important seed crop, but unlike other cool-season perennial grass seed crops such as perennial ryegrass (*Lolium perenne* L.) and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.], seed yields in have not increased over time so there is considerable room for improvement. Research suggests that seed yield increases in orchardgrass may be possible with plant growth regulators (PGRs) such as trinexapac-ethyl (TE) and chlormequat chloride (CCC). Field trials were conducted at Hyslop Experimental Farm near Corvallis, OR in three crop years (2016-2017, 2017-2018, and 2018-2019) to examine the effects of spring N and PGRs on seed production characteristics in orchardgrass. Spring N was applied at four rates: 0, 112, 157, and 202 kg N ha^{-1} and PGR applications were timed using the BBCH [Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie] scale. The four PGR treatments consisted of : 0 g ai ha^{-1}, 210 g ai ha^{-1} TE applied at BBCH 32, 210 g ai ha^{-1} TE applied at BBCH 51, and a combination of 105 g ai ha^{-1} TE + 1500 g ai ha^{-1} CCC applied at BBCH 32. An interaction of spring N and PGR resulted in increased seed yields in two of the three years while spring N and PGR worked independently in the other year to increase seed yield. The combination of TE and CCC PGRs did not increase seed yield compared to TE alone. Spring N effects on the observed increases in seed yield were largely due to a corresponding increase in seed number m^{-2} evident with spring N application. Increases in orchardgrass seed yield attributable to TE PGR were the result of increased seed number m^{-2} and improved harvest index (HI), but not seed weight. This study suggests that the combination of spring N applied at 112 kg N ha^{-1},

and TE PGR application at 210 g ai ha^{-1} would be the best practice to increase seed yield in orchardgrass.

3.1 Introduction

Orchardgrass (*Dactylis glomerata* L.) is an important seed crop in Oregon, a global leader in seed production of this crop. Unlike other cool-season perennial grass seed crops such as perennial ryegrass (*Lolium perenne* L.) and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.], seed yields have not increased over the past 40 years in orchardgrass so there is considerable room for improvement. The review of literature reveals that very little research has been conducted to date on seed production of this valuable crop.

Rampton and Jackson (1969) reported that the best results for N applications in terms of orchardgrass seed yield were observed when the N was placed on the field in spring (March) over fall applications (October) or split between fall and spring. As a result, application of spring N is one of the most important management practices for production of orchardgrass seed crops (Doerge et al., 2000).

Orchardgrass seed yield was increased incrementally with spring N applications from no N to 90 kg N ha^{-1}, but was not further improved with N applications beyond that rate up to 179 kg N ha^{-1} (Rampton and Jackson, 1969). By contrast, Young et al. (1999) found that spring applications of 120 kg N ha^{-1} produced optimum results for seed yield in orchardgrass. Higher rates of spring N application did not increase seed yield over the 120 kg N ha^{-1} rate, but 90 kg N ha^{-1} produced lower seed yields.

The combination of wet soils and spring N present in orchardgrass seed fields results in lodging of the crop because the culm cannot support the increasing weight of the panicle and developing seed. Lodging reduces seed yield in cool-season grasses

(Hebblethwaite et al., 1978). Stem elongation in grasses that leads to lodging is promoted by gibberellic acid (GA), but this elongation can be counteracted by the application of specific plant growth regulators (Rademacher, 2015). There are three different groups of GA-inhibiting plant growth regulators (PGRs). One of these groups of PGRs block GA metabolism by inhibiting cyclase activities in the early stages of GA biosynthesis (onium compounds) while another works in late stages of GA biosynthesis by inhibiting dioxygenase reaction in blocking 2-oxoglutaric acid as a catalyst (acylcyclohexanedione). One widely used example of onium compound PGRs is chlormequat chloride [(2-chloroethyl)-trimethylammonium chloride] and the most widely used acylcyclohexanedione PGR is trinexapac-ethyl [4-(cyclopropyl- α-hydroxymethylene)-3, 5-dioxo-cyclohexanecarboxylic acid ethylester] (Rademacher, 2015).

Trinexapac-ethyl (TE) has been widely adopted for use as a PGR for lodging control and seed yield-enhancing tool in cool-season forage and turf grass seed production (Zapiola et al., 2006; Borm and van den Berg, 2008; Rolston et al., 2010; Chastain et al., 2014a; Chastain et al., 2014b; Chastain et al., 2015). Maximum seed yield increases in these studies have ranged from 38% in strong creeping red fescue (*Festuca rubra* L. subsp. *rubra*) to 45% in perennial ryegrass. Prior to the development of TE as a PGR, chlormequat chloride (CCC) was used commercially in perennial ryegrass seed crops in New Zealand, where the PGR was observed to increase seed yields (Hampton, 1986). The greater seed yield response to TE in comparison to CCC resulted in rapid grower adoption of TE globally. A study in New Zealand has examined the effects of PGR on seed yield in orchardgrass (Rolston et al., 2014). A combination of TE

and CCC PGRs consistently increased seed yield in orchardgrass across multiple cultivars and years.

Since lodging is exacerbated in the high-N environments present in orchardgrass seed production fields in Oregon, work is needed to determine whether there are interactions between spring-applied N and plant growth regulators (Chastain et al., 2014a). The objectives of this study were to determine the effects of spring N applications, PGRs individually and in combination, and possible interactions of N and PGRs on seed production characteristics and seed yield in orchardgrass seed crops.

3.2 Materials and Methods

Field trials were conducted in three crop seasons (2016-17, 2017-2018, and 2018-2019) at Oregon State University's Hyslop Experimental Farm (44° 40' N, 123° 11' 36'' W) near Corvallis, Oregon. The soil type at the research site is a Woodburn silt loam (fine-silty, mixed, superactive, mesic, Aquultic Argixeroll). 'Persist' orchardgrass was planted on 7 October 2015 at a seeding rate of 8 kg ha^{-1} in a 60-cm row spacing. The late fall planting meant that the first seed harvest of the crop was delayed to 2017. Development stages of orchardgrass in relation to management practices, experimental treatments, and the timing of sampling were characterized by using the BBCH scale (Lancashire et al., 1991). Growing degree days (GDD) were used to mark the progress of the crop toward seed development and maturity and were calculated from 1 September of each crop year and were based on air temperatures observed at Hyslop Farm. The base temperature used in the GDD calculations was 5°C.

The experimental design was a split-plot, randomized block design with three replications. Main plots (11.6 m x 13.4 m) were spring-applied N and subplots (3.4 m x 11.6 m) were PGR treatments. Fall N was applied to all plots in October of each year as dry ammonium phosphate-sulfate at 45 kg N ha^{-1} (Doerge et al., 2000). Spring N treatments consisted of dry granular urea (46-0-0), applied prior to stem elongation (BBCH 29; mean = 791 GDD) at four rates: 0, 112, 157, and 202 kg N ha^{-1}. A tractor-mounted orbit-air spreader system was used to make spring N applications while untreated N control plots were driven on by the tractor and spreader system without application of N.

Applications of PGRs were made at two timings: 2-node stage (BBCH 32; mean = 858 GDD) and early panicle (10%) emergence 10% (BBCH 51; mean = 961 GDD).

The PGR subplots consisted of four treatments: an untreated control, 210 g ai ha^{-1} TE applied at BBCH 32, 210 g ai ha^{-1} TE applied at BBCH 51, and a combination of 105 g ai ha^{-1} TE + 1500 g ai ha^{-1} CCC applied at BBCH 32. The PGR was applied at walking speed by using a bicycle-type boom sprayer operated at 138 kPa with XR Teejet 8003VS nozzles. The spray volume used in the PGR application was 194 L ha^{-1}. All other management of the experimental plots were based on common practices for commercial orchardgrass seed production in the Willamette Valley of Oregon.

Two 30-cm^2 samples were harvested at random from each plot at ground level near peak anthesis (BBCH scale 65) in all three years. The number of panicles in each sample was determined. Samples were placed in a dryer at 65˚C for approximately 48 h and then weighed to determine above-ground biomass which was used for the calculation of harvest index (HI).

Harvest for seed yield in orchardgrass was conducted in the two-step process (swathing, followed by combining) that is customary in Oregon. The crop was harvested by swathing with a small-plot swather when seed moisture content reached approximately 420 g kg^{-1} (Silberstein et al., 2010). Dried windrows were threshed in the field with a plot combine when seed moisture content had reached 120 g kg^{-1}. Harvested seed from each plot was weighed in the field for a determination of seed dirt weight (weight of the harvested crop prior to seed cleaning), and two subsamples were collected for laboratory analysis. Seed was cleaned by using an M-2B air-screen cleaner (A.T. Farrell, Saginaw, MI) prior to final clean seed yield determination. Percentage of plot seed dirt weight and plot clean seed weight was used to calculate percent seed cleanout and overall seed yield.

Samples to determine seed weight and seed number were hand cleaned using screens and a blower prior to counting. Two 1,000 seed samples from each plot were counted by an electric seed counter (The Old Mill Company, Savage, MD) and weighed. Seed number m^{-2} was calculated for each plot by dividing the clean seed yield harvested from that plot by the individual seed weight. Harvest index was determined as the proportion of total above-ground dry matter represented by clean seed yield.

Analysis of variance was conducted to test main effects (spring N and PGRs) and interactions. Treatment source terms in the ANOVA model were considered to be fixed effects and none of the data were transformed prior to or after analysis. Treatment means were separated by Fisher's protected LSD values at the 5% level of significance.

3.3 Results

Spring weather conditions (April – June) are important for flowering and seed production in cool-season grasses in Oregon's Willamette Valley. Temperatures during the study period were above long-term averages for the season and site in all but one of the months (Table 3.1). Seasonal precipitation was also above the long-term averages but the distribution of the precipitation was such that April in each of the three years was primarily responsible for this increased precipitation.

Results from the analysis of variance revealed that seed production characteristics in orchardgrass were affected by spring N and PGR, and interactions of spring N and PGR (Table 3.2). An interaction of spring N and PGR influenced seed number and seed yield ($P \leq 0.001$) in 2017, and seed yield ($P \leq 0.05$) in 2019. No other interactions were observed. Overall, PGR effects on seed production characteristics were stronger and more consistent than spring N effects. Characteristics most consistently affected by PGR applications were HI, seed number, and seed yield. Spring N effects were most often observed in seed number and seed yield.

Spring N had no effects on HI across all three years (Table 3.3). The number of panicles produced were increased by spring N in 2019, but not in 2017 and 2018. All three N rates produced similar numbers of panicles which were greater than the untreated control with no spring N except for 2017.

Seed weight was influenced by spring N in 2019, but not in 2017 and 2018 (Table 3.3). Seed weight was increased by the 112 and 202 kg N ha^{-1} rates, but not by the 157 kg N ha^{-1} rate in 2019. Seed number was increased by spring N applications in both 2017

and 2018, but not in 2019. All spring N rates had the same effect on increasing seed number in orchardgrass over the two years.

Application of PGR treatments affected HI in all three years (Table 3.4). The most consistent increases in HI were observed over the three years with the 210 g ai ha^{-1} TE (BBCH 32) and 105 g ai ha^{-1} TE + 1500 g ai ha^{-1} CCC PGR treatments. Panicle production was not influenced by PGR treatments in any of the three years. Seed weight responses to PGR treatments were mixed across the years, but two treatments [210 g ai ha^{-1} TE (BBCH 32) and 105 g ai ha^{-1} TE + 1500 g ai ha^{-1} CCC] produced greater seed weight than the control in 2019. Seed number was increased consistently by PGR treatments over the untreated control in all three years.

Seed yield in orchardgrass was governed by interactions of spring N and PGR in 2017 and 2019 (Figure 3.1). Seed yield was affected independently by spring N and by PGR treatments in 2018, but the responses to these factors are represented as an interaction in Figure 3.1 for ease of comparison among years. The combination of spring-applied N and PGRs worked together to produce the greatest seed yield overall. All rates of spring N increased seed yield over the 0 kg N ha^{-1} control in the absence of PGR except in 2019 where the 112 and 157 kg N ha^{-1} rates were the same as no N without PGR. Seed yield was increased in all three years by PGR treatment at all rates of spring N application, even when no spring N was applied. There was a tendency for the 210 g ai ha^{-1} TE (BBCH 51) treatment to produce somewhat lower seed yields than the other two PGR treatments at the same N application rate, but this was not consistent.

Seed yield was increased in proportion to seed number in all three years (Figure 3.2). There was no significant relationship of seed weight to seed yield evident in this study (data not shown).

3.4 Discussion

Harvest index was not influenced by spring N in orchardgrass over the three years of the study (Table 3.3). In perennial ryegrass and tall fescue seed crops, HI was either reduced or not affected by spring-applied N (Chastain et al., 2014a). Panicle production was increased by spring N in one year of the three harvest years. Young et al. (1999) found that spring-applied N did not increase panicle number in tall fescue. Similar results in panicle production were observed in tall fescue with spring-applied N, but not in spike production in perennial ryegrass where spikes were increased by spring N (Chastain et al., 2014a). Seed yield in orchardgrass was found to increase in proportion to an increasing number of panicles m^{-2} (Chastain and Grabe, 1989). No other studies have investigated the effects of spring-applied N on HI or panicle production in orchardgrass seed crops.

Mixed effects of spring N on seed weight were noted with increases in seed weight observed in one year in three (Table 3.3). By contrast, Rampton and Jackson (1969) reported that seed weight in orchardgrass was increased with spring N applications of 45 kg N ha^{-1} or greater in five harvest years. Spring-applied N consistently increased seed weight in both perennial ryegrass (Young et al., 1998; Chastain et al., 2014a) and tall fescue (Chastain et al., 2014a). Spring N applications increased seed number in two of the three harvest years.

Harvest index provides a measure of how grass seed crop management impacts partitioning to seed in relation to total above-ground biomass production. Harvest index was increased with PGRs in all three harvest years (Table 3.4). This increase in HI was a result of the beneficial modification of the crop canopy by PGRs in reducing stem length,

thereby enabling efficient partitioning of dry matter to seed yield in strong creeping red fescue (Zapiola et al., 2006). Applications of TE PGR mostly increased HI in perennial ryegrass (Chastain et al., 2014a; Chastain et al., 2014b) and tall fescue (Chastain et al., 2015), and even when not significant, the trend was for increased HI with the PGR application. No other studies have examined the influence of PGRs on HI in orchardgrass.

Plant growth regulators did not affect the production of panicles in orchardgrass (Table 3.4). Since the number of panicles in orchardgrass is set prior to the application of the PGRs (BBCH 32 or 51) this is not an unexpected result (Niemeliäinen, 1990). The lack of PGR effects on inflorescence production has been widely observed in other cool-season grass seed crop species (Rolston et al., 2010; Zapiola et al., 2014; Chastain et al., 2014b; Chastain et al., 2015).

Mixed seed weight responses to PGR treatments were evident in orchardgrass (Table 3.4). Borm and van den Berg (2008) and Rolston et al. (2010) found that seed weight was not affected by TE PGR treatment in perennial ryegrass. Chastain et al., (2014a) found that the effects of TE PGR on seed weight for perennial ryegrass and tall fescue were variable; application of the PGR reduced seed weight, increased seed weight, or had no effect on seed weight. Orchardgrass seed number was consistently increased by the application of PGR treatments. This effect of PGRs on increasing seed number has been observed in other cool-season grasses (Zapiola et al., 2014; Chastain et al., 2014a; Chastain et al., 2014b; Chastain et al., 2015). Hampton (1986) found that seed weight in perennial ryegrass was not affected by CCC PGR. No previous work reported the effects of TE or CCC PGRs on seed weight or seed number in orchardgrass.

Interactions of spring N and PGR were influential in seed yield of orchardgrass seed crops in two of the three harvest years (Figure 3.1). Seed yields were greatest with the applications of both spring N and PGRs in each of the three harvest years. Interactions of spring-applied N and TE also governed seed yield of perennial ryegrass in all three harvest years and was evident in tall fescue in two of the three harvest years (Chastain et al., 2014a). Borm and van den Berg (2008) found no interaction of TE and applied N for seed yield in perennial ryegrass but Rolston et al. (2007) reported an interaction of applied N and TE for seed yield in first-year stands of perennial ryegrass.

Notably, PGR treatments increased seed yield in orchardgrass in the absence of spring-applied N. In perennial ryegrass, PGRs had no positive effect on seed yield unless spring N was applied, but in tall fescue PGR-induced increases in seed yield with no spring N were observed in two of the three years (Chastain et al., 2014a). Seed yield was generally increased in orchardgrass with spring N rates at 112 kg N ha^{-1} but there was no benefit of greater spring N rates. These results confirm the findings of Rampton and Jackson (1969) and Young et al (1999) for spring N applications in orchardgrass seed crops. The PGR use did not affect the need for spring N by the plant.

Timing of PGR application (BBCH 32 or 51) did not have an impact on seed yield in orchardgrass. Application timing of TE PGR had no effect on seed yield in tall fescue and perennial ryegrass as long as the applications were made between BBCH 32 and BBCH 51 (Chastain et al., 2014b; Chastain et al., 2015). Timing of PGR applications before or after these stages resulted in reduced seed yield.

The combination of TE and CCC PGRs did not increase seed yield over TE alone applications in orchardgrass in this study. The 210 g TE ha^{-1} + 1500 g CCC ha^{-1} PGR

combination consistently produced the greatest seed yield in orchardgrass (Rolston et al., 2014), although it should be noted that the 105 g ai ha^{-1} TE + 1500 g ai ha^{-1} CCC combination used in this study had one-half of the rate of TE. This study did not include a CCC alone treatment but in concurrent OSU research with PGR combinations in tall fescue, PGR-induced seed yield increases were due solely to TE contained in the combination and CCC by itself had no effect on seed yield (Hudgins, Anderson and Chastain, unpublished).

Seed number was increased by the interaction of spring N and PGR in one of three years, but seed number was increased by PGR in all years (Table 3.2). This positive effect of PGRs and in one instance by the interaction on seed number was influential in the seed yield increases observed in orchardgrass (Figure 3.2). Similar results of PGR-induced increases in seed number in turn causing seed yield increases have been observed in other grass seed crops species (Rolston et al., 2010; Zapiola et al., 2014; Chastain et al., 2014a; Chastain et al., 2014b; Chastain et al. 2015). No effects of seed number on seed yield have been previously reported in orchardgrass.

The results of this study indicate that spring N can be used in combination with TE PGR to increase seed yield in orchardgrass. The practice of applying PGRs in orchardgrass has not been widespread in Oregon and their use did not result in the need for additional spring N. The timing of TE application does not affect seed yield when applications begin with early stem elongation (BBCH 32) and end by early panicle emergence (BBCH 51). Other timings may or may not affect seed yield. There is not enough evidence to recommend that CCC be used in combination with TE as part of a combination of PGRs. Seed yield increases in response to spring N and PGRs were

mostly attributable to increases in seed number. For best results, TE should be applied in conjunction with the recommended rate of spring N for orchardgrass.

Table 3.1. Spring monthly and seasonal average temperature and precipitation totals in comparison to the 130-year means for Hyslop Farm, Corvallis Oregon.

		Month			
Climatic factor	Year	April	May	June	Seasonal Mean
Temperature (°C)	2017	9.5	14.1	17.4	13.7
	2018	10.5	14.9	16.7	14.0
	2019	11.1	14.3	17.0	14.0
Long-term Mean		10.2	13.2	16.1	13.1
Precipitation (mm)					Seasonal Total
	2017	105	44	39	188
	2018	134	5	20	159
	2019	165	49	19	233
Long-term Mean		67	50	31	148

Table 3.2. Analysis of variance for spring nitrogen (N) and plant growth regulators (PGR) effects on seed production characteristics in orchardgrass.

Year/Source of variation	HI	Panicle m^{-2}	Seed weight	Seed m^{-2}	Seed yield
2017					
N	ns†	ns	ns	*	***
PGR	***	ns	*	***	***
N x PGR	ns	ns	ns	***	***
2018					
N	ns	ns	ns	***	***
PGR	***	ns	ns	***	***
N x PGR	ns	ns	ns	ns	ns
2019					
N	ns	***	*	ns	ns
PGR	***	ns	***	***	***
N x PGR	ns	ns	ns	ns	*

*$P \leq 0.05$.
**$P \leq 0.01$.
***$P \leq 0.001$.
†Not significant.

Table 3.3. Spring-applied nitrogen effects on seed production characteristics in orchardgrass.

Year	Spring N	HI	Panicles m^{-2}	Seed weight	Seed m^{-2}
	kg ha^{-1}	%	no.	mg	no. x 10^3
2017	0	6.6 a†	526 a	0.921 a	73.9 a
	112	7.1 a	448 a	0.914 a	89.2 b
	157	7.6 a	446 a	0.931 a	88.2 b
	202	6.8 a	474 a	0.921 a	96.9 b
2018	0	4.0 a	888 a	0.911 a	70.1 a
	112	3.7 a	1077 a	0.912 a	97.7 b
	157	3.9 a	1005 a	0.915 a	94.4 b
	202	3.8 a	1025 a	0.907 a	96.5 b
2019	0	11.8 a	246 a	0.916 a	39.7 a
	112	8.3 a	389 b	0.959 b	52.9 a
	157	9.4 a	384 b	0.940 ab	56.8 a
	202	8.6 a	422 b	0.947 b	60.0 a

†Means within years followed by the same letter are not significantly different by Fisher's protected LSD values ($P = 0.05$).

Table 3.4. Plant growth regulator (PGR) treatment effects on seed production characteristics in orchardgrass.

Year	TE Rate	CCC Rate	Timing	HI	Panicles m^{-2}	Seed weight	Seed m^{-2}
	g ai ha^{-1}	g ai ha^{-1}	BBCH	%	no.	mg	no. x 10^3
2017	0	0	-	4.8 a†	470 a	0.921 ab	61.4 a
	210	0	32	7.6 b	462 a	0.929 b	94.9 bc
	210	0	51	7.1 b	543 a	0.935 b	93.7 b
	105	1500	32	8.6 c	419 a	0.902 a	98.3 c
2018	0	0	-	3.2 a	1037 a	0.908 a	70.7 a
	210	0	32	4.3 b	969 a	0.919 a	96.7 bc
	210	0	51	3.5 a	1031 a	0.914 a	91.4 b
	105	1500	32	4.4 b	958 a	0.902 a	100.1 c
2019	0	0	-	6.9 a	386 a	0.920 a	44.0 a
	210	0	32	11.1 b	347 a	0.967 c	54.1 b
	210	0	51	8.7 a	398 a	0.933 ab	53.3 b
	105	1500	32	11.4 b	310 a	0.942 b	58.1 b

†Means within years followed by the same letter are not significantly different by Fisher's protected LSD values ($P = 0.05$).

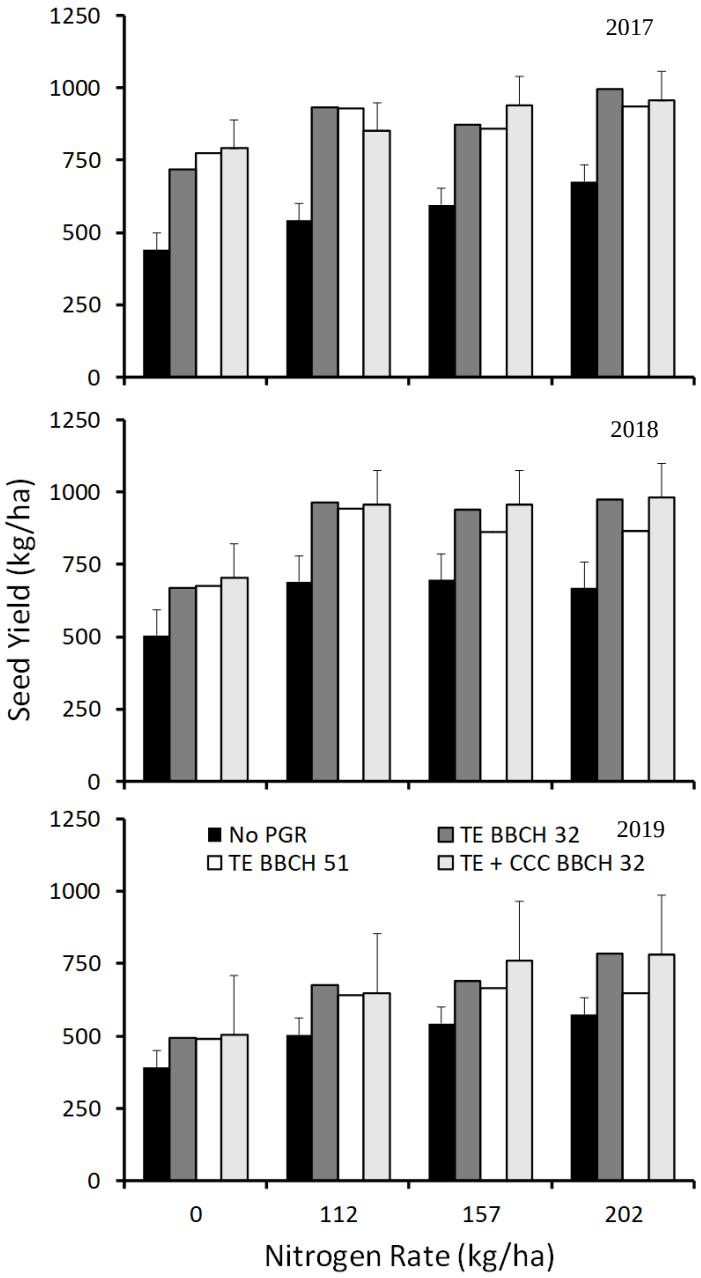

Figure 3.1. Interaction of spring N and PGR treatments on seed yield of orchardgrass. Small bars represent LSD values for comparison of PGR means within spring N levels while large bars represent LSD values for comparison of means among spring N levels (*P* = 0.05).

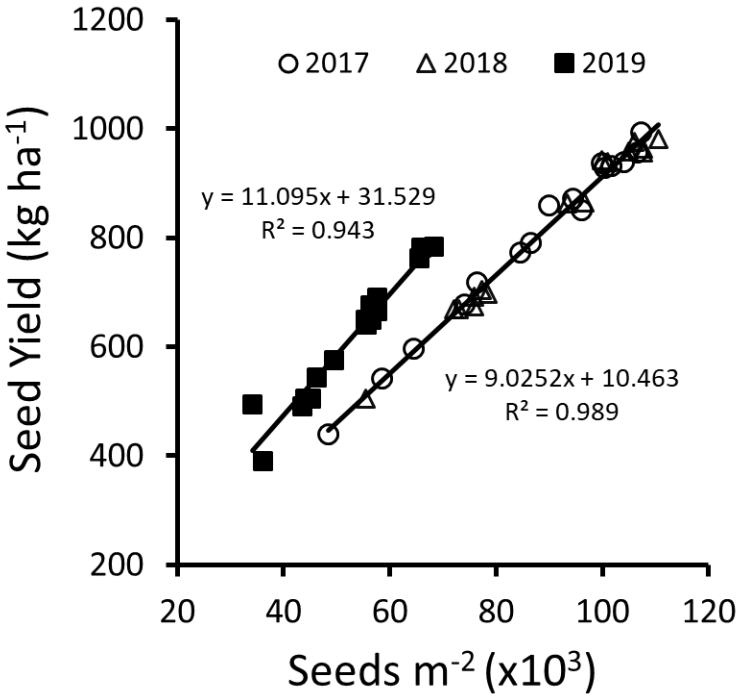

Figure 3.2. Relationship of seed number and seed yield in orchardgrass in 2017, 2018, and 2019.

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Chapter 4: Carbon and Nitrogen Deposition in the Development of Field-grown Orchardgrass (*Dactylis glomerata* L.) Seed.

Abstract

The deposition of carbon and nitrogen in the seed is essential for the synthesis of starch and proteins during seed development and in the formation of seed yield. Plant growth regulators (PGRs) such as trinexapac-ethyl (TE) and spring nitrogen increase seed yield in grass seed crops through their effects on increasing seed number but it is not known whether these agronomic practices affect the deposition of C and N in the seed. Field trials were conducted in two growing seasons (2017-2018, 2018 - 2019) at Hyslop Experimental Farm, Corvallis, OR, to test the effects of TE PGR and spring N application on C and N seed deposition. Four treatments were used; control, TE (210 g ai ha^{-1}), spring N (112 kg ha^{-1}), and TE + N. Regression analyses were used to aid in understanding the deposition of C and N in seed and the effects of treatments. The content of C and N in seed increased over growing degree days ($P \leq 0.001$) and followed a segmented model except for N content in the N treatment. Both C and N content of seed were increased by spring N application. The N treatment reached peak C deposition earliest and had the shortest duration of C filling in seed among the four treatments. The peak deposition of C and N preceded physiological maturity of the seed. During seed development, flag leaf chlorophyll declined in treatments with no N and either declined or were constant with N application. There were no consistent effects of TE PGR on the deposition of C or N in the seed.

4.1 Introduction

Seed development in cool-season grasses such as perennial ryegrass (*Lolium perenne* L.) begins with a short and rapid initial cell division phase with no discernable change in seed weight and is followed by a longer linear phase that is accompanied by large increases in seed weight (Anslow, 1964; Stoddart, 1968). This increase in seed weight is mostly accounted for by starch that is accumulated in the endosperm. Carbon and nitrogen are partitioned to the seed as sucrose and amino acids and in the seed serve as the building blocks for the synthesis of starch and proteins. During the linear phase, the uptake and deposition of C and N in seeds is important to support the activity of seed growth during seed development (Weber et al., 1998). The assimilation of C and N in the seed as starch and proteins is required at adequate levels for the successful synthesis of these essential components of the seed. The metabolic pathways for assimilation of C and N are interconnected in seed development (Egli et al., 1998). Once maximum dry weight is attained at physiological maturity, the growth of the seed ceases and as a result, the assimilation of C and N reaches an endpoint. These essential assimilates contribute to the increase in seed number and seed weight that in turn accounts for increases in seed yield.

The concentration of N in seed increases in a bi-phasic pattern over the course of the seed development process in wheat (*Triticum aestivum* L.) (Dalling et al., 1976). This initial phase consists of a linear increase over about two-thirds of the seed development period while the final phase is a plateau. A two-phase pattern of N concentration increase was also observed in perennial ryegrass seed over the course of seed development but the linear increase phase is much shorter in duration (Warringa et al., 1998).

Warringa et al. (1998) reported different N deposition rates among spikelet positions in perennial ryegrass. Nitrogen concentration was higher in proximal seed than in other spikelet positions. Starch concentration increased over the course of seed development and reached a maximum concentration at 26 days after anthesis in proximal seeds within the spikelet. No information is available on the nature of C and N assimilation dynamics during seed development in orchardgrass (*Dactylis glomerata* L.) or most other cool-season grasses for that matter. Moreover, both wheat and perennial ryegrass have spike-type inflorescences while orchardgrass has a panicle-type inflorescence and it is not known whether seed development processes differ with inflorescence type in the grasses.

Plant growth regulators (PGRs) have been widely adopted in grass seed crop production across the globe (Zapiola et al., 2006; Borm and van den Berg, 2008; Rolston et al., 2010; Chastain et al., 2014a; Chastain et al., 2014b; Chastain et al., 2015), and has been found to increase seed yield in orchardgrass (Rolston et al, 2014). The most commonly used PGR in cool-season grass seed production is trinexapac-ethyl [4-(cyclopropyl- α-hydroxymethylene)-3, 5-dioxo-cyclohexanecarboxylic acid ethylester]. Chastain et al. (2014b) found that trinexapac-ethyl (TE) PGR had influenced seed partitioning in perennial ryegrass by increasing the number of seeds produced on each spikelet. But it is not known whether there is an increase in C and possibly N that are available to fill this increased number of seeds. No work to date has been conducted in orchardgrass or any other species of grass on the potential effects of TE on C and N assimilation. Therefore, the objectives of this study were to determine the effects of

spring N application, TE PGR application, and possible interactions of N and TE on seed carbon and nitrogen depositions during seed development in orchardgrass.

4.2 Methods and Materials

4.2.1 Overview

Field trials were conducted in two crop seasons (2017 - 2018, 2018 - 2019) at Oregon State University's Hyslop Experimental Farm (44° 40' N, 123° 11' 36'' W) near Corvallis, Oregon. The soil type at the research site is a Woodburn silt loam (fine-silty, mixed, superactive, mesic, Aquultic Argixeroll). 'Persist' orchardgrass was planted on 7 October 2015 at a seeding rate of 8 kg ha^{-1} in a 60-cm row spacing. Development stages of orchardgrass in relation to management practices, experimental treatments, and the timing of sampling were characterized by using the BBCH scale (Lancashire et al., 1991). Growing degree days (GDD) were used to mark the progress of the crop toward seed development and maturity and were calculated from September 1st of each crop year and were based on air temperatures observed at Hyslop Farm. The base temperature used in the GDD calculations was 5°C.

The experimental design was a split-plot, randomized block design with three replications (Chapter 3). Main plots (11.6 m x 13.4 m) were spring-applied N and subplots (3.4 m x 11.6 m) were PGR treatments. Four treatments were chosen for the C and N deposition study from the treatments listed in chapter 3. These treatments were: control (no N, no TE), TE (210 g ai ha^{-1} TE), N (spring N at 112 kg N ha^{-1}), and TE + N (both TE and N). Fall N was applied to all plots in October of each year as dry ammonium phosphate-sulfate at 45 kg N ha^{-1} (Doerge et al., 2000). Spring N treatments consisted of dry granular urea (46-0-0), applied prior to stem elongation (BBCH 29; mean = 791 GDD). A tractor-mounted orbit-air spreader system was used to make spring

N applications while untreated N control plots were driven on by the tractor and spreader system without application of N.

Applications of trinexpac-ethyl PGR were made at the 2-node stage of early stem elongation (BBCH 32; mean = 858 GDD. The PGR was applied at walking speed by using a bicycle-type boom sprayer operated at 138 kPa with XR Teejet 8003VS nozzles. The spray volume used in the PGR application was 194 L ha^{-1}. All other management of the experimental plots were based on common practices for commercial orchardgrass seed production in the Willamette Valley of Oregon.

Five panicles were collected periodically from random locations within each plot starting at BBCH 65 (peak anthesis) and continuing until BBCH 85 (seed harvest) in two growing seasons – 2018 and 2019. The panicle samples were used for determination of floret number, seed number, floret weight, seed weight, and seed moisture content (Chapter 2). Seed and florets were obtained from distal, central, and proximal positions within spikelets located in the apical, medial, and basal region of the panicle in 2018 and from only the medial position in 2019. The remaining seed and florets were collected in bulk.

Bulk seed and florets were placed in a metal sample container and weighed prior to oven-drying. The sample containers were placed in a laboratory air-oven and dried at 130°C for 2 hours. After drying was complete, the cover was placed on each sample container. The container was cooled to room temperature prior to weighing in a desiccant-containing chamber. Seed dry weight was determined after oven drying. Proximal seed samples (50 mg) were collected from bulk samples that were not used for

seed weight analysis (Chapter 2) and were used in the determination of C and N assimilation in orchardgrass seed. Proximal and central seed (50 mg each) were collected after harvest to determine C and N content. Total N and C content of the seed at each sampling date were determined by the Dumas combustion method (Jones and Case, 1990) by using an automated macro analyzer. Seed C and N filling periods were determined by subtracting the start point of the filling periods from the GDD where maximum C and N seed content was recorded.

Chlorophyll content is often used as an index of crop maturity with a decline in chlorophyll indicating relative progress toward reproductive maturity and seed harvest as well as to evaluate N status of crops. Chlorophyll meter readings were made periodically on flag leaves by using a portable Minolta SPAD 502 chlorophyll meter (Follett et al., 1992). The chlorophyll meter readings were made twice per week starting at BBCH 50 (initiation of panicle emergence) through BBCH 85 (seed harvest).

4.2.2 Data Analysis and Interpretation

To describe seed development processes in orchardgrass, regression analysis was conducted to describe the nature of relationships of carbon and nitrogen seed deposition over the course of seed development. The relationship of seed growth (weight) throughout the seed development process is often graphically represented with seed weight plotted on the ordinate and time plotted on the abscissa. The same relationship was used for carbon and nitrogen seed content with time plotted on the abscissa. Instead of time, the more biologically relevant GDD is probably a better replacement on the

abscissa (Chynoweth and Moot, 2017). Thus, GDD (base 5°C) was used in curve-fitting as the abscissa for both carbon and nitrogen content relationships.

Regression analysis was conducted to describe spring N and TE effects on seed C and N deposition in the seed using PROC GLM and PROC NLIN in SAS (SAS 9.4). A segmented model was chosen if the data would fit a linear or a quadratic function as a first segment then a plateau as a second segment. When using PROC GLM, the data were fitted to linear and quadratic models and compared for goodness of fit. If the quadratic term was significant, the data were fitted into a segmented model where a quadratic function was the first segment and a plateau is the second segment. If the quadratic term was not significant, the data were fitted into a segmented model where a linear function was the first segment and a plateau would be the second segment. To fit the data to a segmented model, the method used was MARQUARDT for PROC NLIN. Initial parameters used in the segmented model were chosen from the quadratic functions found in PROC GLM results.

The effect of the four treatments (Control, TE, N, and TE + N) were compared using regression analysis for differences in maximum C and N contents, GDD date of reaching maximum contents, and carbon and nitrogen accumulation rate. Carbon and nitrogen accumulation relationships with GDD over the course of seed development were fit with logistic, Gompertz, segmented piecewise, and other appropriate models. These procedures were used to test relationships of seed C and N content with GDD over the course of seed development. The goodness of fit of models with observed data were determined using the coefficient of determination (R^2) and the best model was used.

4.3 Results

4.3.1 Carbon and Nitrogen Deposition in Seed

Carbon and nitrogen content accumulation were different during seed growth where carbon accumulation exhibited a quadratic function to a plateau in the segmented model, while nitrogen accumulation followed a linear to plateau segmented design (Figure 4.1; Figure 4.2).

Carbon content in orchardgrass seed increased over GDD ($P < 0.0005$) during seed development (Table 4.1; Figure 4.1). Among the four treatments tested, the deposition of C was not statistically different (Analysis of Covariance, $P > 0.05$). The maximum seed carbon content for the treatments ranged from 451 to 456 mg g^{-1}. The control treatment had a C accumulation rate of 0.559 mg g^{-1} GDD^{-1} (559 μg g^{-1} GDD^{-1}) and the peak carbon content was reached at 1452 GDD. The seed carbon accumulation rate was 0.518 mg g^{-1} GDD^{-1} (518 μg g^{-1} GDD^{-1}) with the TE treatment and the accumulation of C peaked at 1462 GDD. The deposition of C peaked early at 1415 GDD with the N treatment. The rate of C accumulation for the N treatment was 0.887 mg g^{-1} GDD^{-1} (887 μg g^{-1} GDD^{-1}). The rate of C content increase was 0.515 mg g^{-1} GDD^{-1} (515 μg g^{-1} GDD^{-1}) with the TE + N treatment and the accumulation peaked at 1457 GDD. The highest rate of C accumulation was with the N treatment while other treatments had a lower but similar rate of C accumulation. The N treatment not only had the highest rate of C accumulation but also reached the peak C content earliest among the four treatments.

The duration of C filling in orchardgrass seed varied among the four treatments (Table 4.3). For the control treatment, the duration of C filling in seed was 187 GDD while for the TE treatment, the duration of C filling was 212 GDD. The N treatment had a C filling duration of only 149 GDD, whereas the C filling duration for the TE + N treatment was 198 GDD. The TE treatment had the longest C filling duration while the N treatment had the shortest duration of the four treatments. Physiological maturity in orchardgrass seed varied with treatment in the proximal spikelet position, ranging from 1453 GDD to 1498 GDD (Chapter 2). The peak accumulation of C in the seed in the proximal spikelet position ranged from 1415 GDD to 1462 GDD and corresponded with the attainment of physiological maturity. Thus, the peak of C accumulation in the seed preceded the attainment of physiological maturity (maximum dry weight of the seed).

The final C content of orchardgrass seed collected from both central and proximal seed varied among treatments in 2018 and 2019 (Figure 4.5). Seed in the central spikelet in the N and TE + N treatments tended to have higher C content than the control and TE treatments in 2018. The treatments with N application (N and TE + N) and TE exhibited elevated levels of C in seed from both proximal and central spikelets compared to the control in 2019. There was no consistent effect of TE on C content in seed of orchardgrass.

Nitrogen content increased over GDD with all treatments ($P \leq 0.001$). The deposition of N in seed over GDD with the TE + N treatment followed a linear function whereas the deposition of N in the other three treatments followed a segmented model (linear to plateau) (Table 4.2; Figure 4.2). With the control treatment, the rate of N deposition in seed was 0.0637 mg g^{-1} GDD^{-1} (63.7 μg g^{-1} GDD^{-1}) with a peak of N

accumulation at 1457 GDD. The peak N content for the control treatment was 29.7 mg N g^{-1}. The rate of N deposition in the TE treatment was 0.0759 mg g^{-1} GDD^{-1} (75.9 μg g^{-1} GDD^{-1}) and the peak N content in seed was observed at 1438 GDD at a seed N content of 25.6 mg g^{-1}. With the N treatment, the rate of N deposition in seed was 0.0860 mg g^{-1} GDD^{-1} (86.0 μg g^{-1} GDD^{-1}) and the peak N content was 31.1 mg N g^{-1} at 1439 GDD. The TE + N treatment produced a rate of N deposition in seed of 0.0494 mg g^{-1} GDD^{-1} (49.4 μg g^{-1} GDD^{-1}). The rate of seed N deposition tended to be greatest in TE and N treatments and lower in control and TE + N treatments.

There was variation among the treatments in the duration of N filling in the seed (Table 4.3). For the control treatment, the duration of N filling was 192 GDD. The duration of N filling in the TE and N treatments were 173 GDD and 174 GDD, respectively. The peak of N filling in orchardgrass seed ranged from 1438 GDD to 1457 GDD in proximal spikelets. These peaks in N accumulation in the seed took place before physiological maturity of the seed in proximal spikelets at 1453 GDD to 1498 GDD.

The final seed N content in proximal and central spikelets differed among treatments in 2018 and 2019 (Figure 4.6). The N content of seed in both seed positions was higher in N-containing treatments (N and TE + N) than in the control or TE treatments in 2018 and 2019. There was no independent effect of TE on N deposition in seed of orchardgrass.

4.3.2 Flag Leaf Chlorophyll

The flag leaf chlorophyll meter readings (SPAD) data revealed two basic response patterns over GDD in 2018 and 2019 – treatments with N (N and TE + N) and treatments

without N (control and TE) (Figure 4.3; Figure 4.4). The four treatments were combined into N and no-N and then analyzed together in each of the two years. There was a linear decrease in flag leaf chlorophyll meter readings over GDD in 2018 for both the N and the no-N treatments (Figure 4.3) and for the no-N treatments in 2019. But flag leaf chlorophyll meter readings did not change appreciably over GDD for the N treatments in 2019.

In 2018, leaves were observed to senesce earlier than in 2019 and as a result, the flag leaf measurements ended prior to when 1450 GDD was reached. The rate in flag chlorophyll meter reading decline in 2018 for the N treatments was -0.0778 units GDD^{-1} and the rate of decline for no-N treatments was -0.0806 units GDD^{-1} (Table 4.3). In 2019, flag leaf chlorophyll meter readings did not decrease in N treatments, but for no-N treatments the rate of decrease was -0.0465 units GDD^{-1}.

4.4 Discussion

The C content of orchardgrass seed located in proximal positions in the spikelet increased over GDD during seed development but there was no consistent effect of the treatments in this study on the rate of C deposition in seed (Table 4.1; Figure 4.1). The C content in orchardgrass seed increased over GDD corresponding to the increase in starch accumulated in the endosperm as observed in perennial ryegrass (Warringa et al., 1998). The increase in starch was the largest single contributor to the increase in dry weight of perennial ryegrass seed during seed development and was likely to be the greatest contributor to seed weight increase observed in orchardgrass (Chapter 2). The starch concentration in perennial ryegrass reached maximum levels ranging from 300 mg g^{-1} to 360 mg g^{-1} depending on spikelet position. While starch concentration was not measured in this study, it is likely that the starch concentration was similar in orchardgrass and as a result, starch would most likely account for much of the gain in C content in orchardgrass seed (maximum C content ranged from 451 mg g^{-1} to 458 mg g^{-1}). The pattern of C deposition observed in orchardgrass seed over GDD was similar to the pattern of starch accumulation reported in perennial ryegrass but the C content of seed was not included in their account of biochemical changes in seed during development (Warringa et al., 1998). No other studies have reported the C content and dynamics of C deposition of orchardgrass seed over the course of seed development.

The peak C deposition in seed was reached earlier in the N treatment than for the other treatments and the filling duration of C was also shortest for this treatment (Table 4.1; Table 4.3). The seed filling duration for all treatments was longer than the duration of C deposition in the seed. For all treatments, the peak C deposition in the seed

preceded physiological maturity. Similar results were found in perennial ryegrass where peak starch accumulation in the seed preceded physiological maturity (Warringa et al., 1998). In their study, source material (sucrose) import in the seed ended after maximum starch accumulation of the seed suggesting that the growth of seed in perennial ryegrass was limited by the sink (seed) rather than by C source availability. This finding cannot be confirmed in orchardgrass as C source materials such as sucrose were not measured, but this work in orchardgrass does confirm that the accumulation of C in the seed ends prior to physiological maturity implying a sink limitation for C deposition in the seed.

The N content of orchardgrass seed located in the proximal position in the spikelet increased over GDD during seed development (Figure 4.2; Table 4.2). There were differences among the four treatments in the dynamics of the deposition of N in seed over GDD with the TE + N treatment exhibiting a linear function whereas the deposition of N in the other three treatments followed a segmented model. Seed N deposition also followed a bi-phasic segmented model in perennial ryegrass and wheat over the course of the seed development process (Dalling et al., 1976; Warringa et al, 1998). The increases in N content deposited in the seed over GDD during seed development corresponds to the increase in seed proteins in the embryo and protein bodies in the endosperm (Huber and Grabe, 1987). The rate of deposition of N in seed varied among treatments but tended to be greatest in TE and N treatments and lower in control and TE + N treatments. As observed for C accumulation in seeds, the peaks in N accumulation in the seed took place before physiological maturity of the seed. There are no other studies that have investigated the deposition of N in orchardgrass seed.

The seed N concentration values in orchardgrass varied over the course of seed development, and were mostly in the lower end (12.4 to 29.2 mg N g seed dry weight^{-1}) of the range when N was not applied (control, TE) and in the upper end (15.6 to 32.0 mg N g seed dry weight^{-1}) when N was applied (N, TE + N) (Figure 4.2). These values were in the same range as wheat seed over the seed development period where low N (15.5 to 21.5 mg N g seed dry weight^{-1}) and high N (23.5 to 35.3 mg N g seed dry weight^{-1}) nutrition was employed (Morris and Paulsen, 1985). Similar values in seed N contents over the course of seed development were found in perennial ryegrass (Warringa et al., 1998). Seed N concentration increased in wheat and perennial ryegrass over the course of seed development as was observed in orchardgrass in this study. This is the only known report of seed N content in orchardgrass seed.

In contrast to the seed filling duration observed for orchardgrass seed in the proximal spikelet position (Chapter 2), the duration of C filling in the seed was ≤ 212 GDD and was ≤ 192 GDD for seed N filling duration (Table 4.3). Thus, the period of deposition of these important components in the seed was nearly always less than the seed filling duration at ≥ 207 GDD. The greatest treatment effects on deposition of C and N assimilates in the seed of orchardgrass were found with the N-containing treatments (N and TE + N) (Figure 4.5; Figure 4.6). The highest final seed content of both C and N were found in the N and TE + N treatments. The TE treatment only affected C content in 2019 but was otherwise without impact on either C or N content in the seed. The rate or duration of C or N deposition was not tied to the weight of seed as observed in Chapter 2. No other studies have researched the impact of TE PGR on the C or N composition of seed.

Flag leaf chlorophyll meter readings declined over the seed development period in treatments with no N application and either declined or were constant over the seed development period with N application (Figure 4.3 and 4.4). Chlorophyll meter readings have been found to be related to tissue N concentrations in flag leaves of wheat (Reeves et al., 1993). Thus, the decline in chlorophyll meter readings is indicative of reductions in tissue N concentration during seed development in orchardgrass flag leaves except in 2019 where no decline was observed with N application. Guitman et al. (1991) reported that flag leaf chlorophyll concentration declines over seed development in wheat with slower reductions found with N applications than were found without N application. This decline in flag leaf chlorophyll concentration corresponded to reductions in flag leaf N concentration and the accompanying remobilization of the N to the seed in wheat. Similar remobilization of flag leaf N to the developing seed is also likely in orchardgrass and this might account for some of the N gain evident in the seed of orchardgrass (Figure 4.2).

The primary effects of TE PGR application in increasing seed yield appear to be through increasing seed number (Chapter 3) but there does not seem to be any consistent influence of TE on the deposition of C or N in the seed. However, there were consistent effects of N application on the content of C and N deposited in the seed – both were increased with N application.

Table 4.1. Function parameters estimated from PROC NLIN to describe carbon deposition in orchardgrass seed in proximal spikelets over growing degree days (x = GDD-1000), where y is the estimate mean for carbon (mg/g), B_1 is the rate of carbon deposition (milligram per growing degree day), and j is the breakpoint where maximum carbon is reached.

Fitted Curve: If $x < j$ then $y = B_\circ + B_1 x + B_2 x^2$

if $x \geq j$ then $y = B_\circ + B_1 j + B_2 j^2$

Treatment		**Fitted values and standard errors**								
TE (g a.i. ha^{-1})	N (kg ha^{-1})	B_o	s.e.	B_1	s.e.	B_2	s.e.	j	s.e.	R^2
0	0	325.5	31.2061	0.5587	0.1751	-0.00062	0.000238	452.0	34.3719	0.932
210	0	334.4	26.2363	0.5183	0.1448	-0.00056	0.000193	462.3	31.7221	0.937
0	112	272.5	48.5029	0.8872	0.2853	-0.00107	0.000409	415.4	26.9473	0.926
210	112	337.6	21.8081	0.5151	0.1212	-0.00056	0.000163	457.1	25.9286	0.995

Table 4.2. Function parameters estimated from PROC NLIN to describe nitrogen deposition in orchardgrass seed in proximal spikelet positions over growing degree days (x = GDD-1000), where y is the estimate mean for nitrogen (mg/g), B_1 is the rate of seed nitrogen deposition (milligram per growing degree day), and j is the breakpoint where maximum nitrogen is reached.

		Fitted Curve: If $x \leq j$ then $y = B_\circ + B_1 x$ if $x > j$ then $y = B_\circ + B_1 j$						
Treatment		**Fitted values and standard errors**						
TE (g a.i. ha^{-1})	N (kg ha^{-1})	B_o	s.e.	B_1	s.e.	j	s.e.	R^2
0	0	-1.4551	4.4381	0.0637	0.0128	456.9	30.0272	0.909
210	0	-7.6320	5.9607	0.0759	0.0172	438.4	29.4530	0.859
0	112	-6.5993	2.9491	0.0860	0.00850	439.0	12.9025	0.970
210	112	3.86	2.20	0.04936	0.00527	-	-	0.917

Table 4.3. Seed C and N filling duration in proximal spikelet positions in orchardgrass seed in the crop season (2018 – 2019) under the four treatments (Control, TE, N, TE + N). Seed C and N filling duration were calculated by subtracting the startpoint of filling (1265.04 GDD) from the time maximum seed nutrient achieved (GDD).

Treatment		**Seed C Filling Duration**	**Seed N Filling Duration**
TE (g a.i. ha^{-1})	N (kg ha^{-1})	(GDD)	(GDD)
0	0	186.96	191.86
210	0	211.56	173.36
0	112	148.76	173.96
210	112	198.16	N/A

Table 4.4. Function parameters estimated from PROC GLM to describe SPAD reading over growing degree days (x = GDD) in two growing season (2018 and 2019), where y is the estimate mean for SPAD reading, B_1 is the rate of SPAD decrease (unit per GDD)

Treatment		**Fitted Curve: $y = B_\circ + B_1 x$**				
		Fitted values and standard errors				
Year	Treatment	B_o	s.e.	B_1	s.e.	R^2
2018	Control	133.703	18.253	-0.0806	0.0135	0.748
	Nitrogen	140.038	32.495	-0.0778	0.0240	0.467
2019	Control	93.537	18.691	-0.0465	0.0133	0.468
	Nitrogen	45.424	20.231	-0.0047	0.0143	ns

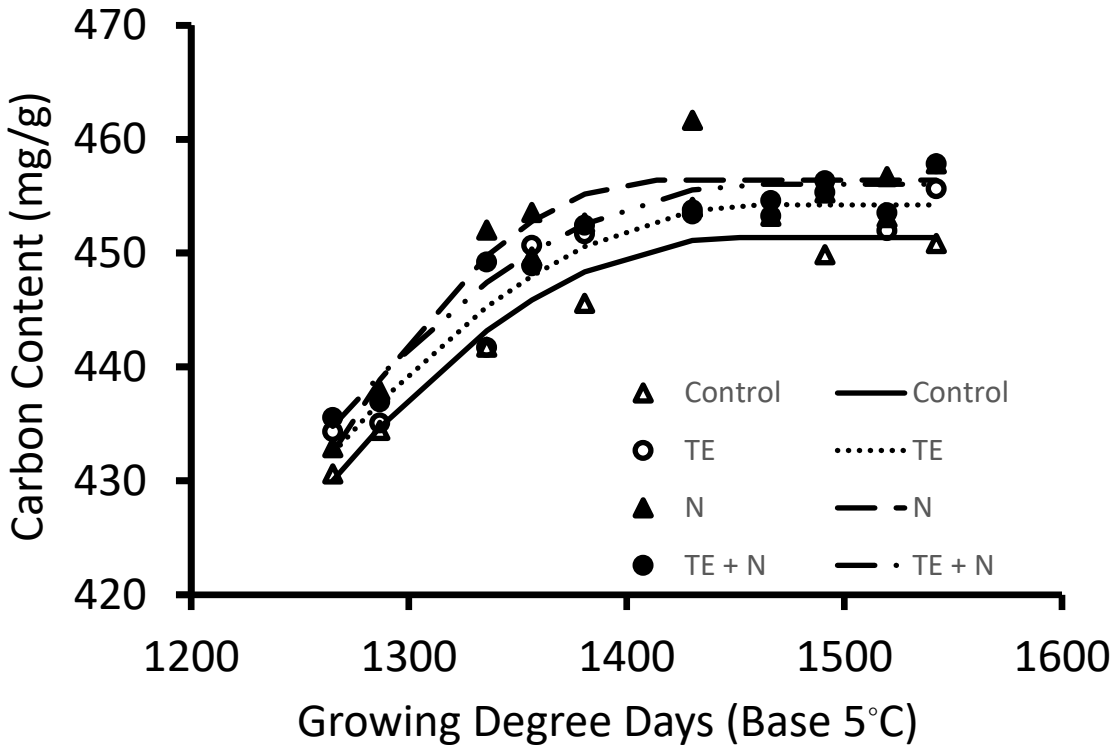

Figure 4.1. Relationship of growing degree days and seed carbon content over the course of seed development in orchardgrass in 2019. Seed carbon was determined on seed collected from proximal spikelet position. Lines represent regression function for each treatment.

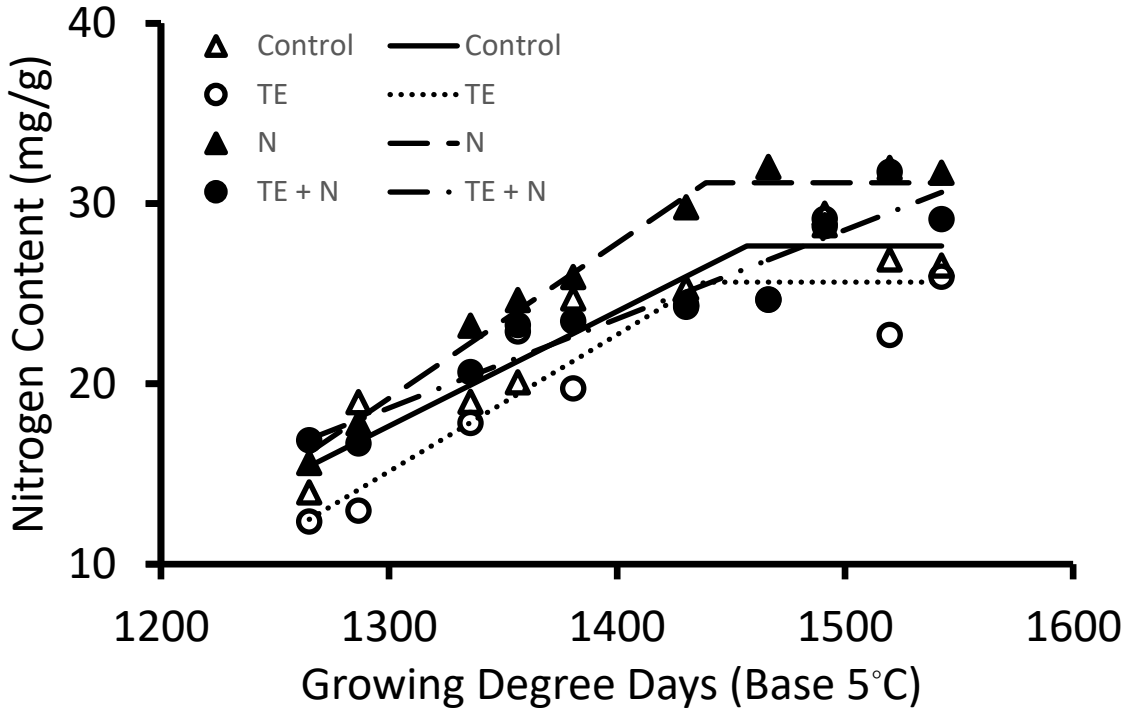

Figure 4.2. Relationship of growing degree days and seed nitrogen content over the course of seed development in orchardgrass in 2019. Seed nitrogen was determined on seed collected from proximal spikelet position. Lines represent regression function for each treatment.

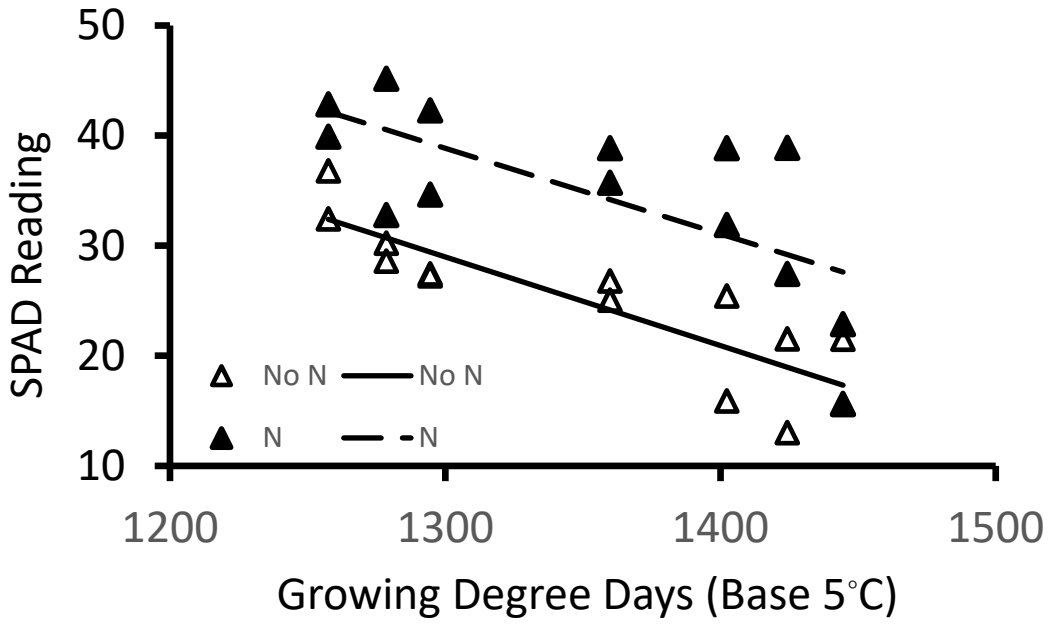

Figure 4.3. Relationship of growing degree days and flag leaf chlorophyll meter readings over the course of seed development in orchardgrass in 2018. Lines represent regression function for each treatment.

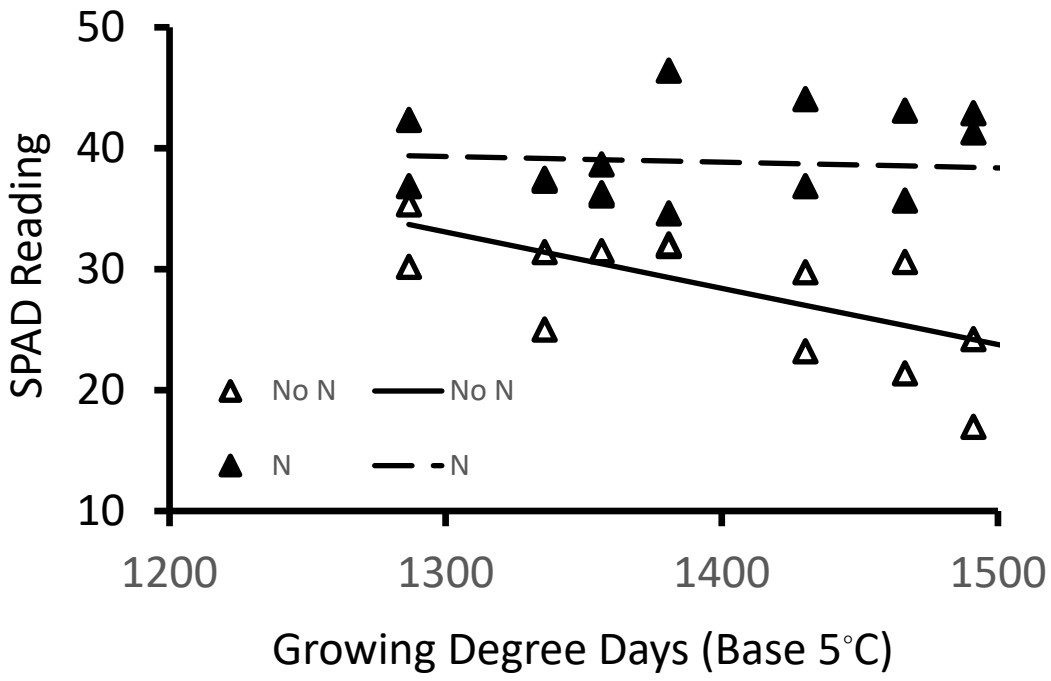

Figure 4.4 Relationship of growing degree days and flag leaf chlorophyll meter readings over the course of seed development in orchardgrass in 2019. Lines represent regression function for each treatment.

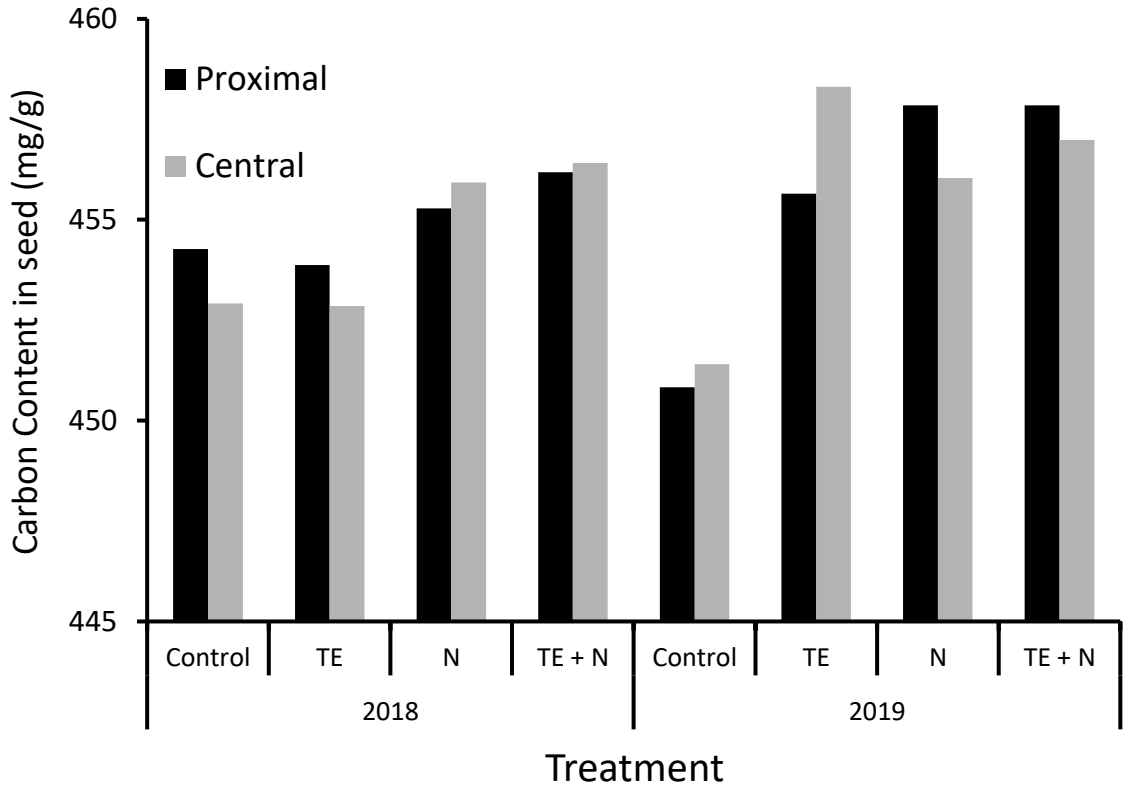

Figure 4.5 Final seed carbon content in proximal and central orchardgrass seed. Seed was collected at 19 June 2018 and 19 June 2019 (GDD 1533 in 2018 and GDD 1542 in 2019).

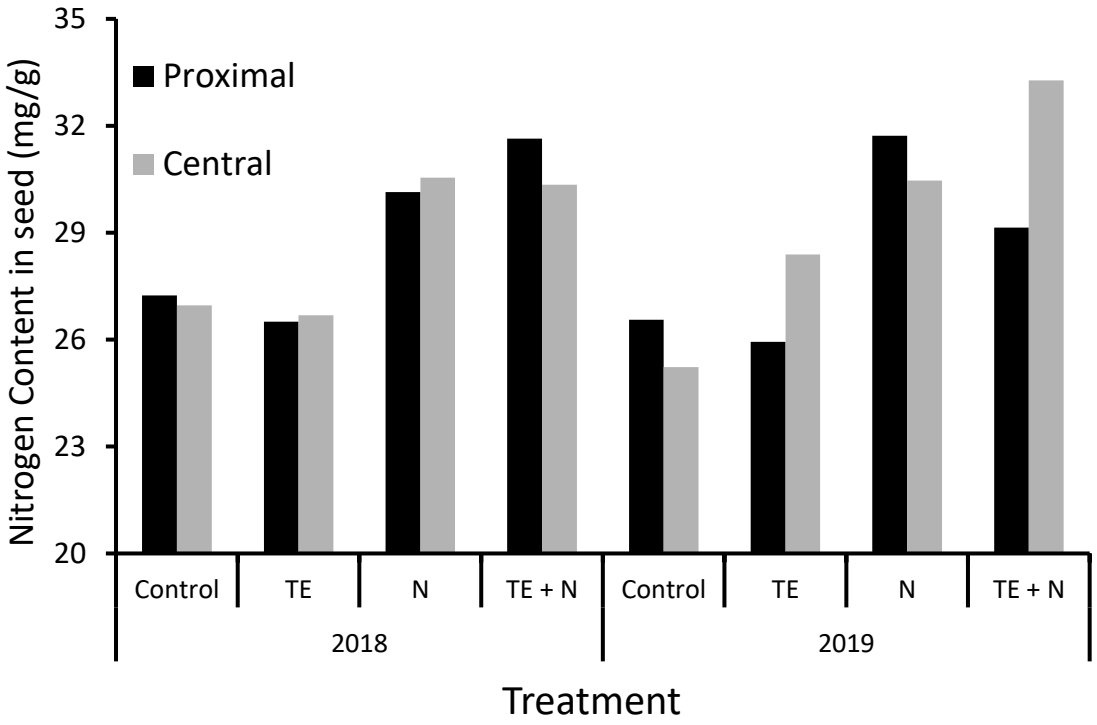

Figure 4.6 Final seed nitrogen content in proximal and central orchardgrass seed. Seed was collected at 19 June 2018 and 19 June 2019 (GDD 1533 in 2018 and GDD 1542 in 2019).

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Appendix

A. PROC GLM code for linear regression

```
proc sort data = data;
by trt;
run;
proc glm data = data plots = diagnostics;
by trt;
model y = x;
run;
proc glm data = data plots = diagnostics;
by trt;
model y = x x*x;
run;
```

B. PROC NLIN code for linear to plateau regression

```
proc nlin data=data method=marquardt plots=fit;
by trt;
parameters a=-90 b=0.5 j=400; /* parameters determined from PROC GLM
and initial j can be chosen based on data inspection*/
/* left linear segment*/
if x <= j then do;
 model y = a + b*x;
   end;
/* right linear segment*/
else do;
 model y = a + b*j;
   end;
   estimate 'plateau'    a + b*j;
   estimate 'breakpoint'  j;
output out=newdata predicted=yhat;
ods output ParameterEstimates=parm;
run;
proc print data=parm; run;
```

C. PROC NLIN code for curvilinear to plateau regression

```
proc nlin data=data method=marquardt plots=fit;
title 'Segmented model - carbon content ';
by trt;
   parms alpha=337.0489087 beta=0.4892755 gamma=-0.0005164; /*
parameters determined from PROC GLM and initial j can be chosen based
on data inspection*/
   x0 = -.5*beta / gamma;
   model y = (x <x0)*(alpha+beta*x +gamma*x*x) +
             (x>=x0)*(alpha+beta*x0+gamma*x0*x0);
             estimate 'plateau'    alpha + beta*x0 + gamma*x0*x0;
             estimate 'breakpoint' -0.5*beta / gamma;
             output out=newdata2 predicted=yhat2 ;
                   ods output ParameterEstimates=parm2;
run;
proc print data=parm2; run;
```