ABSTRACT

Herbicide resistant weeds are a serious problem plaguing the agricultural community today. Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.)) is a major weed affecting winter wheat (*Triticum aestivum* L.) production in the southeastern United States, causing large reductions in grain yield and slowing harvesting operations. Resistances to diverse chemistries such as ACCase inhibitors (Hoelon and Axial XL) and ALS inhibitors (Osprey and Powerflex) have been identified and are common. The growing presences of herbicide resistant Italian ryegrass biotypes have increased interest from the North Carolina State University Small Grains Breeding Program to develop weed suppressive wheat cultivars. The objective of this study was to identify quantitative trait loci (QTL) associated with Italian ryegrass suppression in the AGS 2000 x NC-Neuse soft red winter wheat recombinant inbred line (RIL) population. A mapping population containing 140 F5-derived RILs generated from the cross between AGS 2000 x NC-Neuse was planted in a randomized complete block design with two replications per location at Salisbury, Kinston and Plymouth, North Carolina in 2014 and 2015. RILs and parents were evaluated in 3 sq. m plots over seeded with 175 Italian ryegrass seeds in a 1.5 sq. m section in the center of each plot. Ryegrass head counts (Zadoks GS 80), estimates of early plant vigor (Zadoks GS 29-30) and late plant vigor (Zadoks GS 55) plus three plant height measurements (Zadoks GS 29-30, 55 and 70-80) were collected during the growing season to identify morphological traits and molecular markers correlated with weed suppression. After all height measurements were collected, an area under the height progress curve (AUHPC) was calculated to evaluate the accumulation of growth throughout the season. The morphological
traits with the strongest correlation with ryegrass head counts were AUHPC, height at Zadoks GS 29-30 (HT1), early vigor (Zadoks GS 29-30), and late vigor (Zadoks GS 55). This indicated that ryegrass suppression was most closely associated with taller, more vigorous plants starting early in the growing season and maintained through head emergence. The plant measurements were subsequently utilized to identify putative QTL associated with weed suppressive ability. Multiple interval mapping (MIM) was performed using Windows QTL Cartographer v2.5 and a previously developed map with twenty seven linkage groups containing over 1800 single nucleotide polymorphisms (SNP), simple sequence repeat (SSR), and diversity array technology (DArT) markers. Five putative QTL were identified in multiple environments and/or traits and therefore considered reproducible. The QTL mapped to chromosomes 2B, 5B, 6A, and 7D.2. These QTL’s were co-localized with major effect genes, such as Ppd-B1 located on chromosome 2B, Vrn-B1 located on chromosome 5B, a Rht genes located on chromosome 6A, and Vrn3 gene located on chromosome 7D. This research indicated that selecting lines containing the AGS 2000 allele at both the Vrn-B1 and Vrn-D3 loci, while selecting for either parental allele at the Ppd-B1, Qrgj.nc-5B-2, and Qrgj.nc-6A loci, combined with selection for tall plants at GS 29-30, with vigorous, erect growth providing both ground cover and increased plant height accumulation throughout the entire growing season with a medium heading date, should improve the ryegrass suppressive ability in North Carolina adapted wheat cultivars.

by
Matthew Clyde Granberry

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APPROVED BY:

_______________________________
Dr. J. Paul Murphy
Committee Chair

_______________________________
Dr. S. Chris Reberg-Horton
_______________________________
Dr. Gina Brown-Guedira
BIOGRAPHY

Matt Granberry was raised in Salisbury, North Carolina. He grew up on a small family farm where his love for agriculture began as a small child. Matt graduated from West Rowan High School in the spring of 2007 and entered college in the fall of 2007 at the University of North Carolina at Asheville, majoring in Chemical Engineering. After two years of engineering school, Matt had to return home and help with the family farm, due to illness in the family. He enrolled at Rowan-Cabarrus Community College while at home to continue taking classes. While home, his love for agriculture grew more and he decided to change his major from Chemical Engineering to Plant and Soil Science. After a year at RCCC, he transferred to North Carolina State University and majored in Plant and Soil Science. During his time at NCSU as an undergraduate, Matt joined FarmHouse Fraternity, worked as a summer/part-time worker for the NCSU Small Grains Breeding program, USDA Soybean Breeding Program, and the NCSU Plant Pathology Program (with corn). In December of 2012, he graduated, with honors, with a B.S. in Plant and Soil Science-Crop Production and minored in Agricultural Business Management. In the fall of 2013, Matt began his master’s degree in Plant Breeding and Genetics under Dr. Paul Murphy in the North Carolina State University Small Grains Breeding Program. After graduation, Matt will begin his position as the Cotton Field Biologist for PhytoGen Cotton, a subsidiary of Dow Chemical, located in Leland, Mississippi.
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Literature Review
**Introduction**

Common wheat (*Triticum aestivum* L) is a staple food and feed crop grown and consumed throughout the world. World wheat production has been on a continuous increase over the past decade, with a total world production in 2013-2014 of over 728 million tonnes (FAOSTAT, 2015). From 1961-2014, the United States saw an increase in wheat production from over 33 million tonnes to over 55 million tonnes. Wheat yields throughout the world have increased at an overall average rate of 2.24 percent per year over the past 50 years (Shiferaw et al., 2013). Developing countries had a larger average yield growth rate of 2.8 percent, whereas, developed countries had a 2.2 percent yield growth rate. Wheat accounts for one fifth of the global food supply and ranks second in source of calories for developing country diets (Singh et al., 2011). In developing countries, wheat provides roughly twenty percent of food calories and protein in the diet. By 2050, demand for wheat is projected to increase by 60 percent, so wheat producers around the world will need to produce roughly 1.2 billion tonnes of wheat annually by that date to meet this demand (Singh et al., 2011).

With the introduction of wheat cultivars containing dwarfing (*Rht*) genes, the abundant use of nitrogen fertilizers and implementation of good agriculture practices since the Green Revolution, wheat producers throughout the world have continued to produce higher yields. Despite the use of new cultivars and good agricultural practices, wheat production is still limited by both abiotic and biotic stresses affecting grain yield. Common biotic stresses are viral and fungal diseases, and insect infestations. However, the most important biotic stress is weed competition which limits the space, water and nutrients available for the wheat plant (Shiferaw et al., 2013). The severity of weed competition has prompted wheat breeders to start identifying traits that can express weed suppressive ability in new cultivars.
The major abiotic stresses for wheat producers are salinity, heat and drought stress. With climate change becoming a big factor impacting global wheat production, breeding for both biotic and abiotic stress has become an important focus of the agriculture community (Oritz et al., 2008). To continue increasing wheat yields throughout the world, wheat breeders must continue developing new cultivars containing desired traits such as milling and baking quality, grain yield, test weight, maturity, heat and drought tolerance, disease and insect resistance, and weed suppression ability.

**Origin and Evolution of Wheat**

Wheat was domesticated roughly 10,000 years ago in the Fertile Crescent, an area in the Middle East spanning present day Lebanon, Jordan, Israel, western Syria, southeastern Turkey and along the Tigris and Euphrates Rivers into Iraq and western Iran (Salamini et al., 2002). Wheat is a self-pollinated allohexaploid (2n = 6x = 42), composed of three genomes (AABBDD) contributed from related wild species. Roughly 500,000 years ago, wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides* Körn. ex Asch & Graebn.) (AABB; 2n = 4x = 28) arose from an interspecific cross between two related diploid species and subsequent chromosome doubling. The A genome was donated by *T. urartu* Tum. Ex Grand (Dvorak et al., 1993). The B genome donor is unknown, but the B genome of common and emmer wheat shares strong synteny with the S genome of *Ae. speltoides* (Tausch.) (Talbert et al., 1991; Wang et al., 1997). Hexaploid common wheat is the outcome of a spontaneous chromosome doubling event following hybridization between the tetraploid emmer wheat and diploid *Ae. tauschii*, the D genome donor (McFadden and Sears, 1946; Dvorak et al., 2012).
Wheat in North Carolina

The U.S. Wheat Associates (http://www.uswheat.org/) divides wheat into six different market classes: hard red winter, hard red spring, soft red winter, soft white, hard white, and durum. The six wheat classes are determined by color, kernel hardness and growing seasons giving customers a variety of options when selecting quality characteristics for specific end uses and products. In North Carolina and most areas east of the Mississippi River, soft red winter wheat is the market class best suited for production. Soft red winter wheats contain ideal characteristics for products such as crackers, cookies, pretzels, cakes and other pastries. Wheat from North Carolina is used for human as well as animal consumption, with the relative proportions varying annually based on factors such as the relative prices of wheat and maize (Zea mays L.), and state production of wheat and maize.

Wheat is used as an off-season crop in the maize and short-season soybean (Glycine max) rotation commonly used in North Carolina. Wheat is normally double-cropped with short-season soybeans, typically planted from mid-October through late-November, starting in the western part of the state and moving east as the planting season progresses (Weisz, 2013). Wheat harvest commonly starts in late-May, early June and continues into early-mid July. Once the wheat has been harvested, producers plant the short-season soybeans using a no-till system, and harvest the soybean crop between October and November. In the following spring, producers plant maize to complete the wheat-soybean-maize rotation.

The USDA National Agricultural Statistics Service (2015) reported that North Carolina wheat producers planted 327,795 ha (810,000 acres) in 2012, 400,638 ha (990,000 acres) in 2013, and 335,889 ha (830,000 acres) in 2014, while harvesting over 91 percent of hectares planted each year. In 2012 and 2013, North Carolina produced an averaged wheat yield of 3.8
tonnes/ha and in 2014 the average wheat yield had increased to 3.9 tonnes/ha. The large increase in wheat planted during 2013 resulted from the record high prices of wheat at $267.13 tonne\(^{-1}\) ($7.27 bu\(^{-1}\)) (NCDA&CS, 2013).

**Organic Wheat**

The USDA defines organic agriculture as “the process of producing products using methods that preserve the environment and avoid most synthetic materials, such as pesticides and antibiotics” ([www.usda.gov](http://www.usda.gov)). Since 2004, organic cereal hectares throughout the world have more than doubled from 1,233,801 ha to 3,309,788 ha in 2013 (Willer and Lernoud, 2015). Of the over three million hectares planted in 2013, 36 percent was planted in wheat. Wheat continues to be the top organic cereal, based on hectares planted, from year-to-year throughout the world. The United States continues to be a world leader in organic cereals production, with 330,000 ha, only behind China with 600,000 ha produced in 2013. Since the 1990’s, the organic industry in the United States has seen a double-digit increase in consumer demand every year with sales increasing from $3.6 billion in 1997 to $39 billion in 2014 ([www.ota.com](http://www.ota.com)). With the double-digit increase in consumer demand each year, it has become difficult for organic producers to answer the demand from consumers. North Carolina has seen an increase in the amount of hectares planted in organic wheat from 28 ha in 2000 to 704 ha in 2011 ([www.ers.usda.gov](http://www.ers.usda.gov)).

In order to obtain an organic certification in the United States, producers must implement and follow the rules and regulations developed by the USDA: National Organic Program (NOP) ([www.ams.usda.gov](http://www.ams.usda.gov)). With a continuing increase in demand for organic wheat for human consumption, there has been an increase in the demand for organic meats as
well. To comply with the organic regulation outline by NOP, any animals labeled organic have to be fed certified organic feed, except for vitamins and trace minerals as needed for the animal’s nutritional requirements. This demand for organic animal feed has continued to rise, adding to the increased demand placed on organic wheat producers.

**Organic Wheat Breeding**

Organic producers are continuously battling to achieve high crop quality and yield while employing environmentally acceptable farming practices (Murphy et al., 2007). The majority of organic producers today are burdened by a lack of cultivars suitable for organic production. Producers are left selecting cultivars that were developed in one of three types of breeding programs: (1) conventional breeding programs that, by luck, have cultivars that perform tolerably in organic systems, (2) hybrid conventional / organic breeding programs that, for economic reasons, use conventional methods for early generation selection (F₁-F₅) then conduct advanced stages under organic conditions and (3) organic breeding programs that conduct all stages of cultivar development under organic conditions (Wolfe et al., 2008). Murphy et al. (2007) compared grain yield and test weight of wheat cultivars evaluated in both conventional and organic systems. They found a significant genotype by system interaction, expressing the need for utilizing an organic breeding program whose goal is to produce high quality, high yielding organic wheat cultivars.

Despite a continuous increase in hectares planted and double-digit increase in consumer demand every year, organic wheat production still contributes a small amount to the overall wheat production in North Carolina ([www.ers.usda.gov](http://www.ers.usda.gov)). Because organic wheat production is contributing a small amount of total hectares planted, it would not be efficient to add an
organic wheat breeding program to the current Small Grains Breeding Program at North Carolina State University (NCSU) at this time. However, a greater understanding of the morphological traits important in organic wheat cultivars and the genetic control of those traits would be beneficial to the wheat breeders interested in serving both the conventional and organic communities.

Important traits for organic agriculture include increased nutrient-use efficiency, weed competitive ability and resistance to disease and insect damage (Murphy et al., 2007). Because these traits are traditionally provided for, or supplemented by, synthetic fertilizers, herbicides and pesticides in conventional agriculture, organic breeders have to focus their programs on these characteristics as well as addressing the standard grain yield, test weight, end-use quality, and genotype by environment (GxE) interactions (Lammets van Bueren and Meyer, 2012; Murphy et al., 2007). Organic programs will have to look at expanding the adaptation of organic cultivars across more environments or breed cultivars for each unique environment.

To produce high yielding wheat in organic systems, management of soil fertility is very important. If soil pH is too high, manganese deficiencies can occur, causing wheat plants to have a discolored, stunned growth that could lead to plant death if not properly addressed (Crozier et al., 2013). Whereas, if soil pH is too low, wheat plants will have poor growth and development resulting in reduced grain yield. Nitrogen is one of the many key macro- and micro-nutrients needed to produce a healthy, high yielding wheat crop. Because most nitrogen is supplied from synthetic sources for conventional systems, organic producers are limited to use of organically approved sources such as compost, manure, or incorporated cover crops such as legumes (Weisz et al., 2013). With such great importance placed on soil fertility to aid
in the ability of organic produces to maintain high yield, an emphasis has been put toward breeding for increased nutrient-use efficiency in organic cultivars.

Another priority area for organic cultivar development is utilization of genetically based disease and insect resistances. Disease and insect infestations can reduce grain yield and quality just as much as poor soil fertility. The Small Grains Breeding Program at NCSU currently evaluates cultivars for major disease and pests problematic to North Carolina producers such as Cereal Leaf Beetle (*Oulema melanopus*), Hessian fly (*Mayetiola destructor*), Stripe rust (*Puccinia striiformis* f. sp. *tritici*), Leaf rust (*Puccinia triticina* f. sp. *tritici*), Powdery Mildew (*Blumeria graminis* f. fp. *tritici*), Fusarium Head Blight (*F. graminearum*), *Stagonospora nodorum* Blight, Barley Yellow Dwarf Virus, and Soil Borne Wheat Mosaic Virus. Though the NCSU Small Grains Breeding Program is not tasked with producing organic cultivars, cultivars from programs like this can provide organic breeders a source of resistance to integrate into future organic cultivars. With the prohibition on the use of synthetic insecticides and fungicides, organic producers have few options to combat the infestation and damage caused by these biotic stresses other than inherent genetic resistances.

Weed infestation is a widespread problem affecting organic wheat producers. With limited herbicide options approved for organic use (such as acetic acid (distilled vinegar), corn gluten meal products, clove oil and boiling water) accompanied with the high cost of certified organic herbicides, developing cultivars with weed suppression would greatly increase organic wheat production (Reberg-Horton et al., 2013). In North Carolina, wheat producers most commonly encounter annual broadleaf weeds such as henbit (*Lamium amplexicaule* L.) and chickweed (*Stellaria media* (L.) Vill.), along with perennials such as curly dock (*Rumex crispus* L.), wild garlic (*Allium vineale* L.) and the most problematic, Italian ryegrass
(Lolium perenne L. ssp. multiflorum (Lam.)) (Everman and Jordan, 2013). With the increased frequencies of herbicide-resistant weeds plaguing producers in both conventional and organic systems, developing cultivars containing weed suppressive abilities will greatly benefit both production systems.

**Weed Competition**

Due to the rapid increase in herbicide resistant weeds which plague the agricultural community today, the production of varieties containing weed suppressive traits has become an expanding area of research that is garnering recognition among breeding programs. Some breeding programs have begun evaluating and selecting for weed suppression traits within their breeding nurseries, alongside evaluating and selecting for traditional traits such as yield, test weight, and disease and insect resistance.

With this continued improvement of traits within modern cultivars, such as increased yield and test weight, the practice of applying herbicides to breeding nurseries may have decreased the ability of a program to select for cultivars with weed suppressive ability (Worthington and Reberg-Horton, 2013). There has been research proposing that older or “historical” cultivars actually contained superior weed suppressive ability over modern cultivars (Mason et al., 2007; Murphy et al., 2008; Wolfe et al., 2008; Wicks et al., 2004; Lemerle et al., 2001; Bertholdsson, 2004). Research in many countries around the world has identified the desired morphological traits breeders can use to evaluate and select for weed competitiveness in rice (Oryza sativa L.), wheat, and barley (Hordeum vulare L.). The traits identified were end of season plant height for wheat in Nebraska (USA), Canada, Australia, Washington (USA) and for rice in Arkansas (USA) (Challaiah et al. 1986; Blackshaw 1994; Lemerle et al. 1996; Mason et
al. 2007; Murphy et al. 2008; Gealy and Moldenhauer 2012). Leaf angle and canopy structure were found to be important for wheat in Canada, Australia, the United Kingdom, and Germany and in barley in the United Kingdom (Huel and Hucl 1996; Lemerle et al. 1996; Seavers and Wright 1999; Korres and Froud-Williams 2002; Drews et al. 2009). Tillering capacity was a key trait in wheat in Nebraska (USA), Canada, Australia and the United Kingdom (Challaiah et al. 1986; Blackshaw 1994; Lemerle et al. 1996; Coleman et al. 2001; Korres and Froud-Williams 2002; Wicks et al. 2004; Mason et al. 2007; Mason et al. 2008). Early vigor was identified as an important trait for weed suppression for wheat in Nebraska (USA), Argentina, and Canada, as well as for rice in the Philippines (Wicks et al. 1986; Huel and Hucl 1996; Acciaresi et al. 2001; Zhao et al. 2006a; Mason et al. 2007). Maturity was found to be an important trait for wheat in Canada (Huel and Hucl 1996; Mason et al. 2007).

Worthington et al. (2013) identified morphological traits that were highly correlated with weed suppression in soft red winter wheat grown in North Carolina environments (measured by relative numbers of rye grass head counts at GS 70-80). The traits identified were three plant height measurements recorded at Zadoks Growth Stage (GS) 29-30 (HT1), GS 55 (HT2) and GS 70-80 (HT3) (Zadoks et al., 1974). Subsequently, an Area Under the Height Progress Curve (AUHPC) was calculated to create a comprehensive measurement of height accumulation throughout the growing season. In addition, two visual ratings of early and late vigor were recorded at GS 29-30 (Vig1) and GS 55 (Vig2). For the North Carolina environments, the ideal plant suggested for weed suppression was a tall, vigorous plant starting growth early following the completion of vernalization and maintaining vigorous growth through head emergence. It would exhibit a medium, or mid-season, heading date to avoid late spring freeze events that are common in North Carolina.
**Italian Ryegrass**

Italian ryegrass, also known as annual ryegrass, is native to Europe (Lamp et al., 2001). It is a diploid member of the *Lolium* family, along with perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Lolium arundinaceum* Schreb.) (www.ogtr.gov.au).

Italian ryegrass is an annual or biennial ryegrass species that survives one to two years before re-seeding is needed (www.nrcs.usda.com). It has a wide adaptation area, preferring darker soils with a milder climate and does not grow well under hot, dry conditions. Plants grow in a bunch form, with multiple leaves that are a green to dark-green color with hairless, evenly ribbed upper leaf surface with a smooth, green, waxy lower surface and are rolled within the bud prior to emergence. The flower seed heads are spikes that run along the main stem, with each spike containing multiple spikelets. Italian ryegrass is a wind-pollinated, highly out-crossing species sexually reproduced through seed production. Because Italian ryegrass is classified as an annual, it is supposed to only reproduce sexually, but it is possible for this species to reproduce asexually, especially in areas that are grazed or mowed regularly.

Italian ryegrass is a turf and forage grass in most areas of the world such as Australia, New Zealand, South Africa, North and South America (Lamp et al., 2001). It is an ideal plant for pastures and erosion control due to its persistent growth, quick establishment and ability to withstand rotationally grazing. For turf applications, it does not contain the desired characteristics for high quality turf, such as fine leaf texture, density, and ability to withstand low cutting heights, but it is an inexpensive alternative that can be easily maintained making it excellent for low profile locations such as schools, parks, and some home lawns (http://www.turffiles.ncsu.edu). In both pasture and turf situations, Italian ryegrass is a useful
over-seeded winter cover crop producing green, living plants when warm-season cultivars have senesced due to the cold temperatures.

Italian ryegrass has some beneficial applications, but it has quickly become a detrimental invasive weed for the row crop agriculture community. Although it has been problematic for the entire agriculture community, this weed has particularly become a nuisance in conventional wheat production and even more so for organic wheat producers. Heap et al. (2014) ranked Italian ryegrass 11th on the top-20 worst herbicide-resistant weeds globally in wheat production. Italian ryegrass has developed resistance to five different herbicide site-of-action (SOA) groups: ACCase Inhibitors (Group 1), ALS Inhibitors (Group 2), EPSP Synthase Inhibitors (Group 9), Glutamine Synthetase (Group 10), and Long-Chain Fatty Acid Inhibitors (Group 15) (www.takeactiononweeds.com). Herbicide-resistant Italian ryegrass has plagued wheat producers in the United States since it was first discovered in Oregon in 1987 and North Carolina in 1990 (Heap, 2016). The two main SOA groups used by North Carolina wheat producer are ACCase and ALS Inhibitors. A typical herbicide management strategy for post-emergence control of Italian ryegrass is obtained through a tank-mixture of Mesosulfuron-Methyl (Osprey, Bayer CropScience LP, Research Traingle Park, NC) or Pyrozsulam (Powerflex HL, Dow AgriSciences LLC, Indianapolis, IN) with Pinoxaden (Axial XL, Syngenta, Greensboro, NC) (Everman and Jordan, 2013). For additional broad-spectrum broadleaf weed control a producer would apply Thifensulfuron-methyl plus Tribenuron-methyl (Harmony® Extra, Dupont, Wilmington, DE) post-emergence. The problem producers face with this popular herbicide rotation is Italian ryegrass has resistance to both SOA’s for these chemicals, because Osprey and Powerflex HL are ALS Inhibitors and Axial XL are ACCase Inhibitors. This places wheat producers at a significant disadvantage in controlling Italian
ryegrass. If herbicide-resistance is present, producers are left to use mechanical cultivation, hand-pulling weeds, or in extreme cases abandoning infested fields.

Organic wheat production does not have the luxury of synthetic herbicides to control Italian ryegrass. There are some herbicides available for organic producers, such as acetic acid (distilled vinegar), corn gluten meal products, clove oil and boiling water, but these products are exceedingly expensive and have a lower efficacy on weeds (Reberg-Horton et al., 2013). Organic weed management is largely dependent on cultural and mechanical control. Cultural control is management through proper crop rotation, cultivar selection, seed quality, planting date, seeding rate, managing soil fertility and use of off-season cover crops. Mechanical control is management through tillage, such as blind cultivation using tine weeders, springtooth harrows, chain link harrows, or rotary hoes; between-row cultivation using rolling cultivators or low residue cultivators; flame weeding, or hand weeding which is extremely costly in time and labor.

Appleby et al., (1976) conducted a two-year study to estimate the amount of ryegrass present in a winter wheat field in year two based on the amount of Italian ryegrass control implemented in year one. If there was insufficient Italian ryegrass control in year one the potential for ryegrass infestation in year two was higher especially if followed by another season of winter wheat. The main detrimental effect of poor ryegrass control in year-one was the large increase in amount of Italian ryegrass seed now present in the soil bank. Italian ryegrass is a type I transient seed bank producer (Thompson and Grime, 1979). The characteristics of type I transient seed banks are the ability to take advantage of gaps in between seasonal vegetation cover, large seed size, lack of dormancy mechanisms, the ability to germinate in a wide range of temperatures, and germinate in both light and complete dark...
Due to these characteristics, it is hard to determine the longevity of Italian ryegrass seed. Narwel et al. (2008) investigated the effects of time, depth of seed burial, soil type, and rainfall patterns has on the longevity of Italian ryegrass seeds. It was found that over a short amount of time (several months) rainfall, depth of seed burial and soil type had an effect on the condition and emergence pattern among the seed. Also, it was found that the ryegrass seeds lost all viability, regardless of treatment, after being buried for at least 16 months. Typically, Italian ryegrass will germinate whenever the conditions are suitable and with the wide range of suitable conditions this cycle could continue year-round. For both organic and conventional producers, control of Italian ryegrass is achieved through the synergistic use of multiple weed control methods, not only during the wheat cropping season, but throughout each year of the crop rotation.

**Flowering Time**

In North Carolina the average date for the final killing frost ranges from late-March in eastern North Carolina to mid-May in western North Carolina (Perry, 1996). Heading date is controlled genetically by three important components: vernalization, photoperiod, and narrow-sense earliness (Kitagawa et al., 2012). In most seasons in North Carolina, adapted wheat cultivars have met their vernalization and photoperiod requirements by early- to mid-March when they advance to Zadoks GS 31, the start of reproductive growth. Adapted cultivars typically flower in the April 1st to April 21st period.
Vernalization Genes

Vernalization is defined as extended exposure to cold temperatures causing a transition from vegetative to reproductive growth within plants (Amasino, 2004). Vernalization is a common flowering requirement for many biennial vegetable crops such as beets (*Beta vulgaris*), turnips (*Brassica rapa* subsp. Rapa), carrots (*Daucus carota* subsp. Sativus), kale (*Brassica oleracea* var. sabellica), and cabbage (*Brassica oleracea* var. Capitate), as well as winter wheat, oats (*Avena sativa* L.), and barley.

The Vernalization1 (*Vrn1*) loci in wheat were described previously by Fu et al. (2005). These loci are flowering promoters that are repressed until a required amount of cold temperature is achieved to permit initiation of flowering. It has been shown that *Vrn1* is the main signal indicator for a wheat plant to begin transitioning the apex from vegetative to reproduction growth (Distelfeld et al., 2009). Within the wheat genome, there are homoeologous copies of *Vrn1* loci, designated as *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*, which were previously designated as *Vrn-1*, *Vrn-2*, and *Vrn-3*. The homoeologous copies are located, respectively, on the long arm of chromosomes 5A, 5B, and 5D (Galiba et al., 1995; Law et al., 1976; Worland et al., 1987). The *Vrn1* loci code for a MADA-box transcription factor, that is orthologous to the *Arabidopsis* meristem identity genes APETALA1 (Yan et al., 2003). If a genotype contains homozygous recessive alleles at all *Vrn1* loci, the genotype will have a winter growth habit and if the genotype has a dominant allele at one or more of the *Vrn1* loci, a spring growth habit will result (Stelmakh, 1987). Guedira et al. (2014) found that AGS 2000 contained the short vernalization allele at *Vrn-B1* and NC-Neuse contains the long vernalization allele at *Vrn-B1*. Both parents require six weeks at 4°C to reach full vernalization. When full vernalization was met (6 weeks), there was no difference for HD
between the two parents, whereas when each parent was only partial vernalization (4 weeks), AGS 2000 headed 34 days sooner than NC-Neuse.

The Vernalization2 (Vrn2) gene was described by Yan et al. (2004) and Fu et al. (2005). This gene is a powerful repressor of flowering that is downregulated by vernalization (Yan et al., 2004). It was mapped on chromosome 5A in a small spring x winter diploid wheat T. monococcum F2 population (Dubcovsky et al., 1998). Vrn2 codes for a zinc finger-CCT domain transcription factor with no clear orthologues in Arabidopsis or rice (Yan et al., 2004). It has an opposite mode of action to the Vrn1 loci. If a genotype contains the dominant allele, the genotype will convey a winter growth habit (Fu et al., 2005). If the function of the Vrn2 gene is lost through mutation or deletion, the genotype will have a spring growth habit.

The Vernalization3 (Vrn3) gene was described by Yan et al. (2006). This gene stimulates reproductive development within the apex and is activated by long day lengths while being regulated upstream by the photoperiod gene Ppd-1(Guedira et al., 2014; Yan et al., 2006). It was mapped to the short arm of chromosome 7B (Yan et al., 2006). The Vrn3 gene codes for a RAF kinase inhibitor like protein with high homology to Arabidopsis protein FLOWERING LOCUS T (FT) (Distelfeld et al., 2009; Yan et al., 2006).

Photoperiod Genes

Photoperiod is defined as the interval in a 24 hour period in which a plant is exposed to light (Turner et al., 2005). In winter wheat, the photoperiod may be important during spring growth because it can determine when the transition from vegetative to reproductive growth occurs. In North Carolina, an increasing photoperiod is associated with flowering. In winter wheat production in North Carolina, an important role of genes controlling photoperiod is the
avoidance of damage from a late spring freeze event, and maturation of the plant prior to extremely high temperatures during the summer months.

Photoperiod is controlled at three loci: *Ppd-A1* previously described by Diaz et al., (2012); *Ppd-B1* previously described by Kitagawa et al., (2012); and *Ppd-D1* previously described by Seki et al., (2013). These loci were mapped to the short arm of chromosomes 2A, 2B, and 2D respectively (Scarth & Law, 1983, 1984; Welsh et al., 1973). The *Ppd-A1* locus was previously designated as *Ppd1*, the locus *Ppd-B1* was previously designated as *Ppd2*, and the locus *Ppd-D1* was previously designated as *Ppd3*. The alleles at the *Ppd* loci are characterized with the suffix “a” or with the suffix “b”. The suffix “a” indicates that the allele is photoperiod insensitive, whereas suffix “b” indicates that the allele is photoperiod sensitive (the wild allele). Example nomenclature would be *Ppd-A1a* or *Ppd-A1b* for the photoperiod insensitive and photoperiod sensitive alleles at the *Ppd-A1* locus (Diaz et al., 2012).

Photoperiod sensitive plants are classified as either short day (SD) or long day (LD) (Laurie, 1997). Short day plants flower when the plant is exposed to a short period of light (less than 12 hours) and a long period of dark (more than 12 hours) within a 24 hour time frame. Maize and rice are notable row crops that are SD plants. Long day plants induce flowering when the plant is exposed to a long period of light (more than 12 hours) and a short period of dark (less than 12 hours) within a 24 hour time frame. Wheat, barley and rye (*Secale cereale* L.) are some of the major row crops that are LD plants. Whether a plant is SD or LD, there will be a delay in flowering if grown under non-favorable conditions.
Linkage Maps and QTL Mapping

A linkage map shows the relative positions of genetic loci on chromosomes, determined on the basis of how often the loci are inherited together. A linkage-based map is used to identify DNA markers within genomic regions containing genes or Quantitative Trait Loci (QTL) by comparing the impact of contrasting alleles at the loci with the observed phenotype of the trait of interest. This is known as QTL mapping. A QTL is a region of a chromosome that contains one or more sequence variants that controls the distribution of a trait within an interbreeding population. The position of each gene or marker relative to another gene or marker is expressed in the form of a recombination frequency. The recombination frequency is not an expression of physical distance between genes or markers; rather it is a frequency with which a single chromosomal crossover will occur between two genes or markers during meiosis. Recombination frequencies are expressed in a unit called a centimorgan (cM), where each centimorgan represents a recombination frequency of one percent. Generally, the lower the recombination frequency between two genes or markers the closer together the genes or markers are on the chromosome and the higher the recombination frequency between two genes or markers the farther away on the chromosome the genes or markers are from each other. If genes or markers have a recombination frequency less than 50 percent they are considered “linked” and if the recombination frequency is greater than 50 percent they are considered “unlinked.”

To conduct linkage analysis, a segregating bi-parental population, such as segregating F2, backcross, recombinant inbred lines (RIL), or double-haploid (DH) must be used (Collard et al., 2005). In QTL mapping, DH and RIL populations are the most utilized populations due to the ability of these populations to produce homozygous lines that are easily multiplied
without genetic change. Also, DH and RIL populations can be easily shared for new evaluations and the addition of new markers to existing QTL maps (Paterson, 1996). There are multiple models that can be used to identify QTL within bi-parental populations such as: single marker analysis (SMA), simple interval mapping (SIM) (Lander and Botstein, 1989), composite interval mapping (CIM) (Jansen, 1993), and multiple interval mapping (MIM) (Jansen, 1993).

The first model that can be used to identify QTL is single-marker analysis. It is the simplest model that can be conducted by hand, utilizing a t-test, analysis of variance (ANOVA), and linear regression (Collard et al., 2005). Linear regression is the most commonly used statistical method, because its coefficient of determination ($R^2$) for each marker will explain the phenotypic variation associated with the QTL linked to the marker. There are some disadvantages to this method; the greater the distance between a QTL and a marker, there is less ability to detect the QTL. Recombination can occur between the marker and QTL, causing the effect of the QTL to be underestimated ( Tanksley, 1993). For example, if a large effect QTL is associated with a marker, but it is located at a great distance from the marker, then its observed effect may be equivalent to a small effect QTL closely linked to the marker.

Simple interval mapping allows a set of linked markers to be analyzed at the same time to see their effects on a QTL (Lander and Botstein, 1989). Because this model examines a set of linked markers, recombination between QTL and markers can be compensated for, thus increasing the power to detect a QTL (Collard et al., 2005). Simple interval mapping is better than SMA when markers are 15-35 cM away from each other (Tanksley, 1993). If marker density is less than 15 cM apart, there is no difference and SMA and SIM analysis are basically
the same. If marker density is larger than 35 cM apart, SIM has an inefficient ability to detect QTL between markers.

Composite interval mapping (CIM) merges SIM with linear regression and incorporates other unlinked QTL effects from random markers as cofactors within the model to increase the accuracy (Collard et al., 2005). With the incorporation of the unlinked QTL into the model, the residual variation from other QTL is reduced, causing an increase in power for QTL detection.

Multiple interval mapping (MIM) uses the same method of interval analysis as SIM and CIM, where unlinked QTL are incorporated into the model as cofactors. With MIM, however, instead of having just one QTL model, there are multiple models included that will fit the QTL’s to their maximum likelihood positons (Jansen, 1993). By fitting QTL to their maximum likelihood positons, MIM can evaluate QTL effects with the precision and power of SIM.

Objective

The immediate objective of this research was to identify quantitative trait loci (QTL) associated with Italian ryegrass suppression in the AGS 2000 x NC-Neuse soft red winter wheat recombinant inbred line population. In the long term, the results of this research could impact the use of marker assisted selection to more efficiently develop future wheat cultivars incorporating ryegrass suppression traits alongside other important traits to be utilized by both the organic and conventional wheat producers in North Carolina.
MATERIALS AND METHODS
**Plant Material**

One hundred and forty F$_5$-derived recombinant inbred lines (RILs) generated from the cross between AGS 2000 x NC-Neuse were evaluated for ryegrass suppressive ability in the 2013-14 and 2014-15 growing seasons. AGS 2000 (PI 633037) is a soft red winter wheat released jointly by the University of Georgia and University of Florida (Johnson et al., 2002). This cultivar contains the “$a$” insensitivity allele at the $Ppd-B1$ photoperiod locus, the “$b$” dwarfing allele at the $Rht-D1$ height reducing locus, the short vernalization allele at the $Vrn-B1$ vernalization locus and the 1BL:1RS translocation. NC-Neuse (PI 633037) is a soft red winter wheat released by North Carolina State University (Murphy et al., 2004). This cultivar contains the $Ppd-B1$ null allele at the photoperiod locus, the “$b$” allele at the $Rht-D1$ height reducing locus and the long vernalization allele on the $Vrn-B1$ vernalization locus. In addition, NC-Neuse contains the 2BS:2GS:2GL:2BL translocation chromosome on chromosome 2B, that contains the stem rust resistance gene $Sr36$. The $Sr36$ gene was transferred to hexaploid wheat from timopheevi wheat (*Triticum timopheevii* Zhuck.) (Olson et al., 2009). This translocation is likely the reason for the segregation distortion and reduced recombination on chromosome 2B within this RIL population. Other mapping populations have shown preferential transmission and a reduction in recombination was associated with this 2BS:2GS:2GL:2BL translocation (Nyquist, 1962; McIntosh and Luig, 1973; Tsilo et al., 2008). Both, AGS 2000 and NC-Neuse contain the “$a$” photoperiod insensitivity allele at the $Ppd-A1$ photoperiod locus and the “$b$” sensitivity allele at the $Ppd-D1$ photoperiod locus.
Growing Conditions

The experimental design was a randomized complete block with two replications per location. Although three locations were planted each season, data were collected at only two locations each season due to problems beyond the control of the researchers. Data were obtained during the 2013-14 season at the Piedmont Research Station in Salisbury, NC on a Lloyd Clay Loam (fine, kaolinitic, thermic Rhodia Kanhapludults) and the Caswell Research Station in Kinston, NC on a Johns Sandy Loam (fine-loamy over sandy-skeletal, siliceous, semiactive, thermic Aquic Hapludults). Data was obtained during the 2014-15 season at the Caswell Research Station on a Portsmouth Loam (fine-loamy over sandy-skeletal, mixed, semiactive, thermic Typic Umbraquults) and the Tidewater Research Station in Plymouth, NC on a Cape Fear Loam (fine, mixed, semiactive, thermic Typic Umbraquults). From here out, location and year combinations will be referred to as “SAL” for Salisbury, “KIN” for Kinston and “PLY” for Plymouth followed by the year either “14” for the 2013-14 season and “15” for the 2014-2015 season. Five row plots were planted using a calibrated cone drill. Plot length was 2.4 m and rows were spaced 19.1 cm apart. Standard seeding rates for organic wheat production in North Carolina were used (Weisz et al., 2013) and RILs were seeded at 376 seeds m$^{-2}$, based on the mean seed weight of the parents to achieve an approximate uniform plant density. ‘Annual Ryegrass’ (Pennington Seed Inc., Madison, GA), a commercial turf cultivar, was over seeded at a rate of 175 seed m$^{-2}$ in the center 1.5 m$^{2}$ section of each plot. Ryegrass seeds were sown perpendicular to the planted wheat, in the center of each plot with the calibrated drill at a depth of one-half to one centimeter.

Plots were planted on 15 October 2013 at Salisbury and 25 October 2013 at Kinston during the 2013-14 growing season. At Salisbury, nitrogen was applied pre-plant at a rate of
33.6 kg/ha along with top-dressed potassium, phosphorus and micro nutrients as recommended by soil test results. Thifensulfuron-methyl plus Tribenuron-methyl (Harmony® Extra, DuPont, Wilmington, DE) was applied for broadleaf weed control at a rate of 0.04 L/ha. Spring nitrogen was applied on 3 March 2014 at a rate of 76.2 kg/h. At Kinston, nitrogen was applied pre-plant at a rate of 33.6 kg/ha along with top-dressed potassium, phosphorus and micro nutrients as recommended by soil test results. On 20 December 2013, Thifensulfuron-methyl plus Tribenuron-methyl (Harmony® Extra, Dupont, Wilmington, DE) was applied for broadleaf weed control at a rate of 0.04 L/ha. Spring nitrogen was applied, in liquid form, at a rate of 84.2 L/ha of 30 percent nitrogen solution for a total of 33.6 kg/ha of nitrogen on 10 February 2014 and again on 11 March 2014 at a rate of 201.1 L/ha of 30 percent nitrogen solution for a total of 78.5 kg/ha of nitrogen. On 23 April 2014, Lambda-cyhalothrin1,2 (Warrior II with Zeon Technology, Syngenta, Greensboro, NC) was applied for control of Cereal Leaf Beetle (*Oulema melanopus* L.).

Plots were planted 24 October 2014 at Kinston and 4 November 2014 at Plymouth. At Kinston, nitrogen was applied pre-plant at a rate of 33.6 kg/ha and at Plymouth, in liquid form, at a rate of 187.1 L/ha of 30 percent nitrogen solution for a total of 62.3 kg/ha of nitrogen. Top-dressed potassium, phosphorus and micro nutrients were applied as recommended by soil test results for both locations. On 15 December 2014 in Kinston and 5 December 2014 in Plymouth, Thifensulfuron-methyl plus Tribenuron-methyl (Harmony® Extra, Dupont, Wilmington, DE) was applied for broadleaf weed control at a rate of 0.04 L/ha and 0.05 L/ha. Spring nitrogen was applied, in liquid form, at a rate of 336.7 L/ha of 24 percent nitrogen solution for a total of 100.9 kg/ha of nitrogen on 3 March 2015 in Kinston and at a rate of 187.1 L/ha of 30 percent nitrogen solution for a total of 62.3 kg/ha of nitrogen on 11 March 2015 in Plymouth. On 23 April 2014,
Lambda-cyhalothrin\textsuperscript{1,2} (Warrior II with Zeon Technology, Syngenta, Greensboro, NC) was applied to control Cereal Leaf Beetle in Kinston.

**Morphological Traits**

Worthington et al. (2013) identified morphological traits correlated with ryegrass suppression in winter wheat in North Carolina. Protocols outlined in this previous study were utilized in this experiment.

Three plant height measurements were recorded at Zadoks Growth Stage (GS) 29-30 (HT1), GS 55 (HT2) and GS 70-80 (HT3) (Zadoks et al., 1974) at each location in both 2014 and 2015. Each height measurement was taken within the 1.5 m\textsuperscript{2} ryegrass planted section of each plot. Before head emergence (Zadoks GS 25-30), plant height was estimated as the distance from ground level to the top of the canopy. Plant heights during and after head emergence (Zadoks GS 55 and 70-80), were estimated as the distance from ground level to the tip of the average head, excluding awns. After collection of the three height measurements, an area under the height progress curve (AUHPC) (Worthington et al. 2013), was calculated to measure the height accumulation throughout the growing season:

\[
\text{AUHPC} = \sum_{i=0}^{n} [(H_{i+1} + H_{i})/2][X_{i+1} - X_{i}]
\]

where, \(H_{i}\) = height at the \(i\)\textsuperscript{th} observation, \(X_{i}\) = time, in Julian days, at the \(i\)\textsuperscript{th} observation, and \(n\) = the total number of observations. It was assumed that on Julian day one (January 1\textsuperscript{st}), all genotypes had equal height within each location.

Visual ratings for Early Vigor (Vig1) were recorded at Zadoks GS 29-30 at each location in both 2014 and 2015 growing seasons. Vigor ratings were based on a 1 to 9 scale that combined estimates of the percent ground cover by wheat foliage and plant height taken in the center 1.5
m² section of each plot. The genotypes with the combination of greatest ground cover and height received a rating of 1, whereas the genotypes with the combination of least ground cover and height were given a rating of 9. Late vigor (Vig2) was recorded at Zadoks GS 55 at each location in both 2014 and 2015 growing seasons. The same protocol as described above for Early Vigor was utilized in the visual ratings. At each rating, only the 1.5 m² ryegrass planted section of each plot was evaluated. The ability to distinguish between ryegrass and wheat canopies was facilitated by the fact that Italian ryegrass plants have narrow leaf blades, no hairs and a glossy green color due to the waxy surface of the leaf blades. Wheat plants, in contrast, had much wider leaves with lighter green to grey coloration.

Heading dates for the RILs were recorded at the Lake Wheeler Road Field Laboratory in Raleigh, NC during the 2012, 2013 and 2014 growing seasons (Petersen, 2015). Each RIL was planted in 1.2-m long rows with a seeding rate of 40-60 seeds per row, using a random complete block design with two replications. Heading dates were recorded for each RIL when 50 percent of the heads in the row have emerged from the sheath. Significant differences among genotypes (p < 0.01) were found previously by Petersen (2015). Adjusted means were calculated from these data and used in this study.

Ryegrass head counts are highly correlated with ryegrass biomass (Worthington et al., 2013). Ryegrass head counts were recorded at Zadoks GS 70-80 by counting the number of ryegrass seed heads within a 0.5 m² quadrat placed over the center of the ryegrass planted section of each plot.

**Statistical Analysis**

Each year by location combination was treated as a separate environment. A model including environments, replications within an environment, lines, and the interaction between
genotypes and environment was analyzed for ryegrass counts and morphological parameters using “PROC MIXED” in SAS v9.4 (SAS Institute Inc.). Replications and environments were considered random effects and genotypes where treated as fixed effects to calculate least square (LS) means. Distributions of progeny values for the dependent variables were assessed for normality using “PROC UNIVARIATE”. Least Square means for parents and progeny were generated both within each environment and across all environments for use in QTL analysis. Pearson Correlation Coefficients were calculated for all measured parameters to determine which morphological traits were correlated with ryegrass suppression. A Chi-squared test was calculated for each identified QTL to see if the QTL followed the expected 1:1 segregation ratio.

Heritabilities were estimated on a per-plot basis and on an entry-mean basis for each of the seven traits according to Holland et al. (2002). The SAS code from the website http://www4.ncsu.edu/~jholland/heritability/Inbreds.html was modified to calculate each heritability estimate.

**Genetic Analysis**

A genetic linkage map previously constructed by Maloney (2012) and later updated by Petersen (2015) using MapMaker/Exp version 3.0 (Lincoln et al., 1993) was utilized for the QTL analysis. The linkage map contains twenty seven linkage groups with 1839 makers, where 1452 were single nucleotide polymorphism (SNP), 83 were simple sequence repeats (SSR), 302 were diversity array technology (DArT) markers and two KASP assays. For each morphological trait within and across environments, QTL analysis was preformed using Composite Interval Mapping (CIM) and Multiple Interval Mapping using Windows QTL Cartographer v2.5 (Wang et al., 2004). CIM result files were used to conduct MIM analyses and critical logarithm of odds
(LOD) scores to determine QTL significance ranged from 3.2 to 3.5 based on 1000 permutations for each trait. Only additive effects were estimated.
RESULTS
Phenotypic Evaluations

Data were analyzed from four environments: KIN14, SAL14, KIN15, and PLY15. The RIL population displayed a normal distribution for adjusted means of RG, Vig1, Vig2, HT1, HT2, HT3, and AUHPC across environments. Genotypic effects were significant for all traits in the across environments analyses (Table 1). Across all environments the random-effect factors (environments, replication within environment, genotype, and genotype x environment interaction), were all significant, except for genotype x environment interaction for RG and HT1.

For each individual environment, genotypic effects were found significant for RG in KIN15 and not significant in PLY15, SAL14, and KIN14 (Table 2). For HT1, HT2, and AUHPC genotypic effects were significant in KIN15, SAL14, and KIN14, but not in PLY15. Significant genotypic effects were found for HT3 and Vig2 in all environments. For Vig1, significant genotypic effects were found at KIN14 and KIN15, but not at SAL14 or PLY15.

Entry mean and per plot heritability estimates were calculated for each of the seven traits (Table 1). The per plot heritability estimates ranged from 0.06 for Vig1 and Vig2 to 0.35 for HT1. The entry mean heritability estimates ranged from 0.30 for Vig2 to 0.80 for HT1.

For each trait, parental and progeny adjusted means were used to calculate the least significant differences (LSD) to determine the frequencies of transgressive segregates present among the 140 RILs (Table 3). Each trait with a significant genotypic effect was evaluated within and across environments. The frequencies of transgressive segregates ranged from 6 for HT2 at SAL14 to 112 Vig1 at KIN14. For RG, there were 89 transgressive segregates at KIN15 and 62 across all environments. For HT1, there were 30 transgressive segregates at KIN14, 17 at SAL14, 22 at KIN15, and 31 across all environments. For HT2, there were 17 transgressive segregates at KIN14, 6 at SAL14, 41 at KIN15, and 40 across all environments. For HT3, there
were 69 transgressive segregates at KIN14, 31 at SAL14, 53 at KIN15, 64 at PLY15, and 35 across all environments. For AUHPC, there were 17 transgressive segregates at KIN14, 12 at SAL14, 32 at KIN15, and 13 across all environments. For Vig1, there were 69 transgressive segregates at KIN14, 112 at KIN15, and 61 across all environments. For Vig2, there were 78 transgressive segregates at KIN14, 22 at SAL14, 69 at KIN15, 61 at PLY15, and 50 across all environments.

Pearson Pairwise Correlation Coefficients between the adjusted means for RG verses each trait across all environments were highly significant for all traits (r = -0.45 to 0.29, P < 0.0001) except for HD which was significant (r = 0.06, P < 0.0409) (Table 4). The strongest correlations were between RG and AUHPC (r = -0.45), HT1 and RG (r = -0.37), Vig1 and RG (r = 0.29), and Vig2 and RG (r = 0.27) and the lowest was between RG and HD (r = 0.06) across all environments. To visualize this correlation, scatter plots were created to visualize the relationship between AUHPC and RG, HT1 and RG, Vig1 and RG, and Vig2 and RG (Figure 1). At KIN15, Vig1 and Vig2, were highly significant and the remaining traits were not significant. With vigor ratings taken on a 1 to 9 scale, with 1 being most vigorous and 9 being least vigorous, the positive correlation between RG and both vigor ratings showed that a less vigorous plant had higher amounts of ryegrass present at the end of the season.

**QTL Mapping**

The QTL identified by this research were deemed reproducible if found in more than one environment and/or if identified as associated with more than one trait. Five reproducible QTL for ryegrass suppression, or traits correlated with ryegrass suppression, were mapped to chromosomes 2B, 5B, 6A, and 7D (Table 5). LOD scores for these QTL varied from 3.3 to
21.0 and $R^2$ values from 6.2 to 51.6%. Logarithm of Odds peaks for the five identified QTL are displayed in Figure 2.

Chromosome 2B

The number of RIL’s containing the 2BS:2GS:2GL:2BL translocation chromosome from NC-Neuse was 120 of the 140 RILs. A Chi-square test showed the lack of an expected 1:1 segregation ratio for the translocation in this RIL population (data not shown). This uneven transmission of this translocation has been previously described (Nyquist, 1962; McIntosh and Luig, 1973; Tsilo et al., 2008). *Qrgj.nc-2B* mapped to the *Ppd-B1* region associated with photoperiod sensitivity (Table 5). AGS 2000 contains the insensitivity allele at *Ppd-B1*, while NC-Neuse contains the sensitivity allele. The presence of the AGS 2000 allele at QTL *Qrgj.nc-2B* was associated with increased HT2, HT3 and the related AUHPC, while the NC-Neuse allele at this QTL delayed heading by approximately one day. The AGS2000 allele was not associated with a plant height increase at HT1. Variation explained by this QTL on HT2 and HT3 ranged from 12 to 22 percent. The NC-Neuse allele at *Qrgj.nc-2B* delayed the HD across all environments and explained 7.4% of the variation.

Chromosome 5B

The percentage of RIL’s containing the contrasting parental alleles at the two QTL located on chromosome 5B were not different from the expected 1:1 segregation ratio verified by a Chi-squared test (data not shown). *Qrgj.nc-5B-1* mapped to the *Vrn-B1* region associated with vernalization requirement. AGS 2000 contains the short vernalization allele *Vrn-B1*, while NC-Neuse contains the long vernalization allele at this locus.
The presence of the AGS 2000 allele of the QTL *Qrgj.nc-5B-1* was associated with increased HT1, HT2 and the related AUHPC, while the NC-Neuse allele at this QTL delayed heading date by approximately one day. In contrast to the QTL associated with *Ppd-B1*, the AGS2000 allele at *Qrgj.nc-5B-1* QTL increased HT1 across all environments and explained 51.6% of the variation for this trait across environments. Similar to the QTL associated with *Ppd-B1* it increased HT2 at KIN14. The AGS2000 allele at *Qrgj.nc-5B-1* was associated with increased AUHPC in two environments and explained between 21.5 and 29.4 percent of the variation for this trait. The NC-Neuse allele at *Qrgj.nc-5B-1* delayed the HD across all environments and explained 27.3% of the variation.

The second QTL identified was *Qrgj.nc.5B-2* located in the more distal region of chromosome 5B. The AGS 2000 allele of the QTL *Qrgj.nc-5B-2* decreased Vig1 and Vig2 at KIN15 and explained 6.2% of the variation for Vig1 and 15.3% of the variation for Vig2.

**Chromosome 6A**

The percentage of RIL’s containing the contrasting parental alleles at the QTL located on chromosome 6A was not different from the expected 1:1 segregation ratio verified by a Chi-squared test (data not shown). *Qrgj.nc-6A* was associated with plant vigor and height in KIN14 only. The AGS 2000 allele at the QTL *Qrgj.nc-6A* decreased Vig2 and explained 16.3% of the variation for Vig2. The NC-Neuse allele of the QTL *Qrgj.nc-6A* increased HT3 and explained 14.6% of the variation for HT3.
Chromosome 7D

The percentage of RIL’s containing the contrasting parental alleles at the QTL located on chromosome 7D.2 was not different from the expected 1:1 segregation ratio verified by a Chi-squared test (data not shown). The NC-Neuse allele of the QTL Qrgj.nc-7D.2 increased RG at KIN15 and decreased Vig1 and Vig2 at KIN15. The QTL explained 10.9% of the variation for RG, 21.8% of the variation for Vig1, and 12.6% of the variation for Vig2.
DISCUSSION
Significant genetic variation was observed among RIL in this experiment for all phenotypic traits measured. Worthington et al. (2013) compared the ability of different screening methods for indirect selection for Italian ryegrass suppression and demonstrated a strong correlation between ryegrass-to-wheat biomass and ryegrass seed head counts \( r = 0.94, P < 0.01 \). It was found that increased height throughout the season and early plant vigor had a positive association with weed suppressive ability in wheat. Because ryegrass-to-wheat biomass was highly correlated with ryegrass seed head counts, the phenotypic traits identified by this research with the strongest correlation with reduction in RG were AUHPC, height at Zadoks GS 29-30 (HT1), Vig1 (Zadoks GS 29-30), and Vig2 (Zadoks GS 55). To visualize this correlation, scatter plots were created to visualize the relationship between AUHPC and RG, HT1 and RG, Vig1 and RG, and Vig2 and RG (Figure 1). These results were in agreement with results presented in Worthington et al. (2013); Challaiah et al. (1986); Blackshaw (1994); Acciaresi et al (2001); and Wicks et al. (1986) for wheat. This indicated that ryegrass suppression was most closely associated with taller, more vigorous, and upright plants starting early in the growing season and maintaining these characteristics through head emergence.

Pearson correlations coefficients between RG and each trait were calculated to see which of the morphological traits would confer weed suppression (Table 4). When comparing the correlation between 2014 and 2015, correlations in 2014 were much stronger than 2015. The correlation in 2014 ranged from the strongest between RG and AUHPC \( r = -0.57, P < 0.0001 \) and the weakest between RG and HD \( r = 0.14, P < 0.05 \). In 2015, the strongest correlation was between RG and Vig2 \( r = 0.36, P < 0.0001 \) and the weakest correlation was between RG and HT1 \( r = -0.18, P < 0.01 \). The differences in the correlation from 2014 to 2015 likely can be accredited to environmental factors affecting each location. At KIN15, there were soil fertility
issues caused by un-seasonably cold temperatures over the winter causing a lack of nutrient movement within the soil, accompanied by a fall Hessian fly infestation. With stunted growth from poor soil fertility and a reduction in tillers from the fall Hessian fly infestation, the Italian ryegrass was able to obtain a greater amount of establishment over the struggling wheat within each plot. At PLY15, poor drainage caused a problem with water accumulation within the experiment. Standing water was present within the center part of the field for a large part of the winter causing a reduction in growth, in turn reducing the competitive ability of the wheat. Once the water was able to drain, the wheat had to work harder to maintain competitive ability with the Italian ryegrass that was less affected by the wet conditions over the winter. In SAL14 and KIN14, there were normal, more favorable growing seasons for wheat. This impact of a more favorable environment can be seen in the higher correlation between RG and other traits. The wheat was able to grow throughout the winter and was able to better compete with the Italian ryegrass.

Worthington et al. (2013) recorded three individual height measurements at Zadoks GS 31, 69, and 80 with coefficient of variation (CV) percentages of 7.6 (GS 31), 3.2 (GS 69), and 3.5 % (GS 80). For this research, three individual height measurements were taken at GS 30 (HT1), 55 (HT2), and 70-80 (HT3) yielding similar CV percentages of 12.0 (HT), 7.1 (HT2), and 5.0% (HT3) respectively (Table 1). Worthington et al. (2013) identified early vigor at GS 29 as important to weed suppressive ability in wheat. Early vigor ratings were collected at GS 30 yielding a CV of 32.4%, showing similar results to the early vigor ratings CV of 27.6% from Worthington et al. (2013). The slightly higher CV percentages were expect in this research when compared to the Worthington et al. (2013) results due to a large number of lines in the RIL population, whereas Worthington et al. (2013) evaluated a small number of unrelated,
commercial varieties available to producers. By evaluating a larger number of plots, the amount of human error and intra-replication variation increases, thus causing an increase in CV percentages compared to previous research.

The entry mean heritabilities estimates ranged from 0.30 for Vig2 to 0.80 for HT1 (Table 1). The three height measurements and AUHPC displayed the highest entry mean heritabilities of 0.80, 0.74, 0.65, and 0.78 respectively. Per plot heritability estimates ranged from 0.06 for Vig1 and Vig2 to 0.35 for HT1. Again, the three plant height measurements and AUHPC had the highest per plot heritabilities of 0.35, 0.30, 0.23, and 0.33. These results were expected as plant height is a highly heritable trait in wheat. With early height shown to be correlated with weed suppressive ability and being highly heritable, breeders can use this to advantage when screening genotypes for weed suppression both in early generations in head rows and later generations in replicated trails. Ryegrass head counts (RG), Vig1, and Vig2 displayed lower entry mean heritability’s of 0.41, 0.33, and 0.30 and low per plot heritability of 0.08, 0.06, and 0.06. Thus evaluation of these traits must be done in replicated, multi-location evaluations in later selection generations.

Compared to Worthington et al. (2013) the heritability estimates from this research were lower. The lower heritability estimates were likely due to the large environmental impact on the performance of these traits and the larger number of genotypes assessed which may have resulted in greater random error. Worthington et al. (2013), used plots three meters long, seven rows wide with 17.1 cm row spacing, thus likely experiencing less residual error, and increasing the heritability estimates. Because the use of smaller plots sizes likely decreased the heritability estimates, plot size should not be smaller than the plot length of 2.4 m, with 19.1 cm row spacing.
and over seeded with Italian ryegrass in the center \(1.5 \text{ m}^2\) section of each plot used in this research.

Genotype by environment (GxE) interaction was significant for HT2, HT3, AUHPC, Vig1, and Vig2 traits, but was not significant for both RG and HT1 traits across all environments. The significant GxE interaction for the five traits indicated that each trait had a combination of alleles that allowed the RIL to perform better in some environments and worse in others. Within organic breeding, GxE interaction plays a major role in the ability to produce effective cultivars. Murphy et al. (2007) evaluated the test weight and grain yield of wheat cultivars grown in conventional and organic systems. It was found that grain yield and test weight had a significant genotype by system interaction, suggesting the need for an independent organic breeding program. Even with the continued increase in hectares planted and double-digit increase in consumer demand, the overall small number of hectares produced every year in North Carolina probably does not warrant a separate organic wheat breeding program alongside the Small Grains Breeding Program at NCSU at this time. Due to the limits placed on organic systems, in terms of synthetic chemical usage, organic breeders have to focus their programs toward these characteristics as well as maintaining the standard grain yield, test weight, end-use quality, and deal with the GxE interactions. In order to continue producing organic wheat cultivars, breeders will have to look at expanding the adaption of organic cultivars across more environments or breed cultivars for each unique environment to help address the GxE concerns.

Increased weed suppression, nutrient-use efficiency, and resistance to disease and insect damage are important area of research critical for the continued advancement of organic wheat production (Murphy et al. 2007). This research showed breeders which traits and situations can be used to aid in the development of cultivars with organic weed suppression. Adversely, this
research has shown situations were organic weed suppression is less to non-effective. Unfortunately we did not count ryegrass fall emergence in this research, which could be compared to end of season ryegrass head counts. So the direct comparison of mean RG between locations does not reflect the mean suppression of wheat at different locations. With the differences in environmental factors from 2014 to 2015, it was apparent that weed suppression is dependent, to some extent, on the control of other biotic and abiotic stresses. At KIN15, plots were infested with Hessian fly in the fall followed by poor soil fertility. The Hessian fly infestation caused a reduced number of tillers and stunted growth going into vernalization over the winter months. The winter was cooler than normal and induced soil fertility issues, placing a larger amount stress on the wheat plants. Once temperature began to warm, the Italian ryegrass was able to increase establishment within the plots and reduce the ability of the wheat to compete. This caused an increasing in the amount of ryegrass heads and decreasing wheat biomass within each plot. At PLY15, proper field drainage likely would have helped with weed suppression. Soil was saturated with little to no drainage over the fall and winter months. The water-logged field caused a reduction in fall wheat growth going into vernalization over winter. This reduction in fall growth mirrored the same effects as Hessian fly infestation. Once the temperatures began to warm up and the water drained from the field, the Italian ryegrass was more competitive in comparison to the wheat, thus increasing the number of ryegrass heads.

Weed suppression, nutrient-use efficiency, and resistance to disease and insect are problems that affect both the organic and conventional wheat producers. Conventional producers have an advantage over organic producer due to the ability to use synthetic fertilizers, herbicides, and insecticides to correct production problems that arise whereas organic producers relay on organically approved fertilizers, herbicides and insecticides which may be limited in efficacy
under certain situations. Because organic producer are limited in their ability to use chemicals and with the increase in chemical resistance in conventional systems, having cultivars with genetic weed suppression, nutrient-use efficiency, and resistance to disease and insect will give wheat producers the ability to continue producing high quality, high yielding wheat cultivars. But, while breeders move toward improving these traits, organic producers will have to understand that organic weed suppression is not always feasible in every location, every season and must evaluate their individual situation.

Multiple QTL that were associated with ryegrass suppression traits were identified in this research based upon the phenotypic data collected from four, ryegrass over-seeded wheat trails. Five QTL were significant in multiple environments and/or traits. These QTL were mapped to chromosomes 2B, 5B, 6A, and 7D.2 (Table 5).

_Qrgj.nc-2B_ is an interesting large effect QTL due to it being effective across KIN14, SAL14, KIN15, for HT2, HT3, and AUHPC and across all environments for HD with an R^2_ ranging from 7.4 to 21.9%. AGS 2000 was the contributing parent for larger HT2, HT3, and AUHPC, while NC-Neuse was the contributing parent for later HD. This QTL is co-localized with the _Ppd_B1_ locus that was mapped to the short arm of chromosome 2B (Scath & Law, 1983, 1984; Welsh et al., 1973). Guedira et al. (2014) conducted marker analyses on the AGS 2000 x NC-Neuse population and found a difference between the two parents at the _Ppd_B1_ on chromosome 2B. The NC-Neuse allele of _Ppd_B1_ could not be amplified so no results were given, but it was shown that AGS 2000 contained the _Ppd_B1a_ insensitivity allele that was found in the cultivar Sonora (Diaz et al., 2012). There is distortion in the segregation ratio of the _Ppd-B1_ locus, due to the presence of the 2BS:2GS:2GL:2BL translocation chromosome.
Within this population, 120 of the 140 RILs contained the NC-Neuse allele at Ppd_B1 due to the segregation distortion caused by this translocation.

Selecting at this QTL could enhance the ability of a breeder to incorporate ryegrass suppression into future wheat cultivars. Once vernalization requirements were met, RILs with the Ppd-B1a insensitivity allele from AGS 2000 displayed greater plant heights throughout the season (i.e. HT2, HT3, and AUDPC). With the ability to initiate reproductive growth sooner, the wheat will gain a competitive advantage over the ryegrass. It is important to remember, however, that late spring freeze events are fairly common in North Carolina. Wheat lines lacking photoperiod sensitivity will begin reproductive growth earlier, thus causing earlier heading dates and increasing the risk of damage from a late spring freeze event. By selecting for this QTL, breeders can increase plant heights in turn increasing ryegrass suppression, but they have to maintain awareness of late spring freeze events and try to avoid moving heading date too early. AGS 2000 would be regarded as too early, in general, for routine production in North Carolina.

Qrgj.nc-5B-1 is another large effect QTL due to it being effective in KIN14, SAL14 for HT2 and AUHPC and across all environments for HT1 and HD. It mapped to chromosome 5B with R² ranging from 8.7 to 51.6%. AGS 2000 contributed a large effect allele that increased HT1, HT2, and AUHPC traits associated with large LOD peaks. The NC-Neuse allele contributed to later HD, across all environments. This QTL is co-localized with the Vrn-B1 locus mapped to the long arm of chromosome 5B (Galiba et al., 1995; Law et al., 1976; Worland et al., 1987). Guedira et al. (2016) identified large effect QTLs on chromosome 5B and identified two QTL peaks, labeled Qhd.2W-5B and Qhd.4W-5B. The two QTL peaks were centered over the KASP markers for the Vrn-B1 locus, which coincides with the same position
as the \textit{Qrgj.nc-5B-1} QTL identified in this research. The similarity of the results from both studies, suggested that vernalization requirements play a large role in the ability of a cultivar to convey ryegrass suppression.

By selecting at the \textit{Qrgj.nc-5B-1} QTL breeders can implement ryegrass suppression within future cultivars. The RILs containing the AGS 2000 allele for \textit{Vrn-B1} displayed increased plant heights throughout the season (i.e. HT1, HT2, and AUHPC), due to a decreased vernalization requirement. With the decreased vernalization requirement, the RILs were able to initiate plant growth earlier in the growing season, thus increasing establishment and competitive ability over the ryegrass. However, as with the \textit{Qrgj.nc-2B} QTL, breeders will have to maintain awareness of late spring freeze events. By decreasing the vernalization requirement, the heading date would be moved earlier increasing the chance of damage from late spring freeze. By selecting for this QTL, breeders will be able to increase the plant heights throughout the growing season in turn increasing the ryegrass suppressive ability of the cultivars. Breeders will have to remain vigilant to the potential for late spring freeze events.

\textit{Qrgj.nc-5B-2} mapped to the telomeric end region of chromosome 5B and had $R^2$ ranging from 6.2 to 15.3\%. AGS 2000 contributed the parental allele that decreased plant vigor at this locus in KIN15 only. KIN15 was a fairly unique environment with Hessian fly and fertility issues. Two relevant loci and QTL have been mapped on chromosome 5. The \textit{Rht9} locus is a gibberellin (GA) sensitive height reducing (\textit{Rht}) gene discussed by Worland et al. (1984) and a reduced height QTL \textit{QHt.crc-5B} identified by Haen et al. (2004). Alleles at these two loci may have had a role in this unique environment by impacting plant vigor due to plant vigor being a combination of percent ground cover by wheat foliage and plant height.
Selecting at the $Q_{rgj.nc-5B-2}$ QTL, breeders can improve the vigorous early spring growth of cultivars increasing the ryegrass suppressive ability. The RILs containing the NC-Neuse allele for this QTL displayed an increase in the vigorous growth of wheat throughout the growing season (i.e. Vig1 and Vig2) giving the wheat the ability to out compete the ryegrass within the plots. The vigor ratings were based on a 1 to 9 scale that combined estimates of the percent ground cover by wheat foliage and plant height taken in the center 1.5 m$^2$ section of each plot. With the previous two QTLs, which increase plant heights throughout the growing season, selection for this QTL will allow breeders to increase the plant height and amount of ground coverage from wheat foliage within future cultivars. By increasing the vigorous growth, the wheat will be able to out compete the ryegrass with space, light, and nutrients.

$Q_{rgj.nc-6A}$ mapped to chromosome 6A and had R$^2$ ranging from 14.6 to 16.3%. This QTL was associated with LOD peaks for HT3 and Vig2 at the KIN14 environment only. This environment was deemed a ‘normal’ North Carolina wheat environment. The NC-Neuse allele increased plant height and the AGS 2000 allele decreased plant vigor. Both traits were mapped to within 1 cM of each other, having peaks at 86.4 cM (HT3) and 85.4 cM (Vig2) with these peaks being at marker $wPt_{6951}$. There have been three $Rht$ genes, $Rht14$ from Castelporziano (PI 347731), $Rht16$ from Edmore M1 (PI 499362), and $Rht18$ from Icaro (PI 503555), mapped on the short arm of chromosome 6A (Watanabe, 2008). Würschum et al. (2015) identified a medium effect QTL on chromosome 6A and that was preferentially used in combination with $Rht-D1b$ allele in European winter wheat. So perhaps one or more of these $Rht$ alleles and QTL could have an effect on plant height and plant vigor.

Selecting for the NC-Neuse allele at $Q_{rgj.nc-6A}$ QTL, breeders could help to increase late season plant height and plant vigor at heading (i.e. HT3 and Vig2). With the increased late
season plant height, the wheat will continue to out compete the ryegrass for space, light, and nutrients.

*Qrgj.nc-7D.2* mapped to chromosome 7D.2 and had an $R^2$ ranging from 10.9 to 21.8% for RG, Vig1 and Vig2 at the KIN15 environment only. The NC-Neuse allele at this locus increased the amount of ryegrass seed heads (RG) and decreased plant vigor throughout the growing season (Vig1 and Vig2). The *Vrn-D3* allele is located on chromosome 7D, potentially associated with this identified QTL (Yan et al., 2006). McCartney et al. (2003) identified the *QMat.crc-7D* QTL that is linked to an early maturing allele on the distal end of the short arm of chromosome 7D.

Selecting for the AGS2000 allele at *Qrgj.nc-7D.2* QTL could allow breeder to increase vigorous growth throughout the season and decrease the number of ryegrass heads present. For a breeder, selection at this QTL would help in the implementation of ryegrass suppression in future cultivars by increasing the vigorous growth throughout the season and reducing the amount of ryegrass seed heads present at the end of the growing season.

An examination of the allelic content at the five identified QTL for the top 10 and worst 10 RILs for number of ryegrass heads provided some insights into the best allelic combination to select in this population (Table 6). At both the *Vrn-B1* locus on chromosome 5B (*Qrgj.nc-5B-1*) and at the *Vrn-D3* locus on chromosome 7D.2 (*Qrgj.nc-7D.2*) the AGS 2000 allele was observed in eight of the top 10 RIL for ryegrass suppression. Similar results were found for the worst RIL for ryegrass suppression, except both QTL had the NC-Neuse allele present at most, or all, RIL. The shorter vernalization and associated increase in plant height throughout the season and better plant vigor associated with the AGS alleles appeared to impact ryegrass suppression in these extreme lines.
At the \textit{Ppd\_B1} locus on chromosome 2B (Qrgj.nc-2B), the AGS 2000 allele was present at the highest frequencies in both the best and worst 10 RILs. This suggested that the allelic content at this locus was not as critical for ryegrass suppression in contrast to what was observed at the vernalization loci. AGS 2000 is regarded as too early for routine production in North Carolina and one might have considered it be beneficial to have the NC-Neuse allele at the \textit{Ppd\_B1} locus. But RIL containing the AGS 2000 allele at the \textit{Ppd\_B1}, \textit{Vrn\_B1}, and \textit{Vrn\_D3} loci displayed wide variation for HD, thus there is still considerable scope for selection for HD to avoid damage from late spring freezing even with the AGS 2000 alleles at these three loci. At the \textit{Qrgj.nc-5B-2} and \textit{Qrgj.nc-6A} QTL, both parental alleles were observed at roughly the same frequencies in both the 10 best and worst RILs. This indicated that selection for either parental allele at these QTLs will likely convey similar amounts of ryegrass suppression.

Herbicide resistant weeds are a serious problem plaguing the agricultural community today. The objective of this study was to identify QTL associated with Italian ryegrass suppression in the AGS 2000 x NC-Neuse soft red winter wheat RIL population. Phenotypic traits identified to have the strongest correlation with reduction in ryegrass seed head counts were AUHPC, height at Zadoks GS 29-30 (HT1), Vig1 (Zadoks GS 29-30), and Vig2 (Zadoks GS 55). This indicated that ryegrass suppression was most closely associated with taller, more vigorous plants starting early in the growing season and maintained through head emergence. Five QTL were identified as associated with increased ryegrass suppressive ability. Major effect QTL such as \textit{Ppd\_B1}, located on chromosome 2B, \textit{Vrn\_B1}, located on chromosome 5B, and \textit{Rht} height reducing genes on chromosome 6A were associated with ryegrass suppression or correlated traits. Overall this research indicated that selecting lines containing the AGS 2000 allele at both the \textit{Vrn\_B1} and \textit{Vrn\_D3} loci, while selecting for either parental allele at the
*Ppd-B1, Qrgj.nc-5B-2, and Qrgj.nc-6A* loci, combined with selection for tall plants at GS 29-30, with vigorous, erect growth providing both ground cover and increased plant height accumulation throughout the entire growing season with a medium heading date, should improve ryegrass suppressive ability in North Carolina adapted wheat cultivars.
Table 1. Analysis of variance significance values for AGS 2000, NC-Neuse, and the 140 progeny of an AGS 2000 x NC-Neuse recombinant inbred population evaluated in four field environments for ryegrass counts (RG), heading date (HD), height at growth stage 30 (HT1), height at growth stage 55 (HT2), height at growth stage 70-80 (HT3), area under the height progress curve (AUHPC), vigor at growth stage 30 (Vig1), and vigor at growth stage 55 (Vig2).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>RG</th>
<th>HT1</th>
<th>HT2</th>
<th>HT3</th>
<th>AUHPC</th>
<th>Vig1</th>
<th>Vig2</th>
</tr>
</thead>
<tbody>
<tr>
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<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
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<td>Environment</td>
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<tr>
<td>Genotype*Environment</td>
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<td>0.1779NS</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td>0.0019**</td>
<td>0.0052**</td>
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<td>Entry Mean Heritability ††</td>
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<td>0.78</td>
<td>0.33</td>
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<tr>
<td>Per Plot Heritability ††</td>
<td></td>
<td>0.08</td>
<td>0.35</td>
<td>0.30</td>
<td>0.23</td>
<td>0.33</td>
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</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.20</td>
<td>0.04</td>
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<td>0.04</td>
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</tr>
<tr>
<td>CV %</td>
<td></td>
<td>35.2</td>
<td>12.0</td>
<td>7.1</td>
<td>5.0</td>
<td>6.5</td>
<td>32.4</td>
<td>36.1</td>
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</table>

† Significance levels are indicated as: NS (p>0.05), *(p<0.05), ***(p<0.001).

††Entry mean and Per Plot heritabilities are for each trait across all environments.
Table 2. The significance of genotypic effects for AGS 2000, NC-Neuse, and the 140 progeny of an AGS 2000 x NC-Neuse recombinant inbred population evaluated in each of the four field environments for ryegrass counts (RG), heading date (HD), height at growth stage 30 (HT1), height at growth stage 55 (HT2), height at growth stage 70-80 (HT3), area under the height progress curve (AUHPC), vigor at growth stage 30 (Vig1), and vigor at growth stage 55 (Vig2).

<table>
<thead>
<tr>
<th>Environment††</th>
<th>RG</th>
<th>HT1</th>
<th>HT2</th>
<th>HT3</th>
<th>AUHPC</th>
<th>Vig1</th>
<th>Vig2</th>
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<td>KIN15</td>
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<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
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<td>PLY15</td>
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‡ Significance levels of genotypic effects are indicated as: NS (p>0.05), *(p<0.05), **(p<0.01), ****(p<0.0001).
††KIN15, Kinston, NC 2015; PLY15, Plymouth, NC 2015; SAL14, Salisbury, NC 2014; KIN14, Kinston, NC 2014
Table 3. Adjusted means for parents, AGS 2000 and NC-Neuse, and 140 RILs average, minimum, and maximum trait values for ryegrass counts (RG), height at growth stage 30 (HT1), height at growth stage 55 (HT2), height at growth stage 70-80 (HT3), area under the height progress curve (AUHPC), vigor at growth stage 30 (Vig1), and vigor at growth stage 55 (Vig2) calculated for four individual field environments as well as an across environments calculation.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Environment*</th>
<th>AGS 2000</th>
<th>NC-Neuse</th>
<th>Progeny Min</th>
<th>Progeny Max</th>
<th>Progeny Avg.</th>
<th>LSD (0.05)</th>
<th>CV%</th>
<th>T.S. ‡</th>
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<tbody>
<tr>
<td>RG</td>
<td>KIN15</td>
<td>127.8</td>
<td>131.1</td>
<td>59.0</td>
<td>273.0</td>
<td>126.1</td>
<td>17.4</td>
<td>31.2</td>
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<tr>
<td>RG</td>
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<td>119.1</td>
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<tr>
<td>HT1</td>
<td>KIN14</td>
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Table 3. continued

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<th>NC-Neuse</th>
<th>Progeny Min</th>
<th>Progeny Max</th>
<th>Progeny Avg.</th>
<th>LSD (0.05)</th>
<th>CV%</th>
<th>T.S.††</th>
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†KIN14, Kinston, NC 2014; SAL14, Salisbury, NC 2014; KIN15, Kinston, NC 2015; PLY15, Plymouth, NC, 2015; Across, Across all four environments.

††T.S. indicates the number of Transgressive Segregates present above the high parent and below the low parent combined for each trait for each of the 140 RIL lines.
Table 4. Pearson correlation coefficients between across and within-environments adjusted means for ryegrass counts (RG) with heading date (HD), height at growth stage 30 (HT1), height at growth stage 55 (HT2), height at growth stage 70-80 (HT3), area under the height progress curve (AUHPC), vigor at growth stage 30 (Vig1), and vigor at growth stage 55 (Vig2).

<table>
<thead>
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<th>HT2</th>
<th>HT3</th>
<th>AUHPC</th>
<th>Vig1</th>
<th>Vig2</th>
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† Significance levels of correlation coefficients are indicated as: NS (p>0.05), *(p<0.05), **(p<0.01), ***(p<0.0001).
Table 5. Characteristics of the five QTL detected for ryegrass counts (RG), heading date (HD), height at growth stage 30 (HT1), height at growth stage 55 (HT2), height at growth stage 70-80 (HT3), area under the height progress curve (AUHPC), vigor at growth stage 30 (Vig1), and vigor at growth stage 55 (Vig2).

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<th>QTL</th>
<th>Parameter</th>
<th>Environment</th>
<th>Linkage Group</th>
<th>Location (cM)</th>
<th>Markers</th>
<th>R² (%)</th>
<th>Parental Source</th>
<th>Effect</th>
<th>LOD</th>
<th>LOD threshold</th>
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†Markers located closest on either side of detected QTL peaks. For QTL detected in multiple environments, listed markers flank all regions of detected QTL.

††Thresholds determined through Windows QTL Cartographer 2.5 iterative permutation testing.

†††KIN14, Kinston, NC 2014; SAL14, Salisbury, NC 2014; KIN15, Kinston, NC 2015; PLY15, Plymouth, NC, 2015; ALL, Across all environments

€A positive effect, signifies AGS 2000 donated the parental allele and a negative effect signifies NC-Neuse donated the parental allele
Table 6. The allelic frequencies at the five identified QTL for the best 10 and worst 10 RILs for ryegrass seed head counts (RG) among the 140 recombinant inbred lines (RIL) within the AGS 2000 x NC-Neuse population across all environments.

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<th>Qrgj.nc-5B-1</th>
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† "A" denote the AGS 2000 allele, "B" denotes NC-Neuse allele, and "U" denotes unknown allele
Figure 1. A scatter plot comparing ryegrass head counts (RG) to area under the height progress curve (AUHPC), height at growth stage 30 (HT1), vigor at growth stage 30 (Vig1), and vigor at growth stage 55 (Vig2) across all environments for each of the 140 RILs.
Figure 1. continued

Scatter Plot of HT1 vs RG across all environments
Figure 1. continued

Scatter Plot of Vig1 vs RG across all environments
Figure 1. continued

Scatter Plot of Vig2 vs RG across all environments
Figure 2. The Logarithm of odds (LOD) peaks for the five identified putative quantitative trait loci (QTL) identified through multiple interval mapping (MIM), located on chromosomes 2B, 5B, 6A, and 7D.2.
Figure 2. continued
Figure 2. continued

Chromosome 5B - Qrgj.ne.5B-2

LOD0

Vg1_KIN15
Vg2_KIN15

cM
Figure 2. continued

Chromosome 6A - Oryg i nc-64

- Vig2_KIN14
- HT3_KIN14

LOD0

0.0 25.0 50.0 75.0 100.0 125.0 150.0 175.0 200.0 225.0 251.0
0.0 1.2 2.4 3.6 4.8 6.0

63
Figure 2. continued
Figure 3 The linkage group map for each of the six identified putative quantitative trait loci (QTL) identified through multiple interval mapping (MIM), located on chromosomes 2B, 5B, 6A, and 7D.2.
Figure 3 continued
Figure 3 continued
Figure 3 continued
Figure 3 continued
REFERENCES


Yan, L., D. Fu, C. Li, A. Blechl, G. Tranquilli, M. Bonafede, and J. Dubcovsky. 2006. The wheat and barley vernalization gene VRN3 is an orthologue of FT. P. Natl. Acad. Sci USA. 103(51), 19581–19586.


